ZENECA CENTRAL TOXICOLOGY LABORATORY
ALDERLEY PARK MACCLESFIELD
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PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

by

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Product Toxicologist

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Halogenated Solvents Industry Alliance

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Performing Laboratory - Zeneca Central Toxicology Laboratory
Alderley Park, Macclesfield,
Cheshire, UK

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PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

STATEMENT OF GLP COMPLIANCE

The study described in this report was conducted in accordance with the following Good Laboratory Practice standards.

1. United States Environmental Protection Agency (Title 40 Code of Federal Regulations Part 160 - Federal Insecticide, Fungicide and Rodenticide Act)
2. United States Environmental Protection Agency (Title 40 Code of Federal Regulations Part 792 - Toxic Substances Control Act)
3. United Kingdom Department of Health (Annex to the United Kingdom Compliance Programme, 1989): Compatible with OECD 1982 (Good Laboratory Practice in the Testing of Chemicals - Final Report of the OECD Expert Group on Good Laboratory Practice, ISBN 9264 12367 9)
4. Japanese Ministry of Agriculture, Forestries and Fisheries (59 NohSan No.3850, August 10 1984)

D J Tinston ..... 26 October 1994
Study Director

Endorsed by:

I F H Purchase ..... 31 Oct 94
Director
ZENECA Central Toxicology Laboratory

PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

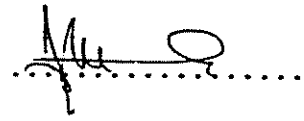
EPA FLAGGING CRITERIA

I have applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of the attached study. This study neither meets nor exceeds any of the applicable criteria.

Submitter: Zeneca Inc

Applicant: Zeneca Inc

Company Agent: G A Wickramaratne
Product Toxicologist



27/10/94

PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT
QUALITY ASSURANCE STATEMENT

In accordance with Zeneca policy and QA procedures for Good Laboratory Practice, this report has been audited and the conduct of this study has been inspected as follows:

Date	Date of QA Report
1 May 92	1 May 92
1 May 92	1 May 92
10 Jun 92	10 Jun 92
25 Aug 92	26 Aug 92
8 Oct 92	8 Oct 92
26 Nov 92	26 Nov 92
3 Dec 92	3 Dec 92
21 Dec 92	21 Dec 92
22 Dec 92	22 Dec 92
4 Feb 93	4 Feb 93
12 Feb 93	12 Feb 93
30 Apr 93	30 Apr 93
1 Oct 93	6 Oct 93
18 Oct 93	21 Oct 93
25 Nov 93	26 Nov 93
7 Dec 93	7 Dec 93
11 Jan 94	12 Jan 94
9 Mar 94	16 Mar 94
10 Mar 94	15 Mar 94
21 Apr 94	21 Apr 94
23 Aug 94	24 Aug 94
13 Oct 94	14 Oct 94
18 Oct 94	19 Oct 94
25 Oct 94	25 Oct 94
25 Oct 94	25 Oct 94

Facilities and process based procedures associated with this study were inspected in accordance with QA Standard Operating Procedures.
So far as can be reasonably established, the methods described and the results given in the final report accurately reflect the raw data produced during the study, RR0580.

G P Fuller (Unit Head, CTL Quality Assurance Unit) ..... G P Fuller ..... 25 Oct 94 ....

PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

I, the undersigned declare that this report constitutes a true record of the actions undertaken and the results obtained in the above study.

D J Tinston (Study Director) David J. Tinston 15 October 1994

PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

The following contributed to this report in the capacities indicated:

K O Rogers	(Study Investigator)	K O Rogers	24 Oct 1994
M Abdy	(Chemical Analyst)	M Abdy	21/10/94
L M Bishop	(Study Pathologist)		20/10/94
I Pate pp	(Statistician)	Pamela Bicknell	25/10/94
Reviewed by: G H Pigott	(Consultant Toxicologist)		25 Oct '94

PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

STATEMENT OF DATA CONFIDENTIALITY CLAIM

This page is not appropriate for this report.

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D J Tinston
Study Director

David J. Tinston ..... 26 October 1994

Endorsed by:

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ZENECA Central Toxicology Laboratory

M Purchase ..... 31 Oct 94

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PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

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PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

SUMMARY

Groups of 24 male and 24 female (F_0 parents) weanling rats were exposed, 6 hours per day, to 0 (Control), 100, 300 or 1000ppm perchloroethylene vapour. The rats were exposed to perchloroethylene 5 days per week for 11 weeks prior to being housed for mating (within their treatment groups) for up to 21 days during which exposure was daily. Following mating, the males continued on daily exposure until termination and the females continued daily exposure up to day 20 of gestation when they were removed from exposure. One litter (F_1A) was produced in the first generation when the dams together with their litters were exposed daily from day 6 to day 29 post partum. The second generation (F_1) parents were selected from the F_1A litters on day 29 post partum and were exposed to perchloroethylene 5 days per week for at least 11 further weeks prior to mating.

Three litters (F_2A, F_2B and F_2C) were produced in the second generation. For the F_2A litters, the dams and litters were exposed from days 6 to 29 post partum (control and 100ppm groups) or days 7 to 29 post partum (300ppm group). The dams and litters in the 1000ppm group were not exposed during lactation for this F_2A litter.

The F_2B litter was generated by mating the males and females in the control, 300 and 1000ppm groups. There was no exposure of the dams and F_2B litters during lactation. The F_2C litter was produced by mating the male controls and the males exposed to 1000ppm with unexposed stock females.

Evidence for toxicity was seen at an exposure level of 1000ppm perchloroethylene as shown by reductions in parental bodyweight gain during the pre-pairing period and lactation in both generations and during pregnancy in the second generation. A similar but less marked effect on bodyweight was also seen in the 300ppm group.

SUMMARY - continued

Histopathological changes which were confined to the kidneys of both sexes in both generations at 1000ppm were also evidence of toxicity, but no significant pathological changes were seen at 100 or 300ppm. A statistically significant dose-related reduction in adult testes weights was seen at 300 and 1000ppm perchloroethylene in the second generation but there were no associated histopathological changes in the testis at 1000ppm or effects on fertility at any exposure level. A marginal reduction in testes weights at 100ppm was not statistically significant.

Exposure to perchloroethylene was also associated with poor growth of the F_1A, F_2A and F_2B offspring at 300 and 1000ppm but reduced pup survival was confined to 1000ppm. This represented a toxic effect which, in part, may have been maternally mediated. A reduction in the proportion of pups born live at 1000ppm was evidence for a reproductive effect at this exposure level. The lack of any effect on the proportion of pups born live, pup survival and growth in the F_2C litters suggests that the changes seen in previous litters were not likely to be male mediated.

The no observed effect levels in this study were considered to be 100ppm perchloroethylene for parental and offspring toxicity and 300ppm for reproductive effects. There were no effects on fertility at exposure levels up to and including 1000ppm.

1. INTRODUCTION

Perchloroethylene is an organic solvent used primarily in dry cleaning.

The purpose of this study was to investigate the effect of inhalation of atmospheres containing perchloroethylene on the propagation of two generations of the Alpk:APfSD (Wistar-derived) strain of rat. The fertility of each generation of parental animals and the clinical condition, survival and subsequent growth of their offspring was determined.

The study design was based on the EPA TSCA guidelines for a two generation study. In each generation, rats of both sexes were exposed to the test substance for at least 11 weeks prior to being housed for mating within their treatment groups. The rats were housed for mating for up to 21 days during which exposure continued. Following mating the males continued on exposure until termination and the females continued exposure up to day 20 of gestation after which they were exposed to air only.

One litter was produced in the first generation (F_1A) when the dams together with their litters were exposed from day 6 to day 29 post partum. Three litters (F_2A, F_2B and F_2C) were produced in the second generation. For the F_2A litters, the dams and litters were exposed from days 6 to 29 post partum (control and low exposure groups) or days 7 to 29 post partum (middle exposure group). The dams and litters in the high exposure group were not exposed during lactation for this F_2A litter.

The F_2B litter was generated by mating the males and females in the control, middle and high exposure levels in order to clarify the changes seen in the F_2A litters. There was no exposure of the dams and F_2B litters during lactation. The F_2C litter was produced by mating the male controls and the males exposed to the high exposure level with unexposed females to provide further clarification of the results from previous litters.

The parameters measured provided information on male and female fertility, offspring viability, mating performance, pregnancy and lactation and included histopathological evaluation of the major reproductive organs of any suspected infertile rat. Histopathology of other organs was limited to the examination of known target organs.

The exposure levels were selected on the basis of previous range finding studies in pregnant and weanling rats conducted in this Laboratory.

The Alpk:APfSD (Wistar-derived) strain of rat was used since background information is available for this strain from reproductive studies performed in this Laboratory. The inhalation route of administration was selected as it is the likely route of exposure to perchloroethylene for man.

Throughout this report, day 1 of gestation was the day that a positive sign of mating was observed and day 1 post partum was the date of birth of the litter.

The study was conducted in the Long Term Inhalation Unit, Zeneca Central Toxicology Laboratory (CTL), Alderley Park, Macclesfield, Cheshire, UK. Exposure of the first generation animals to the test atmospheres started in May 1992, and the final post mortem examinations were carried out on the second generation in May 1993.

All raw data, samples and specimens pertaining to this study are retained in the Archives, Zeneca Central Toxicology Laboratory (CTL), Alderley Park, Macclesfield, Cheshire, UK. A copy of this report is kept in the Report Centre at the same address.

2. MATERIALS AND METHODS

2.1 Test Substance

Perchloroethylene was supplied by ICI Chemicals and Polymers Ltd., Runcorn, Cheshire, UK as a colourless liquid. A single batch (PD299) of perchloroethylene with a purity of 99.9% w/w was used throughout the study and was assigned the CTL reference number Y00207/005. The certificate of analysis is shown in Appendix A.

A bulk supply of the test substance was stored in drums under external ambient conditions. Appropriate aliquots were taken from the drums, transferred to the Inhalation Unit and stored at room temperature in ventilated cupboards until used for atmosphere generation. Re-analysis of the test substance following termination of the study demonstrated satisfactory stability (Appendix B).

2.2 Animals

Male and female Alpk:APfSD rats were obtained from the Specific Pathogen Free (SPF) colony maintained at the Barriered Animal Breeding Unit (BABU) at Zeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK. A total of 28 litters of 4 males per litter and 28 litters of 4 females per litter were delivered as follows:

19 litters of 4 males per litter	- 28 April 1992
19 litters of 4 females per litter	- 28 April 1992
9 litters of 4 males per litter	- 29 April 1992
9 litters of 4 females per litter	- 29 April 1992

All rats were supplied as weanlings (21-22 days old) segregated by sex and litter of origin. They were transported to the Inhalation Unit in sealed containers to maintain their SPF status.

In addition, a total of 52 female rats (approximately 200-290g) were supplied by BABU on 2 April 1993. From these, 48 were randomly allocated to two groups each of 24 rats which were mated with males in Groups 1 and 4 to produce the F_2C litter (see Section 2.5). These females were numbered 201-224 (mated with Group 1 males) and 225-248 (mated with Group 4 males).

2.3 Animal Accommodation and Husbandry

The rats were housed in stainless steel long-term exposure chambers of approximately 3.4m^3 capacity (Doe and Tinston, 1981) for the duration of the study. Each exposure chamber was supplied with conditioned air with a nominal temperature of 20-24°C and a nominal relative humidity of 40-60%. Temperature and relative humidity were generally within the nominal ranges with some deviations of short duration. Temperature, relative humidity, flow rate and pressure were monitored continuously by electromechanical sensors and recorded approximately every 30 minutes using a Honeywell computer (DPS6) system and a hard disk memory on a Tandon Ad-Pac machine. Twelve hour periods of light (6 am - 6 pm GMT/BST) were cycled with twelve hour periods of darkness. Rats were housed by sex in litters on arrival and the cages were fitted with solid cage floors until randomisation. After randomisation (F_0 generation) or selection (F_1 generation) the animals were housed two per cage by sex. During the pairing period one male was housed with one female.

On day 20 of gestation (F_1A, F_2A and F_2B litters) or day 16 of gestation (F_2C litter) females were re-housed in control chambers, the cages fitted with solid cage floors, and paper bedding material was supplied.

The rats were provided with powdered CT1 diet supplied by Special Diets Services Limited (Appendix C) and potable water (via water bottles) ad libitum except during exposure when all food and water were removed. When a female with a litter was scheduled for exposure the cage in which they were housed, complete with floor and bedding material, was replaced in the exposure chamber on day 5 post partum.

2.4 Experimental Design

The study consisted of one control and three treatment groups, with 24 males and 24 females per group. During the acclimatisation period of approximately one week the animals were distributed by litter amongst the four groups by a method which distributed litter mates across each group giving a similar genetic pool in each group (Appendix D). The animals were uniquely identified by ear punching with the number assigned by the experimental design (Table 1).

TABLE 1

Group	Atmospheric Concentration of Perchloroethylene (ppm, v/v)	Identities of Rats	
		Males	Females
1	0	1- 24	25- 48
2	100	49- 72	73- 96
3	300	97-120	121-144
4	1000	145-168	169-192

Each group was housed in a single chamber (one chamber per group) during the first generation pre-mating period. Additional chamber(s) per group were used during subsequent phases as necessary. In each chamber cages were supported on six adjacent levels. The animals were housed two per cage by sex to give the chamber arrangement shown in Appendix E.

An experimental card was placed on each cage identifying the treatment group, study number, sex and individual numbers of the animals. All animals selected for the F_0 generation commenced exposure on the same day whilst those selected for the F_1 generation commenced exposure immediately after selection.

2.5 Exposure Regime

Animals were exposed whole-body in long term exposure chambers to the appropriate concentration of perchloroethylene. Exposure was for 6 hours per day, 5 days per week during the pre-mating periods. During mating and gestation exposure was for 6 hours per day, 7 days per week.

F_1A Litters: Pregnant females were moved to another chamber on day 20 of gestation (after exposure) where they were exposed to air only. Dams and litters in all groups were returned to the exposure chambers at the end of an exposure period on day 5 post partum and resumed exposure on the following day. Dams with their litters were then exposed on a 7 days per week schedule from day 6 post partum until selection for the next generation parents or termination on approximately day 29 post partum. After mating, the males and any non-pregnant females continued to be exposed 7 days per week until termination. The weanling rats selected to be the F_1 parents were exposed to the appropriate concentration of perchloroethylene immediately following selection when exposure for 5 days per week commenced for the pre-mating phase of the second generation. Since the selection phase lasted for approximately 4 weeks, the duration of the pre-mating phase in the second generation was up to approximately 15 weeks.

F_2A Litters: Pregnant females were moved to another chamber at day 20 of gestation (after exposure) where they were exposed to air only. Dams and litters (with the exception of the 1000ppm group) were returned to the exposure chambers at the end of an exposure period on day 5 post partum (control and 100ppm groups) or day 6 post partum (300ppm group) and resumed exposure on the following day. Dams with litters were then exposed 7 days per week from day 6 post partum (control and 100ppm groups) or day 7 post partum (300ppm group) until termination of the litters on approximately day 29 post partum. Following termination of the litters the dams continued exposure 7 days per week. Dams and litters in the 1000ppm group were not exposed during lactation. After mating, the males and non-pregnant females in all groups continued to be exposed 7 days per week.

F_2B Litters: The females in the control, 300 and 1000ppm groups were exposed (7 days per week) for at least 2 weeks prior to mating for the F_2B litter. Exposure of the 100ppm group males and females continued (7 days per week) at this time but they were not mated for this litter. Exposure for the females during gestation was as for previous litters ie days 1 to 20, but there was no exposure of the dams and litters during lactation.

After mating, the males and any non-pregnant females continued to be exposed 7 days per week until termination. The F_2B litters were humanely killed and discarded on approximately day 5 post partum. The females in all groups were also humanely killed at this time and subjected to a post mortem examination.

F_2C Litters: The males in the control and 1000ppm groups were mated with unexposed females to produce the F_2C litter. During mating exposure of the males was daily, but the females were removed from the exposure chamber during each exposure period. The males in the 100 and 300ppm groups continued exposure but an F_2C litter was not derived from these groups. The females were not exposed during gestation or lactation. The females and the F_2C litters were humanely killed and discarded without further examination on approximately day 5 post partum.

An outline of the sequence of events during the study is shown in Appendix G.

2.6 Atmosphere Generation and Analysis

Atmospheres were generated by evaporating liquid perchloroethylene in a heat exchanger warmed to approximately 60°C. The perchloroethylene was metered into the heat exchanger using a peristaltic pump. Clean, dry air was passed through the generation equipment and the vapour/air mixture was then passed into the exposure chamber.

The air flow through each chamber was approximately 700l/min.

Trial generations were carried out prior to the start of exposure in order to determine the requisite compound flow rates to achieve the appropriate concentrations.

The methods used in analysing the test atmospheres are detailed in Appendix F. Typically, the atmospheres were sampled using an automatic air sampling system and analysed automatically using a gas chromatograph equipped with a gas sampling valve and flame ionisation detector. Each

test atmosphere (including control and room air) was analysed at least every two hours during each exposure period, with a small number of exceptions. The analysis system was calibrated using an appropriate range of freshly prepared standards prior to the study, daily during the first week of exposures and at regular intervals thereafter.

2.7 Parental Investigations

2.7.1 Clinical Condition: Prior to the start of the study all rats were examined to ensure that they were physically normal and that they exhibited normal activity. During the study all rats were observed daily for changes in clinical condition and behaviour and once weekly during the pre-mating periods a detailed examination of each rat was made. Subsequent clinical observations were recorded for those weeks when the animals were weighed, the occasions depending on the stage of gestation and lactation. In addition, animals were observed at regular intervals during each exposure. Any abnormalities or the observation of no abnormality detected were recorded. Any rats requiring euthanasia were killed and subjected to a post mortem examination. Any rats found dead were subjected to a post mortem examination as soon as possible after death.

2.7.2 Bodyweight

The duration of the pre-mating period was 11 weeks from the start of the study for the F_0 parents and at least 11 weeks from selection for the second (F_1) generation parents. The bodyweights of all rats were recorded at weekly intervals throughout the pre-mating periods. The initial weights for the F_0 parents were recorded immediately before the first exposure and the initial weights for the F_1 parents were recorded at selection.

After the pre-mating period the males were weighed approximately every four weeks until they were killed prior to post mortem examination. All females were weighed on the first day of the mating period. Subsequently, the females were weighed on presumed days 1, 8, 15 and 22 of gestation (day 1 being the day on which a sperm-positive vaginal smear was seen) and on days 1, 5, 11, 16, 22 and 29 post partum (F_1A and F_2A litters) or days 1 and 5

post partum (F_2B litter). Dams were not weighed during lactation for the F_2C litter. If there was no evidence of successful mating they were weighed at weekly intervals during the mating period as an aid to detection of pregnancy. All rats were weighed at termination.

2.7.3 Food Consumption: Food consumption for each cage of rats was recorded throughout the pre-mating periods and calculated on a weekly basis. Food consumption was recorded for F_1 rats from the time the first animal selected was placed in its cage.

2.7.4 Breeding Programme: In each generation, the females were mated with males of the same group.

After the pre-mating period, one F_0 female was continuously housed with one male from the same group ie from the adjacent cage on the same level in the chamber (odd numbers with odd numbers and even numbers with even numbers) for a maximum pairing period of 21 days. Initial pairing was done in the afternoon. Vaginal smears were taken each morning and examined to determine when mating had occurred (as shown by the presence of sperm). The presence of blood in a vaginal smear, bodyweight gain and abdominal enlargement were also used as evidence of mating, if necessary and the duration of pregnancy estimated where possible. A male showing evidence of mating with a female was separated from the female immediately and individually housed in a separate chamber. The females remained in the same position within the chamber until day 20 of gestation. There was one undetected pregnancy (Female 188, Group 4, F_2A litter) as a result of which the female littered in a cage without a solid bottom. In this case the surviving pups were humanely killed and discarded and the data for the litter were excluded.

Any female which failed to show positive indication of mating after a 21 day mating period, together with any female which did not show the expected weight gain to day 15 of gestation was remated where necessary with a different male from the same treatment group after a rest period of at least three days (so that the paternity of any ensuing litter could be

unequivocally determined). The males used in remating were ones which had shown positive indication of mating with at least one female.

Those females which appeared to be pregnant (i.e. had shown positive indication of mating and bodyweight gain to day 22 of gestation) but failed to litter were killed on day 25 (approximately) of supposed gestation and the uterus examined for the presence of implantation sites.

All F_1A litters were weaned at day 29 post partum and from the F_1 litters a further 24 males and 24 females per group were randomly selected to become the parents for the next generation (litters derived from rematings were included in this process). The parentage of the selected animals was recorded. These animals were maintained for a pre-mating period of at least 11 weeks prior to a 21 day mating period for the F_2A litter.

The breeding programme was continued until the F_2A litters had been weaned.

Mating for the F_2B and F_2C litters was as described above except that the maximum mating period was 14 days for the F_2B litter and 10 days for the F2C litter.

During the course of the study brother/sister matings were avoided for all litters.

From the records of mating and parturition, the reproductive performance of the parents was assessed. The following were examined:

The fertility or otherwise of each male and female was established by the success of each mating. The criterion for a successful mating was the production of a viable litter ie a litter in which at least one pup was found alive at day 1. The method of calculation is shown in Appendix H.

Length of gestation was measured in days from the date of the positive smear to date of birth (but only in females fulfilling the criterion above ie production of a viable litter).

Pre-coital interval, in days between the date of pairing and the date of the positive smear, was measured.

2.7.5 Parent Selection: The F_1A litters were weaned at day 29 post partum and 24 male and 24 females per group were selected to become the next generation (F_1) parents. They were selected from litters containing 2 to 16 pups and included litters derived from remating. Details of the selection procedure are given in Appendix I. Their genealogy was recorded and taken into account during the selection procedure.

2.8 Offspring Investigations

2.8.1 Clinical Condition: Litters were examined for dead or moribund pups at least once daily and any such pups were subjected to a macroscopic post mortem examination. For pups killed or found dead up to and including 18 days of age, abnormalities were recorded and the pups were discarded. Pups over 18 days of age were examined as described in Section 2.9.2. A count of all live and dead pups was made within 24 hours of parturition (day 1) and thereafter at days 5, 11, 16, 22 and 29 post partum for the F_1A and F_2A litters, and day 5 post partum for the F_2B and F_2C litters. The sexes of the pups were also recorded at these times. Any clinical abnormalities seen in the pups were recorded.

The following parameters were calculated for each litter:

$$\text{Percentage of pups live born} = \frac{\text{number of pups born live}}{\text{number of pups born live + number of pups born dead}} \times 100$$

$$\text{Percentage of pups surviving to day 5} = \frac{\text{number of live pups on day 5}}{\text{number of live pups on day 1}} \times 100$$

Pup survival for the F_1A and F_2A litters was also calculated from days 5 to 22 post partum.

2.8.2 Bodyweights: Individual pup bodyweights were recorded within 24 hours of birth (day 1) and at days 5, 11, 16, 22 and 29 post partum for the F_1A and F_2A litters, and day 5 post partum for the F_2B and F_2C litters. Since pups were not individually identified, data were recorded by sex and litter.

The pups selected to be the parents of the next generation were weighed on day 29 post partum to give the initial F_1 parent bodyweight values. Subsequently they were weighed at weekly intervals for the duration of the pre-mating period.

2.9 Pathology

2.9.1 Parents: All rats surviving to scheduled termination and those requiring euthanasia for humane reasons were killed by exsanguination under terminal anaesthesia induced by halothane Ph. Eur. (FLUOTHANE, Zeneca Pharmaceuticals, Macclesfield, Cheshire, UK) vapour. These, and any rat found dead, were necropsied and the following tissues removed and submitted for possible histological examination:

Cervix, epididymis, kidney, liver, mammary gland (adult females only), ovary, pituitary gland, prostate gland, seminal vesicle including coagulating gland, testis, uterus, vagina and macroscopically abnormal tissues.

All tissues were fixed in 10% neutral buffered formol saline except for testis, epididymis, skin (if abnormal) and mammary gland which were fixed in Bouin's fluid, and eye (if abnormal) which was fixed in Davidson's solution.

Tissues for histology were routinely processed, embedded in paraffin wax, and 5µm thick sections were cut and then stained with haematoxylin and eosin.

Initially, histological examination was limited to kidney and liver from the controls and 1000ppm groups, cervix, epididymis, mammary gland, ovary,

prostate gland, seminal vesicle, testis, uterus and vagina from suspected infertile animals in all groups. The criteria adopted for defining suspected infertile animals are shown in Appendices H and J. Subsequently, kidney from the F_0 males and females in the 100 and 300ppm groups, kidney from the F_1 males and females in the 300ppm group and liver from the F_1 males in the 300ppm group were also examined histologically. In addition, testis from the fertile F_1 males in the control and 1000ppm groups were also examined histologically and the testis from the infertile F_1 males were re-examined at this time to ensure consistency. All remaining tissues were stored.

2.9.2 Offspring: All F_1A and F_2A pups surviving to scheduled termination (except those F_1A pups selected to be F_1 parents) were killed by the same method as used for the adults on approximately day 29 post partum. Approximately five males and five females per group from the F_1A litters and approximately ten males and ten females per group from the F_2A litters were selected randomly for a full post mortem examination in which the tissues from the same list as for the parents, but excluding mammary gland, were taken. The pups were selected from those with no clinical abnormalities with the proviso that no more than one pup of each sex from a litter received a full post mortem examination. After selection of pups for full post mortem examination, any pups showing clinical abnormalities were subjected to a macroscopic post mortem examination in which abnormalities only were taken. In addition, two male and two female clinically normal pups were selected at random from each litter, where possible, and given a macroscopic post mortem examination.

Pups killed or found dead intercurrently which were over 18 days of age were subjected to a full post mortem examination and tissues from the same list as for the parents, but excluding mammary gland, were taken.

Fixation and processing of tissues was as for the parents.

The kidney and liver from all pups in the control and 1000ppm groups subjected to scheduled full post mortem examination were examined by light microscopy. All remaining tissues were stored.

2.10 Organ Weights

Testes (left and right separately), kidneys (left and right separately) and liver were weighed from the adult animals terminated as scheduled and from the pups subjected to a full post mortem examination. In addition, these organs were also weighed from approximately 5 F_1A pups per sex per group selected at random from the pups given a macroscopic post mortem examination, so that organs from approximately 10 male and 10 female pups per group from each generation were weighed.

2.11 Statistical Analysis

Bodyweights during the pre-mating period were considered by analysis of covariance on initial (week 1) bodyweight, separately for males and females. In addition, for the F_1 parents, week 1 bodyweight was considered by analysis of variance, separately for males and females.

Food consumption during the pre-mating period was considered by analysis of variance, separately for males and females.

Parental organ weights were considered by analysis of variance and analysis of covariance on final bodyweight, separately for males and females.

Initial (day 1) pregnancy and lactation bodyweights were considered by analysis of variance. Subsequent pregnancy and lactation bodyweights were considered by analysis of covariance on day 1 pregnancy and lactation bodyweight, respectively.

Litter size, mean gestation length, mean pre-coital interval, initial (day 1) mean pup weight and total litter weight were considered by analysis of variance. Subsequent mean bodyweights were considered by analysis of covariance on day 1 mean pup bodyweight.

The proportion of fertile animals (as defined in Appendix H), the proportion of whole litter losses, the proportion of litters with gestation

length <22, 22 and >22 days, and the proportion of litters with pre-coital interval 1, 2, 3, 4 and >4 in each treated group were considered by Fisher's Exact Test.

For live born pups and pup survival the following analyses were carried out:

(1) Percentages were considered by analysis of variance following the double arcsine transformation of Freeman and Tukey (1950).

(2) The proportion of pups born live, the proportion of pups surviving, the proportion of litters with all pups born live and the proportion of litters with all pups surviving were considered by Fisher's Exact Test.

Pup survival was calculated over days 1-5 and, in addition, over days 5-22 for the F_1A and F_2A litters.

Mean pup organ weights were considered by analysis of variance and analysis of covariance on final mean pup bodyweight, separately for male and female pups.

All analyses were carried out in SAS (1989). For Fisher's Exact Test the proportion in each treated group was compared to the control group proportion. Analyses of variance and covariance for pup organ weights did not allow for any experimental design factors. All other analyses of variance and covariance allowed for the replicate structure of the study design and, in addition for the F_0 parents and F_1 litters, with the exception of food consumption, also for the litter of origin of the parents. Least-squares means for each group were calculated using the LSMEANS option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a Student's

t-test, based on the error mean square in the analysis. All statistical tests were two-sided.

The differences from control based on the analysis of pre-mating bodyweight and food consumption are also presented graphically. The centre of each bar represents the mean percentage difference between control and treated group least-squares means, and the top and bottom of each bar represent the upper and lower 95% confidence limits for this difference. If the bar does not cross the zero difference line at a particular week, there is a statistically significant difference between the treated group and the controls at that week. For ease of reference, lines have been added to the bodyweight plots to show differences of ±10%.

3. RESULTS

3.1 Atmosphere Analysis (Table 2, Appendix K)

The daily mean analysed concentrations of perchloroethylene were close to target as shown in Table 2.

3.2 Parents

3.2.1 Clinical Condition and Mortality (Tables 3 and 4): Signs consistent with depression of the central nervous system such as decreased activity and reduced response to sound were seen during each exposure to 1000ppm for the first 2 weeks of exposure in each generation. These signs were not present approximately 30 minutes after the end of each exposure. Other clinical findings associated with perchloroethylene during exposure in both generations included salivation, breathing irregularities, piloerection and tip-toe gait at 1000ppm and piloerection and increased breathing rate at 300ppm. Recovery from these effects at 300 and 1000ppm was also evident by the end of exposure at 300ppm and within approximately 30 minutes after the end of exposure at 1000ppm. When exposure of the dams and litters in the 1000ppm group was resumed on day 6 post partum in the

first generation, sedation of the dams with consequent neglect of their litters was evident.

There were no treatment-related clinical findings in the 100ppm group during exposure.

Other findings recorded during the detailed clinical examinations were low in incidence and of a type normally seen in rats of this age and strain. Mortality was low in each generation and not related to exposure to perchloroethylene.

3.2.2 Bodyweights:

(a) F_0 Parents (Figures 1-6, Tables 5-8): Bodyweights in the 1000ppm males were slightly lower than controls during the first few weeks of the pre-mating phase but from week 6 onwards bodyweights were similar to controls. The largest difference was seen in week 2 where the difference from controls was approximately 5% when adjusted for initial weight. A similar pattern was seen in the 1000ppm females but the reduction was marginal and confined to week 2 when the difference from controls was approximately 2% when adjusted for initial weight. There were no adverse effects on bodyweights at 100 or 300ppm in either sex during the pre-mating phase.

There were no adverse effects on bodyweights during pregnancy at any exposure level of perchloroethylene.

Small but statistically significant reductions in bodyweight were seen during lactation in all groups exposed to perchloroethylene on day 5 post partum the differences from controls being 2, 3 and 4% when adjusted for initial weight for the 100, 300 and 1000ppm groups respectively. Subsequent recovery to control levels was evident in the 300 and 1000ppm groups with an increase over controls at 1000ppm at day 29. Recovery was also seen in the 100ppm group up to day 22 post partum but bodyweights at day 29 post partum were statistically significantly lower than controls.

(b) F_1 Parents (Figures 7-15, Tables 9-12): Initial bodyweights of all groups exposed to perchloroethylene were lower than controls for both sexes the reduction being most marked at 1000ppm where the difference from controls was 26 and 24% for males and females respectively. There was evidence for some subsequent divergence from controls in the 1000ppm group males even after adjustment for initial bodyweight whilst growth of the 1000ppm females was similar to controls. Subsequent growth in the 100 and 300ppm male and female groups was similar to controls.

Initial F_2A pregnancy bodyweights in the 1000ppm group were approximately 9% below controls reflecting the lower bodyweights seen in the pre-mating period. Subsequent growth in the 1000ppm group was similar to controls. There was no evidence for an effect at 100 or 300ppm.

Initial F_2B pregnancy bodyweights in the 1000ppm group were approximately 5% below controls reflecting the lower bodyweights seen previously and remained lower than controls throughout gestation. Marginally lower bodyweights compared with controls were seen at 300ppm.

The F_2C pregnancy bodyweights for unexposed females mated with males in the 1000ppm group were similar to controls.

Initial F_2A lactation bodyweights of the 1000ppm group were approximately 3% below controls. Poor growth was evident to day 5 post partum with recovery to controls thereafter. Initial bodyweights of th 300ppm group were similar to controls but there was poor growth to day 5 post partum with recovery to controls thereafter. There was no consistent effect at 100ppm although day 29 post partum bodyweights were marginally lower than controls.

Initial F_2B lactation bodyweights of the 1000ppm group were approximately 6% below controls and poor growth was evident to day 5 post partum. Initial bodyweights and growth to day 5 post partum of the 300ppm group were similar to controls.

3.2.3 Food Consumption:

(a) F_0 Parents (Figures 16-19, Tables 13-14): The food consumption of the 1000ppm group was slightly lower than controls in both sexes during week 1 of the pre-mating phase but was similar to controls up to week 4. Subsequent food consumption was higher than controls in both sexes. There were no adverse effects on food consumption in the 100 and 300ppm group.

(b) F_1 Parents (Figures 20-23, Tables 15-16): The food consumption of the 1000ppm group was lower than controls in week 1 of the pre-mating phase in both sexes but recovery to control levels was evident by week 6 in males and week 3 in females. Subsequent food consumption was generally similar to controls in males and higher than controls in females. At 300ppm slightly lower food consumption was seen during week 1 in males and weeks 1 and 2 in females but subsequent values were similar to or higher than controls. There were no adverse effects on food consumption in the 100ppm group. The slightly lower food consumption values seen in week 5 compared with previous and subsequent values reflected the introduction of the second (smaller) animal to some cages at the end of the selection process.

3.2.4 Reproductive Performance (Figures 24-31, Tables 17-22):

(a) F_0 Parents: A reduction in pre-coital interval was seen in the 100, 300 and 1000ppm groups compared with controls and this was statistically significant for the 300 and 1000ppm groups. However, on the day prior to mating, it was noted that the light switches in the exposure chambers of all groups were faulty and not switching off at the set time in the evening. These switches had been checked 2 days previously and found to be functioning correctly. The changes in pre-coital interval were probably the result of these alterations to the light cycle rather than an effect of perchloroethylene. It should be noted that pre-coital intervals at 300 and 1000ppm were within the range expected for rats of this strain as shown by the results for the F_1 generation. There were no effects on length of gestation or male and female fertility.

There were statistically significant reductions in the incidence of pups born live and in the incidence of litters with all pups born live in the 1000ppm group compared with controls. There were no effects at 100 or 300ppm.

(b) F_1 Parents: Pre-coital interval, length of gestation and male and female fertility of the treated groups were similar to controls for the A, B and C litters.

The incidence of pups born live and the incidence of litters with all pups born live in the 1000ppm group was lower than controls for the A and B litters although this was only statistically significant for the incidence of pups born live. There were no statistically significant changes at 300ppm for the B litter or at 1000ppm for the C litter.

3.3 Offspring (Figures 32-39, Tables 23-34)

3.3.1 F_1A Litters: Reduced pup survival was seen in the 1000ppm group compared with controls and was statistically significant for days 5-22 post partum. This reduction was evident for both the incidence of pups surviving and for the incidence of litters with all pups surviving. There were no effects at 100 or 300ppm.

Litter size in the 1000ppm group was marginally lower than controls although this was not statistically significant and was the result of two whole litter losses in this group.

Pup bodyweights in the 1000ppm group were approximately 10% lower than controls at birth and, following re-exposure on day 6 post partum, there was a further divergence from controls such that day 29 bodyweights were approximately 20% below controls after adjustment for initial weight. Lower total litter weights than controls, which were statistically significant throughout lactation except for day 1 post partum, reflected these reductions in pup bodyweights.

At 300ppm, a similar but less marked pattern of change was seen, bodyweights on day 11 and day 29 post partum being approximately 11-14% and 7-9% below controls respectively after adjustment for initial weight. Total litter weights were not statistically significantly different from controls. Slightly lower pup bodyweights were seen in the 100ppm group compared with controls probably reflecting the larger litter size in this group, total litter weights being slightly higher than controls, but the differences were generally too small to attain statistical significance.

On day 6 post partum (when the dams and their litters resumed exposure) and on each subsequent day of exposure the pups in the 1000ppm group showed signs of sedation. When the pups were examined after exposure they also showed signs of hypothermia. These signs persisted for up to 2 hours after each exposure on the first two days, 1.5 hours for the next 2 weeks and then 30-60 minutes up to day 29. An increased incidence of clinical findings associated with the poor survival and growth of the pups were also seen in the 1000ppm group and included small and thin appearance. There were no significant clinical findings in the 100 or 300ppm groups.

3.3.2 F_2A Litters: Reduced pup survival was seen in the 1000ppm group compared with controls and was statistically significant for days 1-5 post partum. The reduction in pup survival was evident for both the incidence of pups surviving and for the incidence of litters with all pups surviving. In addition, there was a statistically significant increase in the incidence of whole litter losses. A slight reduction in pup survival was seen for days 5-22 post partum although this was entirely due to one female with a whole litter loss. There were no effects at 100 or 300ppm.

Litter size in the 1000ppm group was statistically significantly lower than controls at birth. A further reduction was seen up to day 5 post partum reflecting the poor survival seen in these litters. Litter size in the 300ppm group was marginally lower than controls although the difference was not statistically significant. Litter size at 100ppm was similar to control.

Pup bodyweights in the 1000ppm group were approximately 5-10% lower than controls at birth and there was a further reduction to day 5 post partum when bodyweights were approximately 21-27% below controls after adjustment for initial weight. Thereafter there was recovery up to day 29 when bodyweights were similar to controls. It should be noted that there was no exposure for the dams or litters during lactation in this group. Total litter weights were statistically significantly lower than controls throughout lactation reflecting the reductions in pup bodyweights and litter sizes. At 300ppm, pup bodyweights were similar to controls at birth but were slightly lower than controls thereafter and the difference was statistically significant after adjustment for initial weight in females on days 5 and 11. Total litter weights were statistically significantly lower than controls on day 16 and 22 post partum reflecting the slightly lower bodyweights and litter sizes. Pup bodyweights and total litter weights in the 100ppm group were similar to controls.

3.3.3 F_2B Litters: A statistically significant reduction in pup survival was seen in the 1000ppm group compared with controls for days 1-5 post partum. This reduction was evident for both the incidence of pups surviving and the incidence of litters with all pups surviving. In addition, there was an increased incidence of whole litter losses. There were no effects at 300ppm.

Litter sizes in the 1000ppm group were statistically significantly lower than controls at birth. A further reduction was seen up to day 5 post partum reflecting the poor survival seen in these litters. Litter sizes in the 300ppm group were marginally lower than controls although the difference was not statistically significant.

Pup bodyweights in the 1000ppm group were approximately 10-13% lower than controls at birth and there was a further reduction to day 5 post partum when bodyweights were approximately 12-16% below controls. Total litter weights were statistically significantly lower than controls on day 5 post partum reflecting the lower bodyweights.

At 300ppm, pup bodyweights were similar to controls at birth but were slightly lower than controls on day 5 post partum and the reduction was statistically significant for the males. Total litter weights were statistically significantly lower than controls on day 5 post partum reflecting the slightly lower bodyweights and litter size.

An increased incidence of clinical findings associated with the poor survival and growth of the pups were seen in the 1000ppm group and included cold, small and moribund appearance. The incidence of clinical findings in the 300ppm group was similar to controls.

3.3.4 F_2C Litters: Pup survival, bodyweights, litter sizes, total litter weights and the incidence of clinical findings in the litters from unexposed females mated with males in the 1000ppm group were similar to controls.

3.4 Organ Weights

3.4.1 Parents (Tables 35-36):

(a) F_0 Parents: Kidney weights (absolute and adjusted for bodyweight) were statistically significantly higher than controls for the males in the 1000ppm group compared with controls. Absolute kidney weights were also statistically significantly higher than controls for the males in the 300ppm group but this was largely a reflection of their higher bodyweights. There were no changes in the kidney weights of males in the 100ppm group or in females at any exposure level. Liver weights (absolute and adjusted for bodyweight) were statistically significantly higher than controls for the males in the 1000ppm group compared with controls. There were no changes in the liver weights of males in the 100 or 300ppm group or in females at any exposure level. There were no dose-related effects on testes weights.

(b) F_1 Parents: Absolute kidney and liver weights for males in the 1000ppm group were slightly higher than controls although the differences were not statistically significant. However, bodyweights in this group

were lower than controls and when kidney and liver weights were adjusted for bodyweight the increases were statistically significant. There were no dose-related changes in males at 100 or 300ppm or in females at any exposure level.

A dose-related reduction in testes weights (absolute and adjusted for bodyweight) was seen at all exposure levels although the reduction at 100ppm was marginal and not statistically significant.

3.4.2 Offspring (Table 37-38):

(a) F_1A Litters: Statistically significant reductions compared with controls were seen in the following organs: kidneys in males and females at 1000ppm and males at 300ppm, liver in males and females at 300 and 1000ppm and females at 100ppm, and testes at 300 and 1000ppm. These were all reflections of differences in final bodyweight since there were no statistically significant changes after adjustment for bodyweight.

(b) F_2A Litters: Statistically significant increases compared with controls were seen in the following organs: kidneys in males at 100 and 1000ppm and liver in males at 100ppm. These were all reflections of differences in final bodyweight since there were no statistically significant changes after adjustment for bodyweight.

3.5 Pathology

3.5.1 Macroscopic Findings (Tables 39-40, 43-44):

(a) F_0 Adults

One control male with malocclusion and one male exposed to 300ppm with a suspected broken nose were killed intercurrently. A variety of common lesions such as staining of the eyelids, pelvic dilatation of the kidney, and deposits in the bladder were observed in these animals.

Three females, one exposed to 100ppm and two to 1000ppm, were removed as a result of dystocia. Associated haemorrhage or discharge in the vagina was observed along with discoloration of kidney, liver, lung, ovary and pituitary (mostly pallor due to blood loss associated with the dystocia).

The incidence of findings in the F_0 adults at termination was low and unrelated to treatment. No macroscopic findings to account for suspected infertility (apart from the cases of dystocia mentioned) were detected in any animal.

(b) F_1 Adults

Two males exposed to 100ppm of compound were removed from the study prior to termination, one with a suspected broken nose and one which was found dead without any previous adverse clinical findings. Discoloration of the lungs only was observed in the animal which was found dead and staining around the eyes and nose and tail damage in the other.

One control female and one female in the 100ppm group with suspected broken noses were killed intercurrently. One female in the 300ppm group which was killed intercurrently having failed to produce a litter had no implant sites and the uterus was distended with abnormal contents. Two females killed with dystocia in the 300ppm group had foetuses present and a variety of non-specific findings was observed in other tissues. In the 1000ppm group one female was found dead with no previous adverse clinical signs and there were no significant macroscopic findings other than firmness and an accentuated lobular pattern to the liver (implantation sites were present). Two females in the 1000ppm group killed because of dystocia had foetuses present in the uterus.

In animals killed at termination, a mass was observed in the kidney of one male exposed to 300ppm and cysts were present in the kidney in one male exposed to 300ppm and in two males exposed to 1000ppm. The incidence of all other macroscopic findings was low and unrelated to treatment with perchloroethylene. No macroscopic findings to account for suspected

infertility were detected in any animal apart from the females with dystocia mentioned above.

(c) F_1A Offspring

The incidence of all macroscopic findings in pups dying or killed intercurrently over 18 days of age and those killed at termination was low and unrelated to treatment with perchloroethylene. Enlarged cerebral hemispheres (hydrocephalus) were observed in one intercurrent male in the 1000ppm group. This is a spontaneous lesion and considered unrelated to treatment.

(d) F_2A Offspring

In this litter there were no pups over 18 days of age which died before scheduled termination. The incidence of all macroscopic findings in pups killed at scheduled termination was low and unrelated to treatment with perchloroethylene.

3.5.2 Microscopic Findings (Tables 41-42, 45-46):

(a) F_0 Adults

Histopathological changes related to treatment with perchloroethylene were observed in the kidney. In animals killed at termination, there was a slightly increased incidence of minimal chronic progressive glomerulonephropathy and an increased pleomorphism within proximal tubular nuclei in all males exposed to 1000ppm. In females in the same group there was a slight decrease in incidence and a more pronounced decrease in severity of intratubular microlithiasis together with increased nuclear pleomorphism in 50% of rats.

A variety of other lesions was observed but none was considered to be related to treatment with perchloroethylene.

No changes were detected to account for infertility in the reproductive organs of animals which failed to produce an F_1A litter.

(b) F_1 Adults

Changes similar to those seen in the F_0 generation were observed in F_1 adults except that a slightly increased incidence of chronic progressive glomerulonephropathy was observed in females exposed to 1000ppm and in most of the males in the control and 1000ppm groups reflecting the greater age of the animals in the second generation. Increased nuclear pleomorphism within proximal kidney tubules was confined to males at 1000ppm in this generation. A decreased incidence and severity of intratubular microlithiasis in females exposed to 1000ppm perchloroethylene was also observed in this generation.

There was a decreased incidence of hepatitis in males at 1000ppm.

The mass observed in one male exposed to 300ppm was a mesenchymal tumour (nephroblastoma). This was an isolated incidence which was considered to be unrelated to treatment. The kidney cysts observed macroscopically in one male in the 300ppm group and two males in the 1000ppm group were not visible in the sections examined microscopically.

Minimal or slight focal unilateral tubular degeneration of the testis was observed in two control males and two males in the 1000ppm group. This is a spontaneous lesion and unrelated to treatment.

No changes were detected to account for infertility in the reproductive organs of animals which were classed as suspected infertile.

(c) F_1A Offspring

Marked vacuolar degeneration of the proximal convoluted tubules in the kidney was observed in the animal with hydrocephalus. This is not a common finding but in view of its isolated incidence it was considered not to be treatment related.

A small number of lesions observed in the kidney was confined to control pups from the terminal kill.

(d) F_2A Offspring

A small number of lesions was observed in the kidney of terminal kill pups but they are commonly observed and considered not to be related to treatment. Minimal hepatitis was recorded in one control pup and slight hepatocyte necrosis was seen in one pup in the 1000ppm group.

3.5.3 Offspring up to and Including 18 Days of Age (Table 47-50):

An increased number of pups with empty, gas filled and distended stomachs was seen in the 1000ppm group for the F_1A, F_2A and F_2B litters reflecting the high pup mortality in this group, the overall incidence of these findings being generally similar in all groups. Other findings were low in incidence and unrelated to exposure to perchloroethylene.

4. DISCUSSION

Significant problems during the study were confined to the early part of the mating period in the first generation where changes in pre-coital interval resulted from alterations in the photoperiod. There were no similar problems in the second generation. The analysed concentrations of perchloroethylene were generally close to target levels. The health status of the rats was generally maintained at a high level and there was no evidence of disease or infection which might have compromised interpretation of the findings.

An exposure level of 1000ppm was associated with clinical signs consistent with depression of the central nervous system during exposure but adaptation was evident since these signs were confined to the early phase of each generation and during lactation when exposure was resumed on day 6

post partum. Other clinical findings noted during exposure but which persisted throughout the study included salivation and breathing irregularities at 1000ppm, and increased breathing rate at 300ppm. These findings may represent slight respiratory irritation at 300 and 1000ppm.

An exposure level of 1000ppm was also associated with reductions in adult bodyweight gain and food consumption which provided evidence that this exposure level was an appropriate top dose level for parental effects. In the first generation these reductions were seen in the early part of the pre-mating period but there was subsequent recovery to control levels. During pregnancy, bodyweights in the exposed groups were similar to controls but during lactation, when exposure was not resumed until day 6, reductions in bodyweight gain were seen in these groups on day 5 but with subsequent recovery. Since the reductions in the 100ppm group were small and within the limits of biological variation, and were not seen in the second generation, the change in this group was considered to be unrelated to treatment.

At 1000ppm, F_1A pup bodyweights were lower than controls at birth and, although there was some recovery up to day 5 post partum, a reduction in weight gain was seen when exposure resumed on day 6. In addition, sedation of the pups was seen during exposure. As a result of these findings there was no exposure for the pups in the F_2A litters at 1000ppm. A similar, but less marked pattern of change was seen at 300ppm but there were no signs of sedation. Slightly lower pup weights at 100ppm were considered to reflect slightly higher litter sizes in this group as shown by total litter weight values and were therefore unrelated to exposure.

In the second generation, lower initial bodyweights in the 100, 300 and 1000ppm groups reflected the lower F_1A bodyweights. At 1000ppm bodyweights remained lower than controls throughout the pre-mating period but recovery was seen at 100 and 300ppm. This reduced growth at 1000ppm continued through pregnancy for the F_2A and F_2B litters. Poor maternal growth to day 5 post partum was seen as in the previous generation in both litters at 1000ppm and in the F_2A litters at 300ppm.

Lower F_2A and F_2B pup bodyweights were seen at 300 and 1000ppm as in the first generation. Recovery to day 29 post partum was seen in the 1000ppm group for the F_2A litter indicating reversibility of the effect following cessation of exposure. An exposure level of 1000ppm was considered to have exceeded the maximum tolerated dose for the offspring.

Exposure to perchloroethylene had no effects on male and female fertility, pre-coital interval or length of gestation at any exposure level of perchloroethylene. However, the incidence of pup mortalities in the 1000ppm group during lactation was higher than controls for the F_1A, F_2A and F_2B litters and it should be noted that there was no exposure during this period for the F_2A and F_2B litters at this exposure level. In addition, the proportion of pups born live at 1000ppm was lower than controls for the F_1A, F_2A and F_2B litters suggesting an effect in utero. There were no statistically significant differences in the proportion of pups born live, mean litter size and survival in the 100 and 300ppm groups compared with controls for all litters.

The lack of any statistically significant change on proportion of pups born live, pup survival and growth in the F_2C litters suggests that the changes seen in previous litters were not likely to be male mediated.

Pathological changes which could be attributed to perchloroethylene were mainly confined to the kidney of adults in both generations at 1000ppm. The most notable lesion, which was subtle and minimal in severity, consisted of an increase in nuclear pleomorphism within proximal kidney tubules. An increased number of very large, irregularly shaped nuclei were present, especially prominent at the cortico-medullary junction in males, and/or changes in chromatin pattern and aberrations of the nucleoli. This change was observed in both sexes in the first generation but only in males in the second generation. In females only, there was a decrease in the incidence and severity of interstitial microlithiasis reflecting lower bodyweights. There was an increase in the incidence of chronic progressive glomerulonephropathy in males in the first generation and in females in the second generation reflecting the greater age of the animals in the second generation.

There were no changes which could be attributed to treatment in the reproductive tract of suspected infertile animals in either generation.

The no-effect level for pathological change in this study was 300ppm.

Increased kidney and liver weights were seen in males only at 1000ppm in both generations and changes of this magnitude are not inconsistent with an adaptive change. There were no changes in kidney and liver weight at 100 or 300ppm in males or in females at any level.

A dose-related and statistically significant reduction in testes weights was seen at 300 and 1000ppm perchloroethylene in adult males in the second generation. However, no functional impairment of male fertility was established and there were no significant histopathological changes in the testes of the males in the 1000ppm group. The significance of these reductions is, therefore, uncertain. There were no changes in the testes weights of adult males in the first generation or in the offspring in either generation when adjusted for bodyweight. It should be noted that exposure of the second generation males was up to 35 weeks compared with 19 weeks in the first generation.

5. CONCLUSIONS

It is concluded that an exposure level of 1000ppm perchloroethylene resulted in reductions in parental bodyweight gain during the pre-pairing period and lactation in both generations and during pregnancy in the second generation. A similar but less marked effect on bodyweights was also seen in the 300ppm group. A reduction in testes weights was seen at 300 and 1000ppm perchloroethylene in adult males in the second generation but there were no associated histopathological changes or effects on male fertility. Histopathological changes were confined to the kidneys of both sexes in both generations at 1000ppm but no significant pathological changes were seen at 100 or 300ppm.

Exposure to perchloroethylene was also associated with poor growth of offspring at 300 and 1000ppm together with a reduction in the proportion of pups born live and reduced pup survival at 1000ppm in both generations.

The no observed effect levels in this study were considered to be 100ppm perchloroethylene for parental and offspring toxicity and 300ppm for reproductive effects. There were no effects on fertility at exposure levels up to and including 1000ppm.

6. REFERENCES

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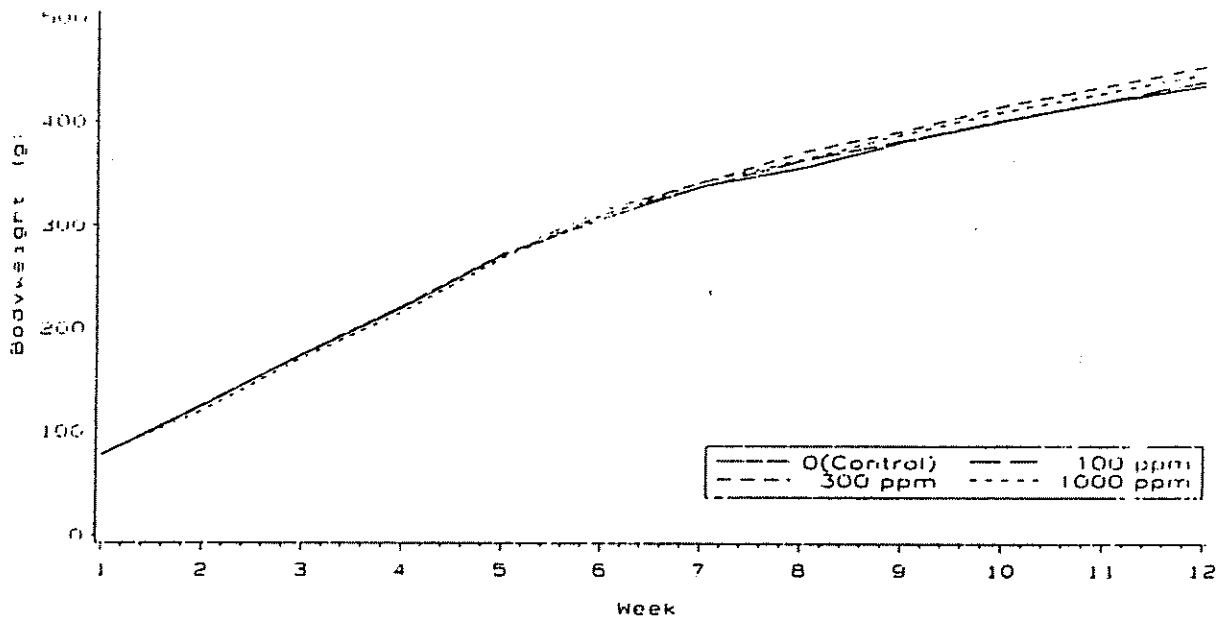
PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 1

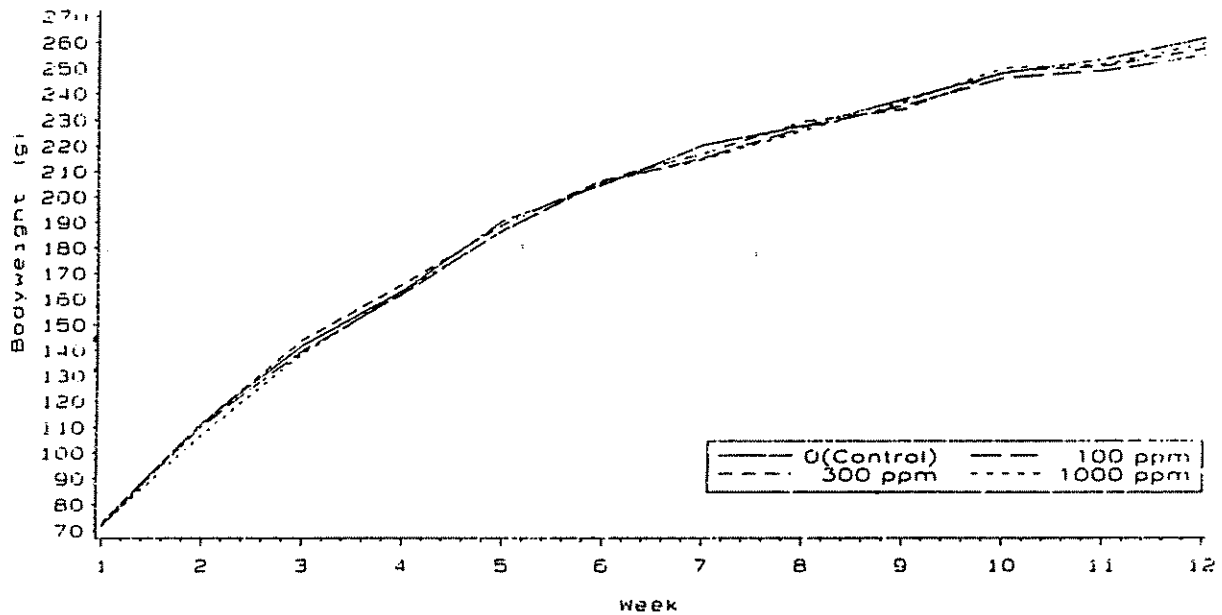
BODYWEIGHTS DURING PRE-PAIRING PERIOD
- F_0 GENERATION - MALES



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 2

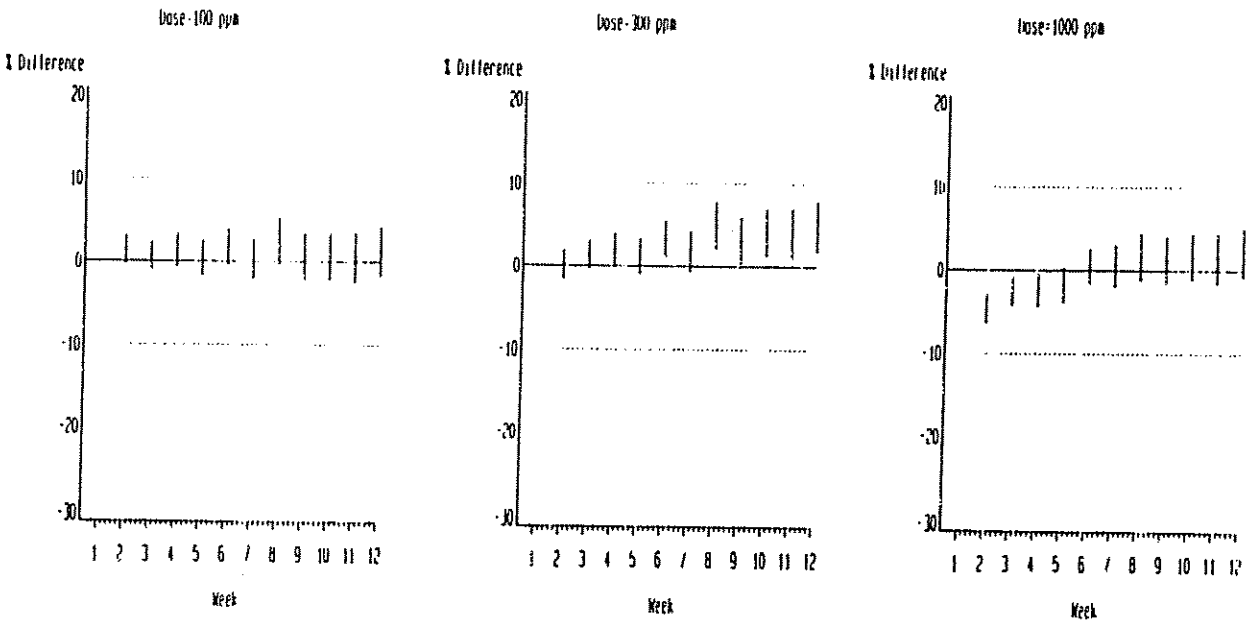
BODYWEIGHTS DURING PRE-PAIRING PERIOD
- F_0 GENERATION - FEMALES



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 3

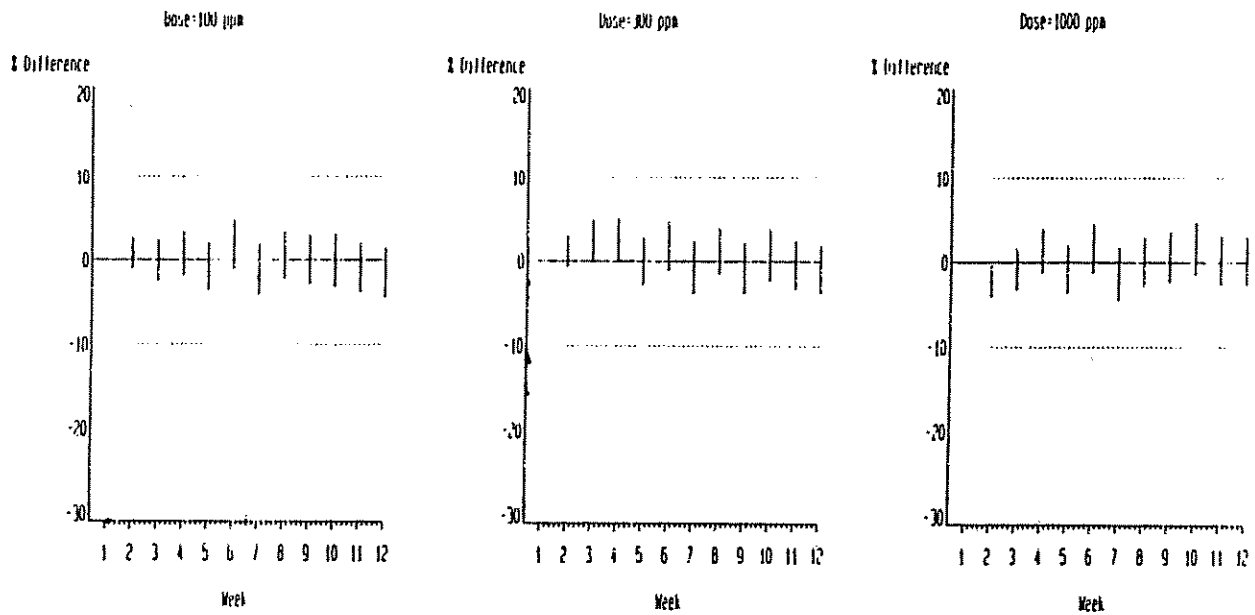
STATISTICAL ANALYSIS PLOTS OF BODYWEIGHT ADJUSTED
FOR INITIAL WEIGHT - F0 GENERATION - MALES



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 4

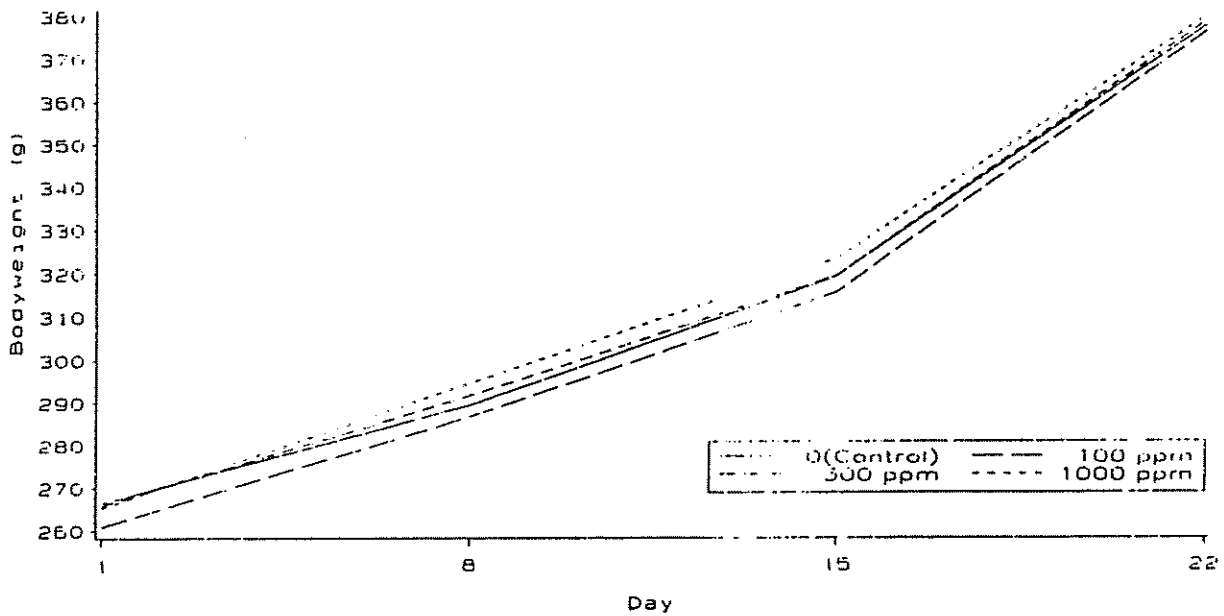
STATISTICAL ANALYSIS PLOTS OF BODYWEIGHT ADJUSTED
FOR INITIAL WEIGHT - F0 GENERATION - FEMALES



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 5

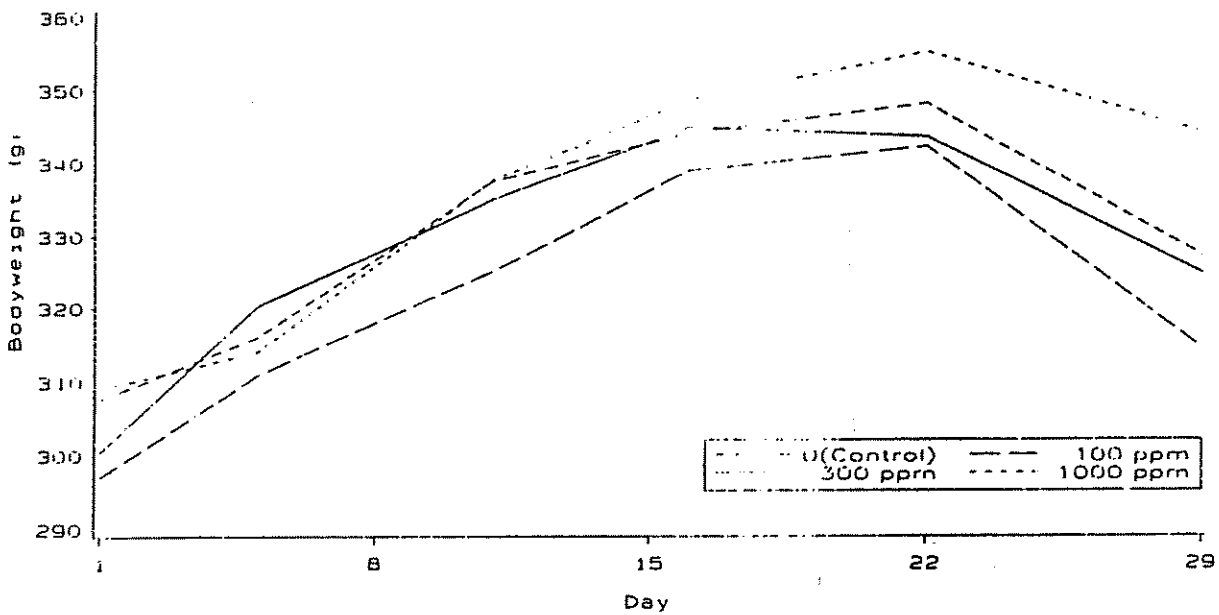
BODYWEIGHTS DURING PREGNANCY
- F_0 GENERATION - F1A LITTER



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 6

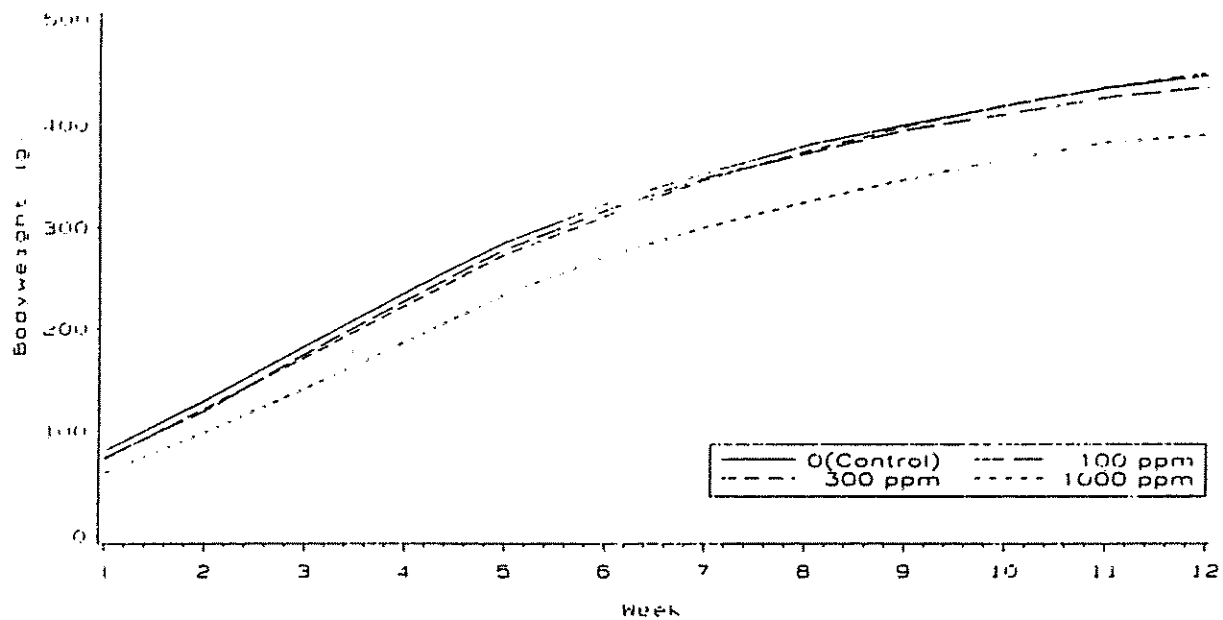
BODYWEIGHTS DURING LACTATION
- F_0 GENERATION - F1A LITTER



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 7

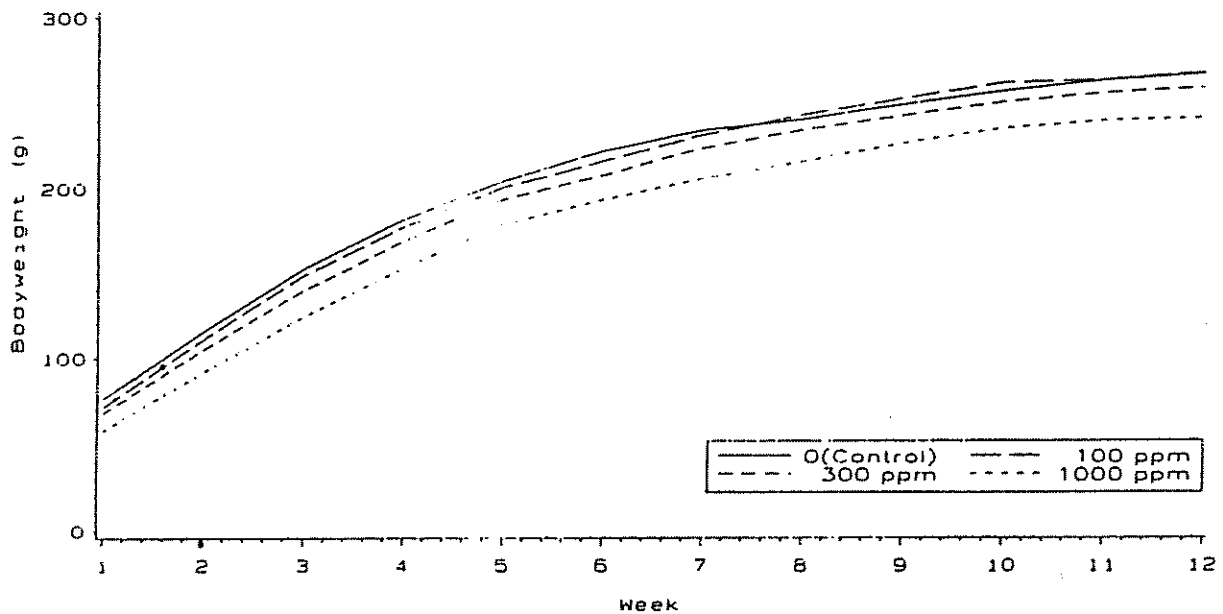
BODYWEIGHTS DURING PRE-PAIRING PERIOD
- F_1 GENERATION - MALES



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 8

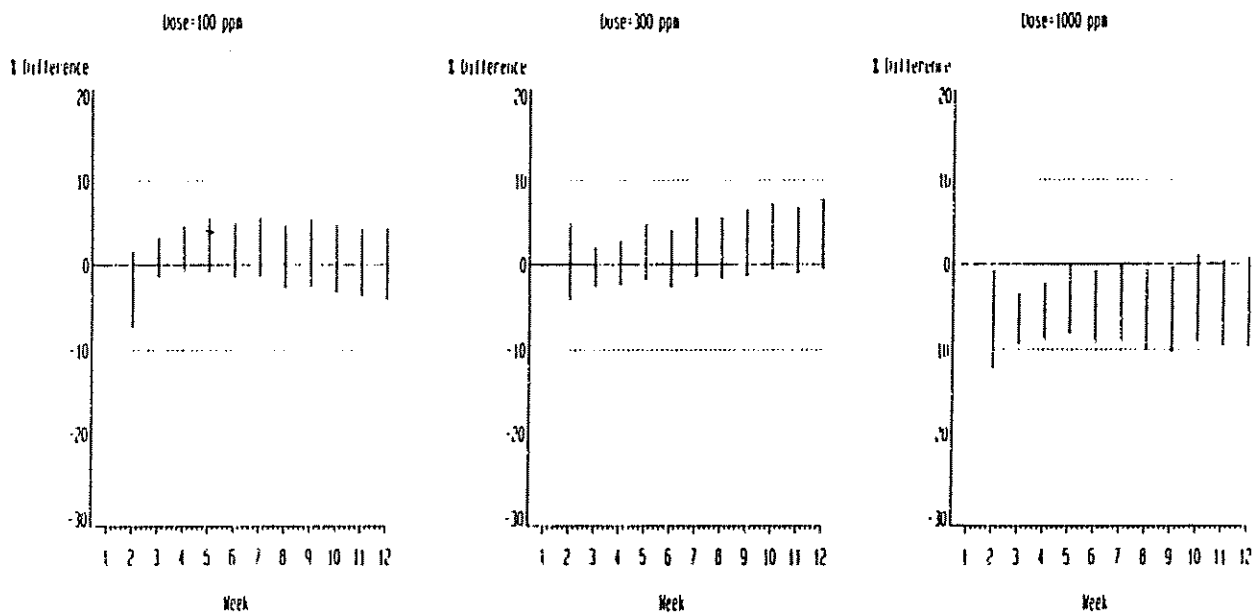
BODYWEIGHTS DURING PRE-PAIRING PERIOD
- F_1 GENERATION - FEMALES



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 9

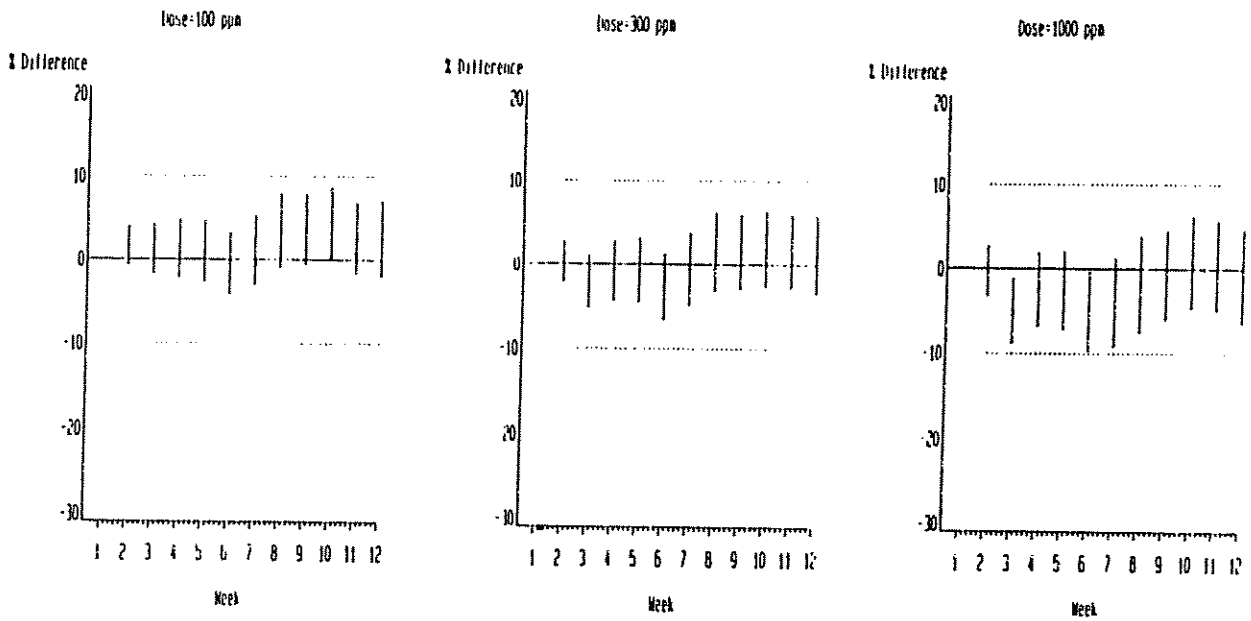
STATISTICAL ANALYSIS PLOTS OF BODYWEIGHT ADJUSTED
FOR INITIAL WEIGHT - F1 GENERATION - MALES



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 10

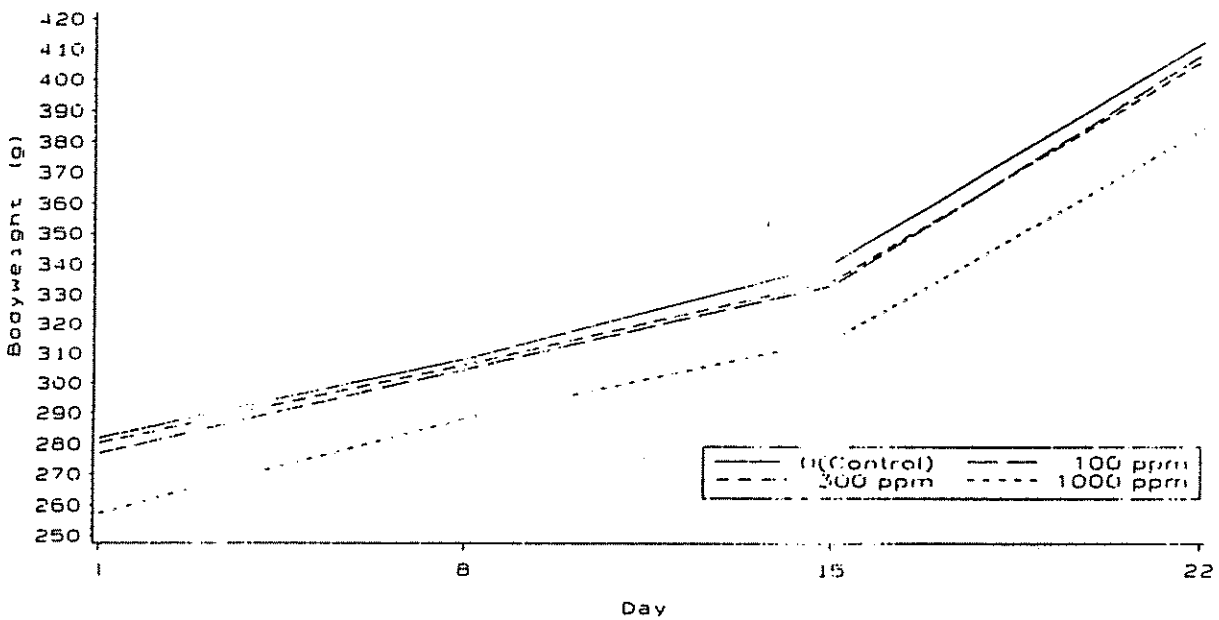
STATISTICAL ANALYSIS PLOTS OF BODYWEIGHT ADJUSTED
FOR INITIAL WEIGHT - F1 GENERATION - FEMALES



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 11

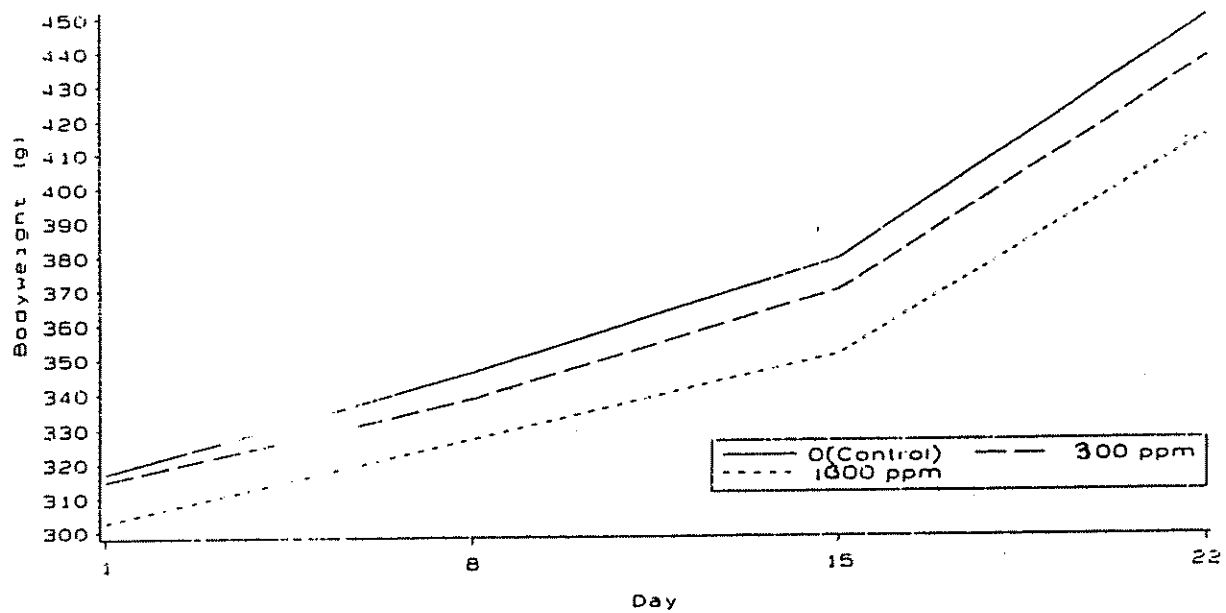
BODYWEIGHTS DURING PREGNANCY
- F_1 GENERATION - F2A LITTER



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 12

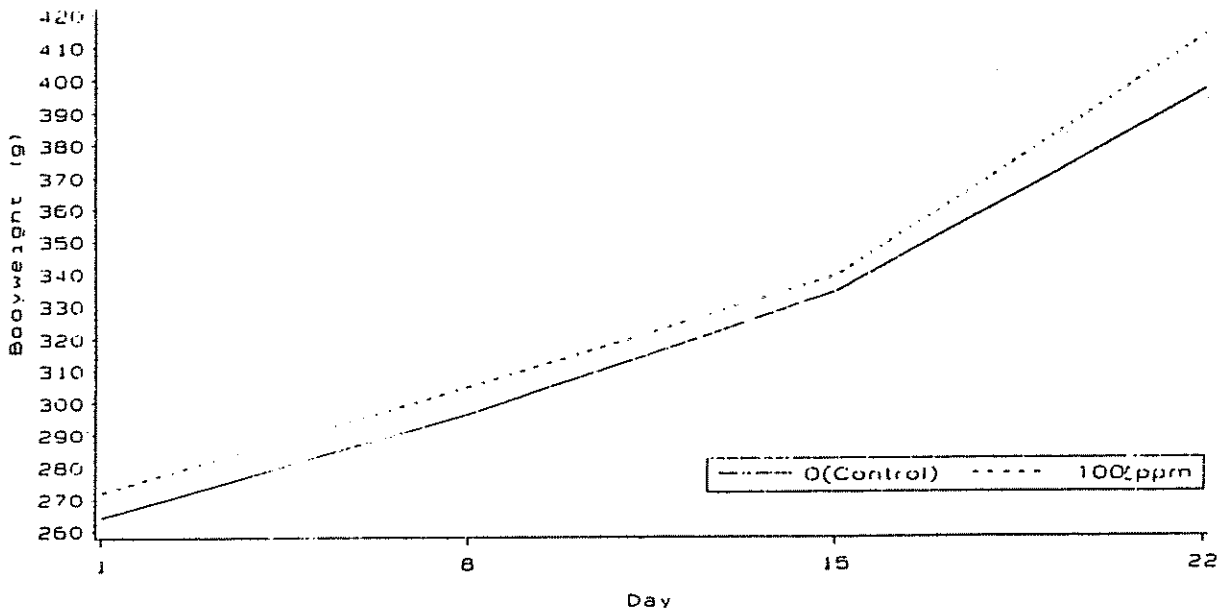
BODYWEIGHTS DURING PREGNANCY
- F_1 GENERATION - F2B LITTER



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 13

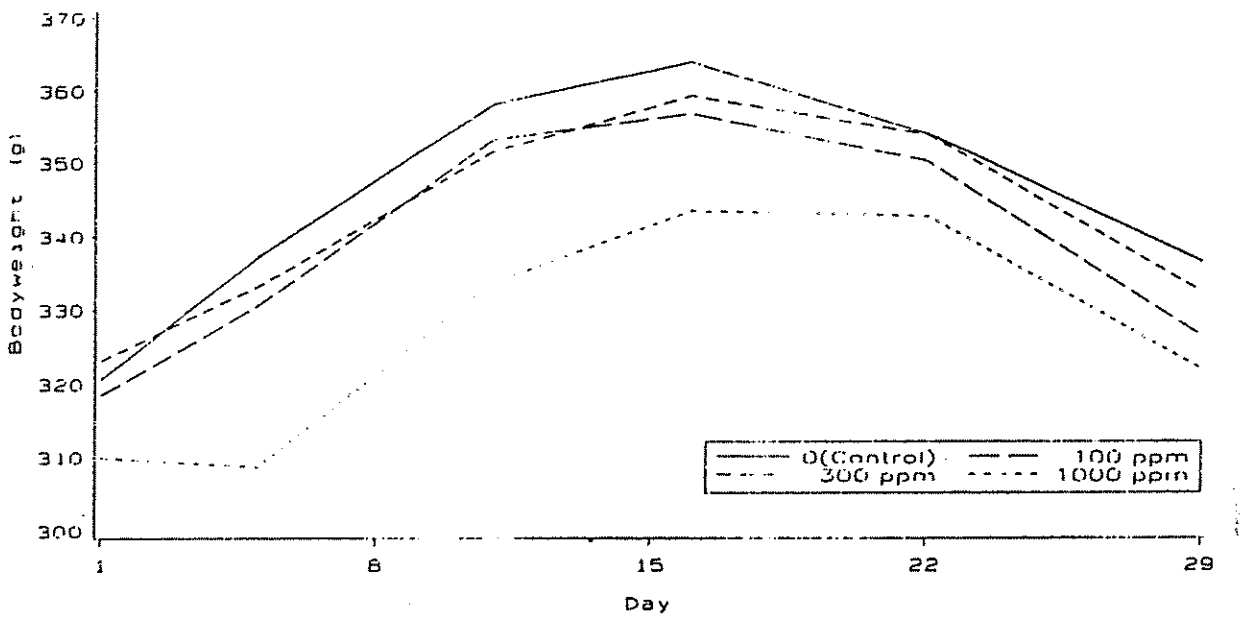
BODYWEIGHTS DURING PREGNANCY
- F_1 GENERATION - F2C LITTER



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 14

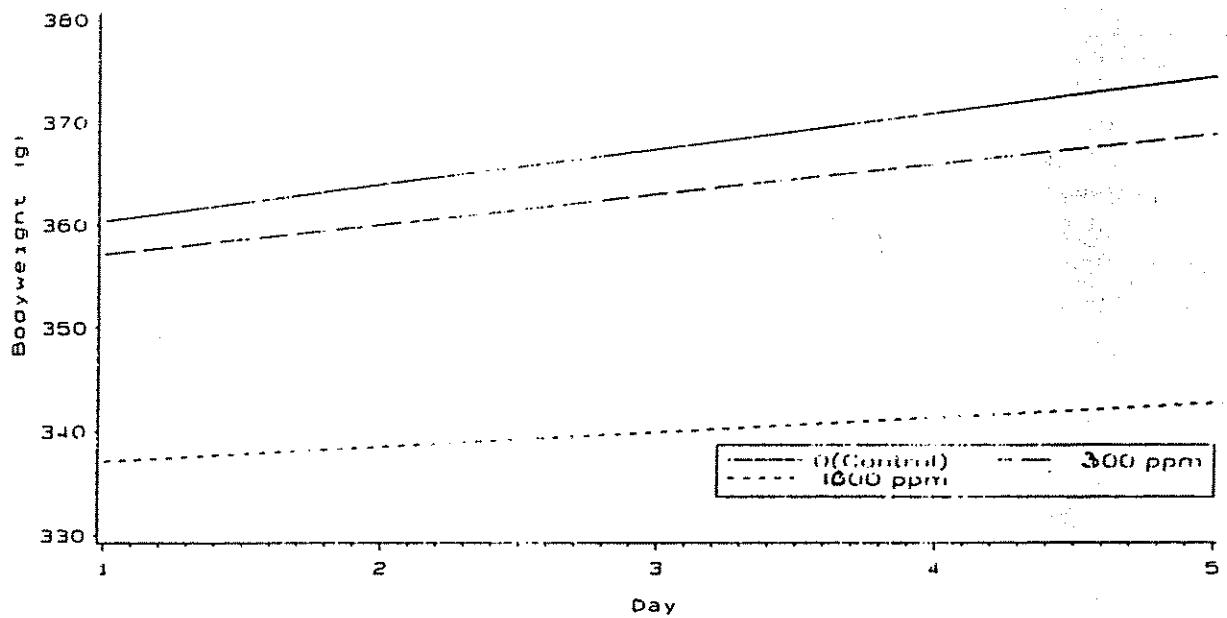
BODYWEIGHTS DURING LACTATION
- F_1 GENERATION - F2A LITTER



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 15

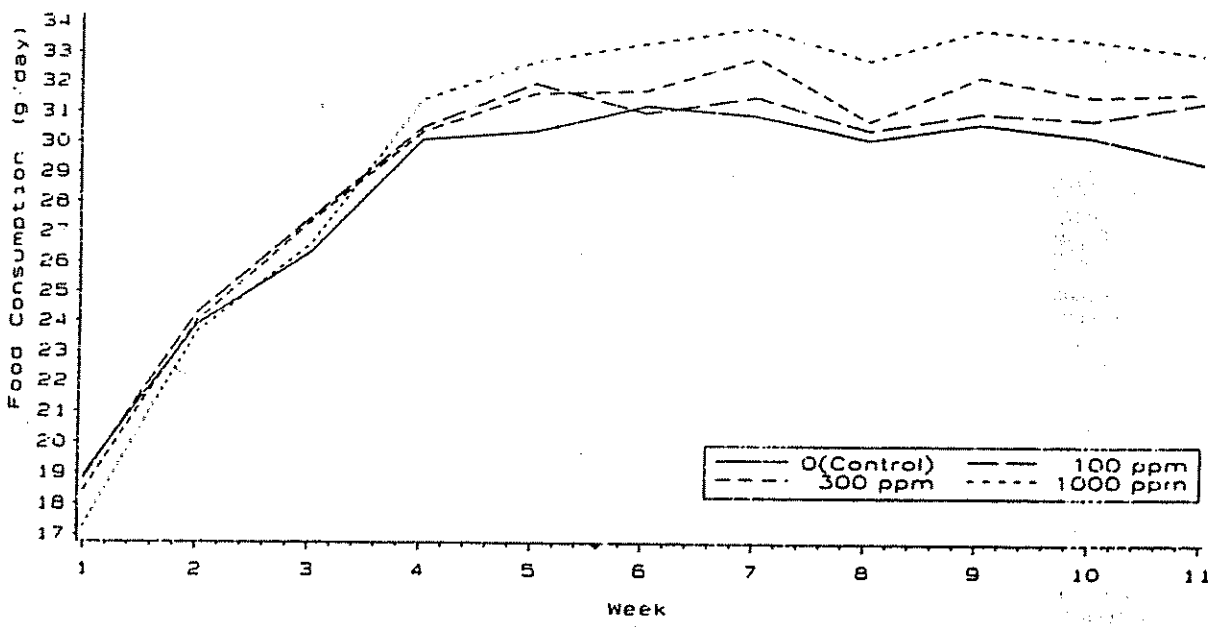
BODYWEIGHTS DURING LACTATION
- F_1 GENERATION - F2B LITTER



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 16

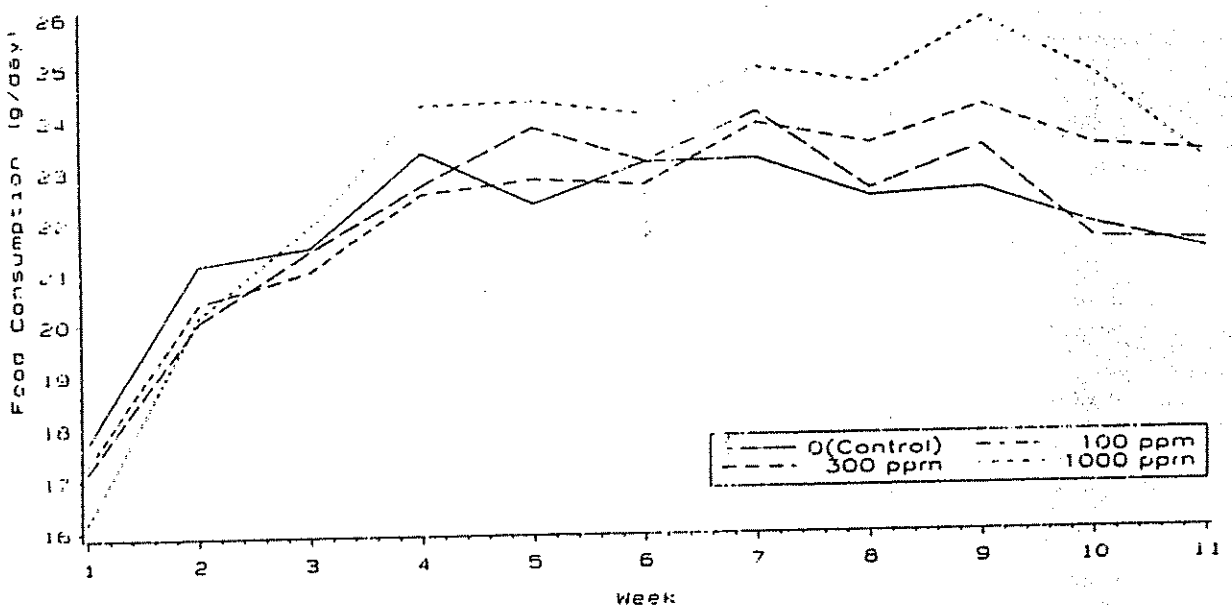
FOOD CONSUMPTION DURING PRE-PAIRING PERIOD
- F_0 GENERATION - MALES



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 17

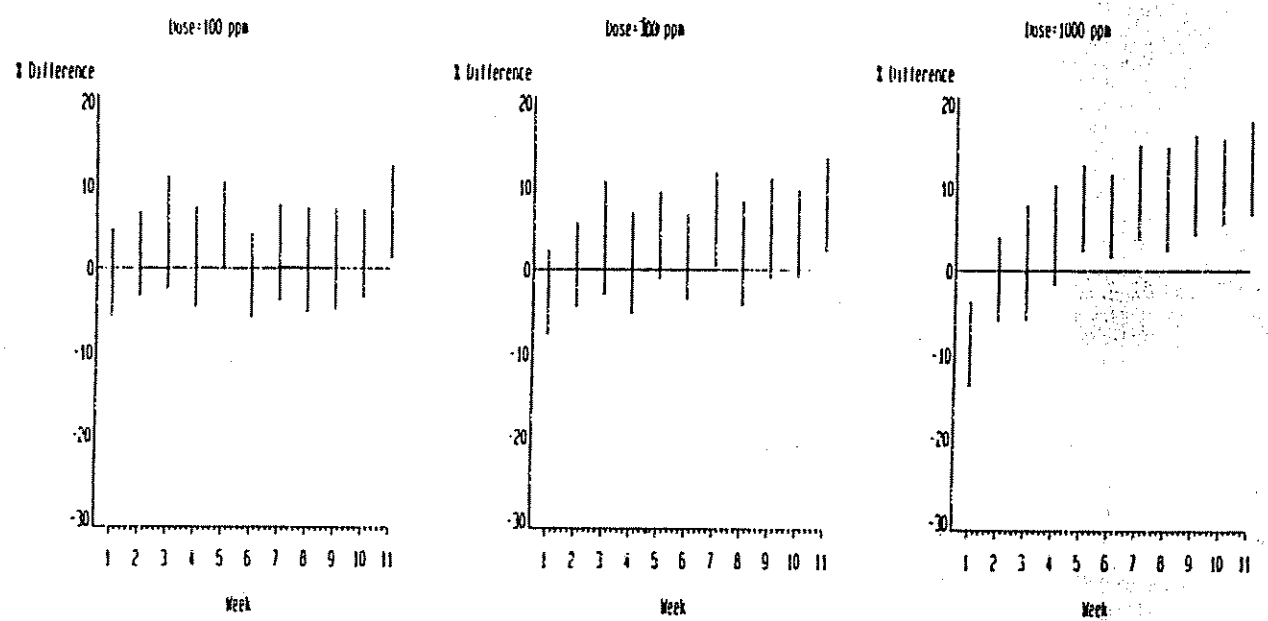
FOOD CONSUMPTION DURING PRE-PAIRING PERIOD
- F_0 GENERATION - FEMALES



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 18

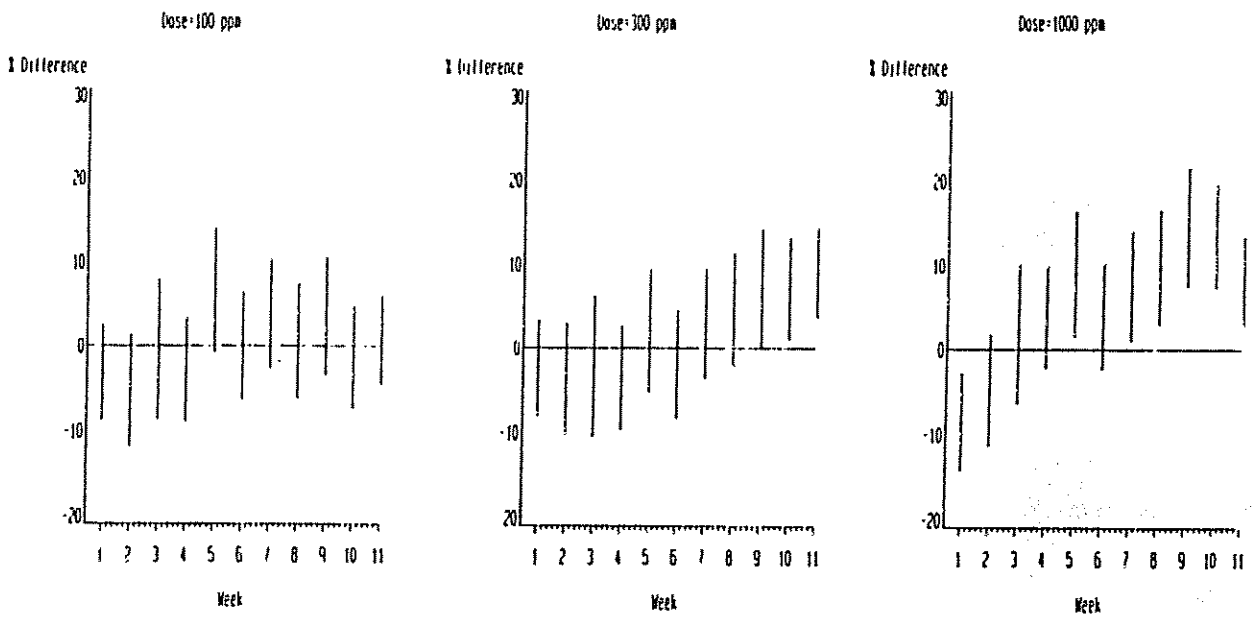
STATISTICAL ANALYSIS PLOTS OF FOOD CONSUMPTION
- F0 MALES



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 19

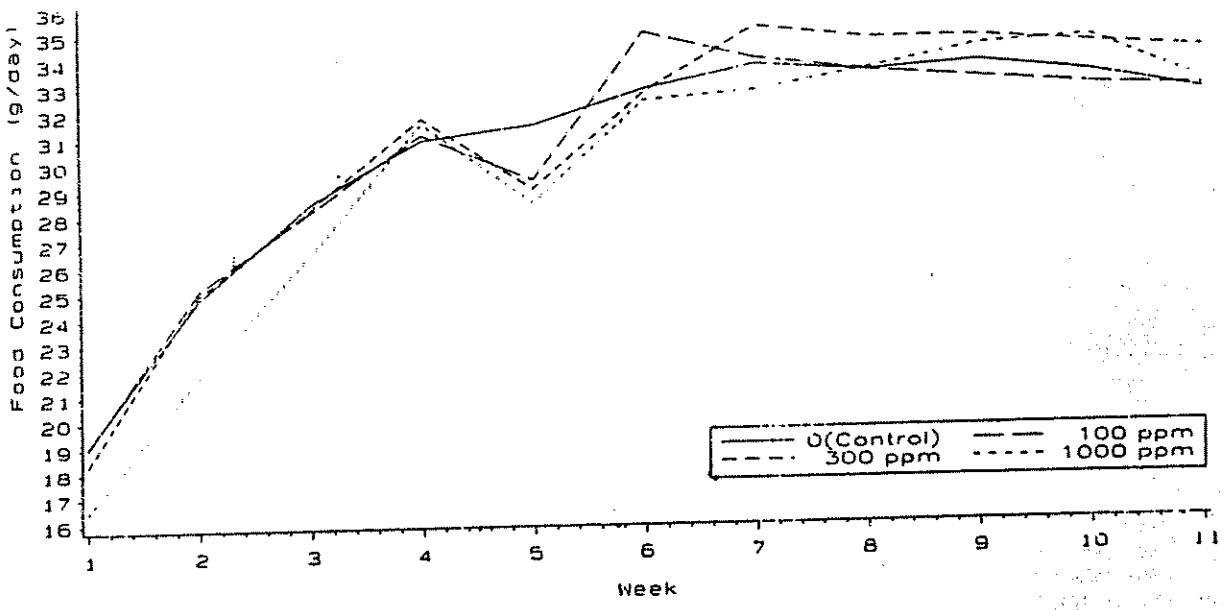
STATISTICAL ANALYSIS PLOTS OF FOOD CONSUMPTION
- F0 FEMALES



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 20

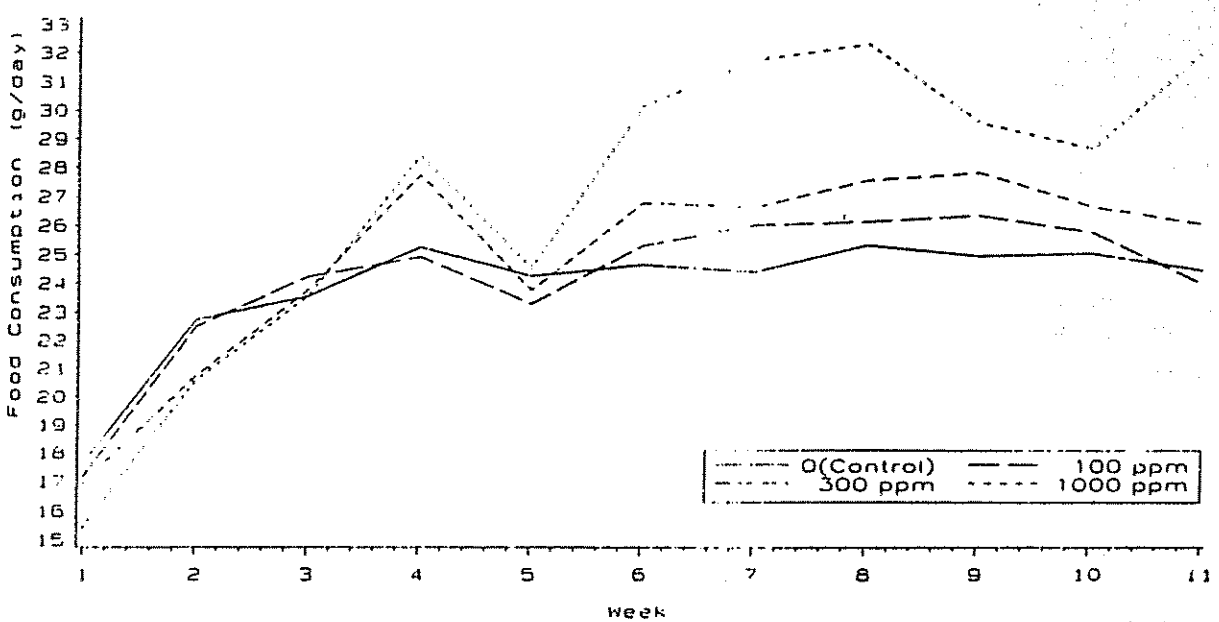
FOOD CONSUMPTION DURING PRE-PAIRING PERIOD
- F_1 GENERATION - MALES



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 21

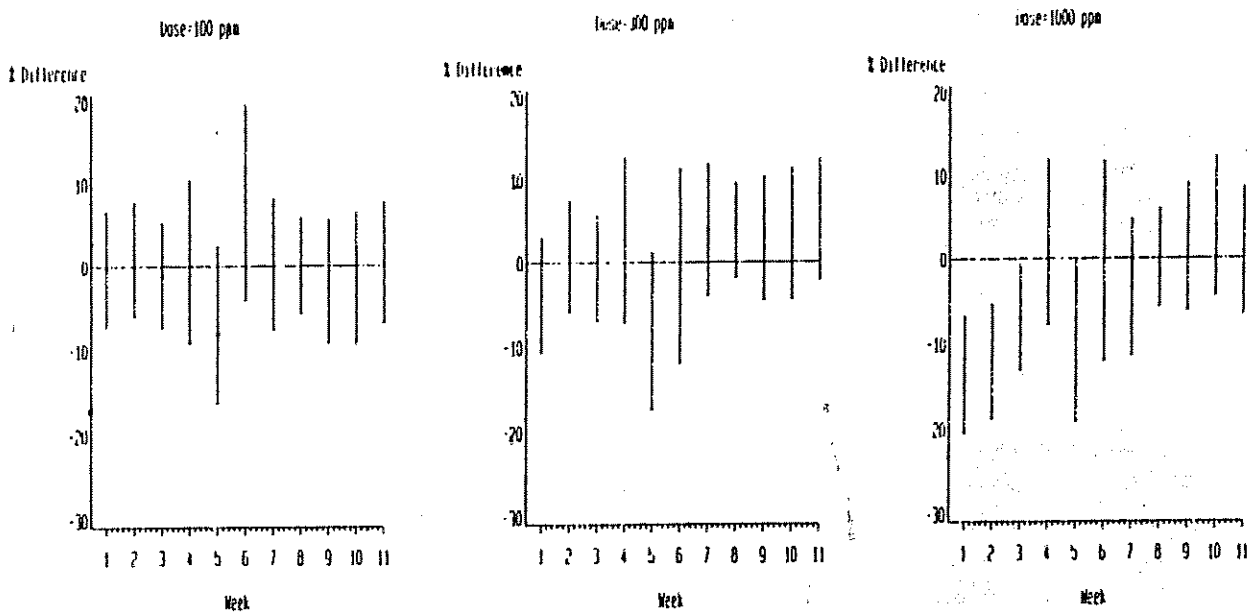
FOOD CONSUMPTION DURING PRE-PAIRING PERIOD
- F_1 GENERATION - FEMALES



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 22

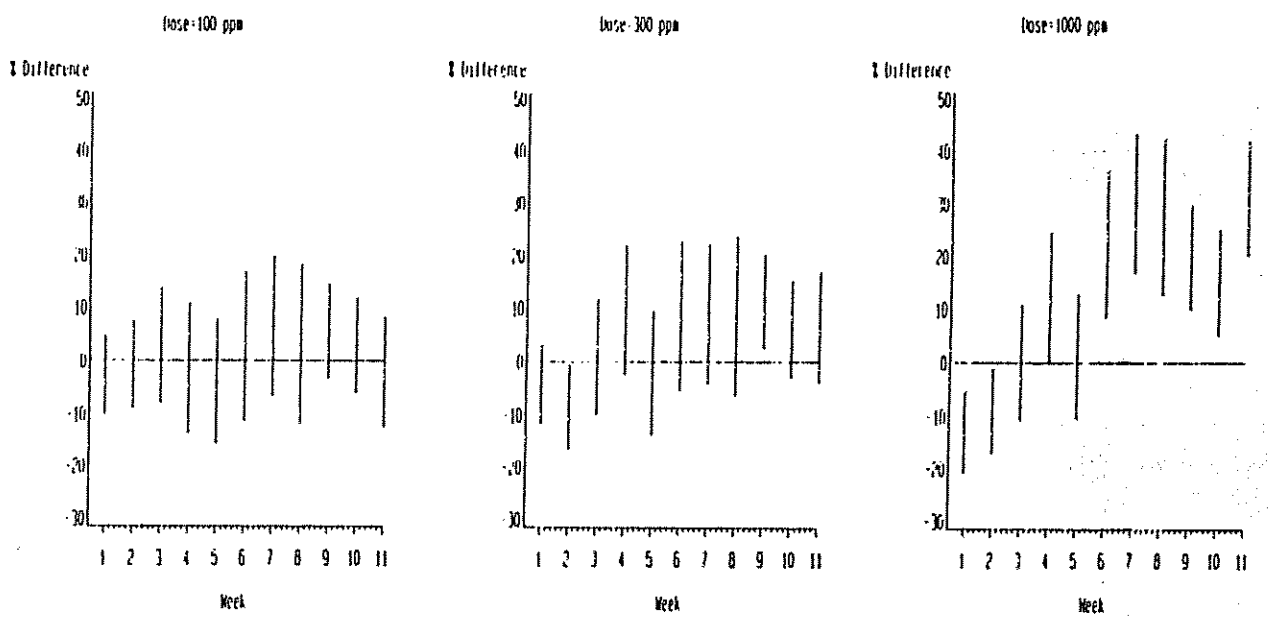
STATISTICAL ANALYSIS PLOTS OF FOOD CONSUMPTION
- F1 MALES



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 23

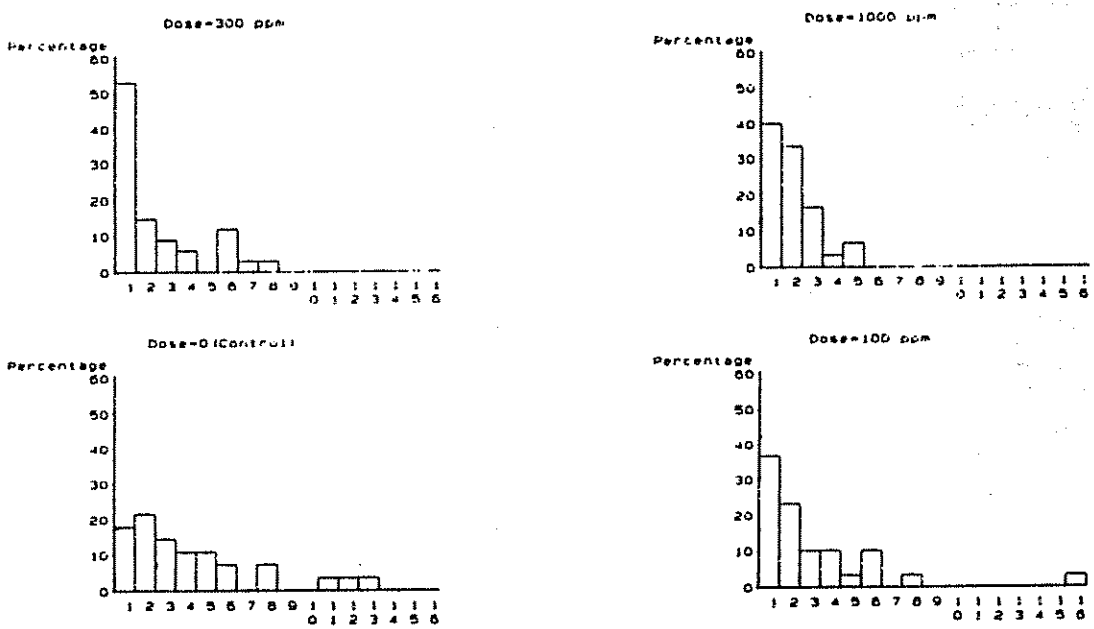
STATISTICAL ANALYSIS PLOTS OF FOOD CONSUMPTION
- F1 FEMALES



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 24

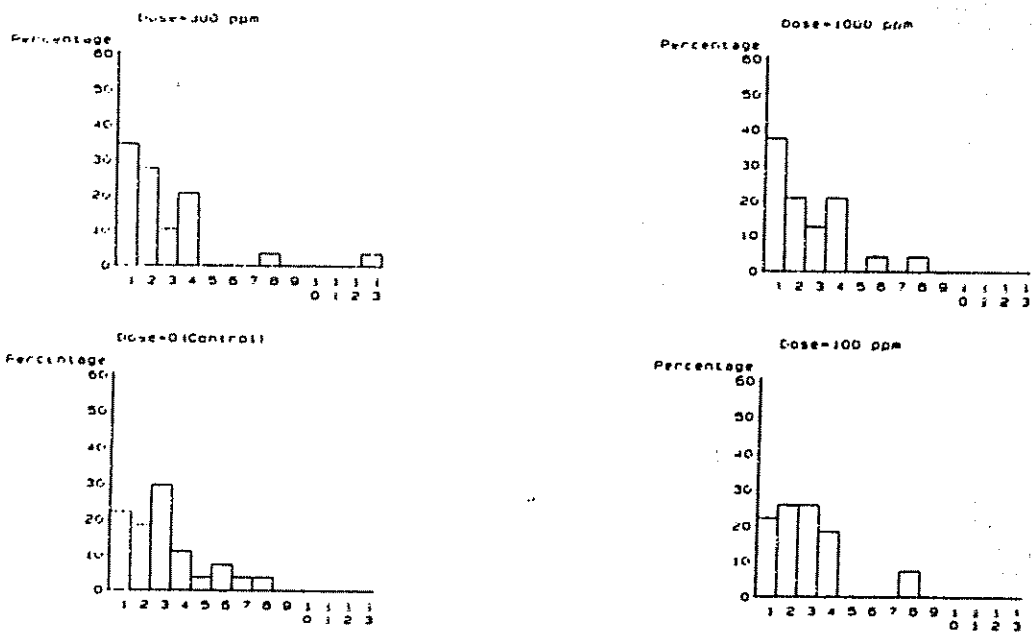
PRE-COITAL INTERVAL (days) - F_0 GENERATION
- F1A LITTER



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 25

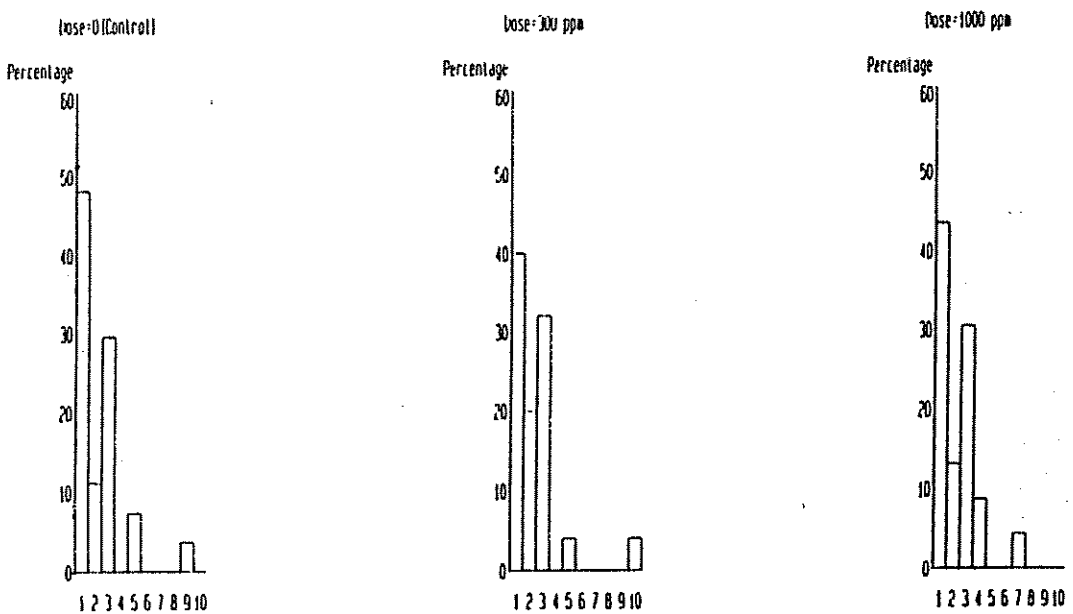
PRE-COITAL INTERVAL (days) - F_1 GENERATION
- F2A LITTER



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 26

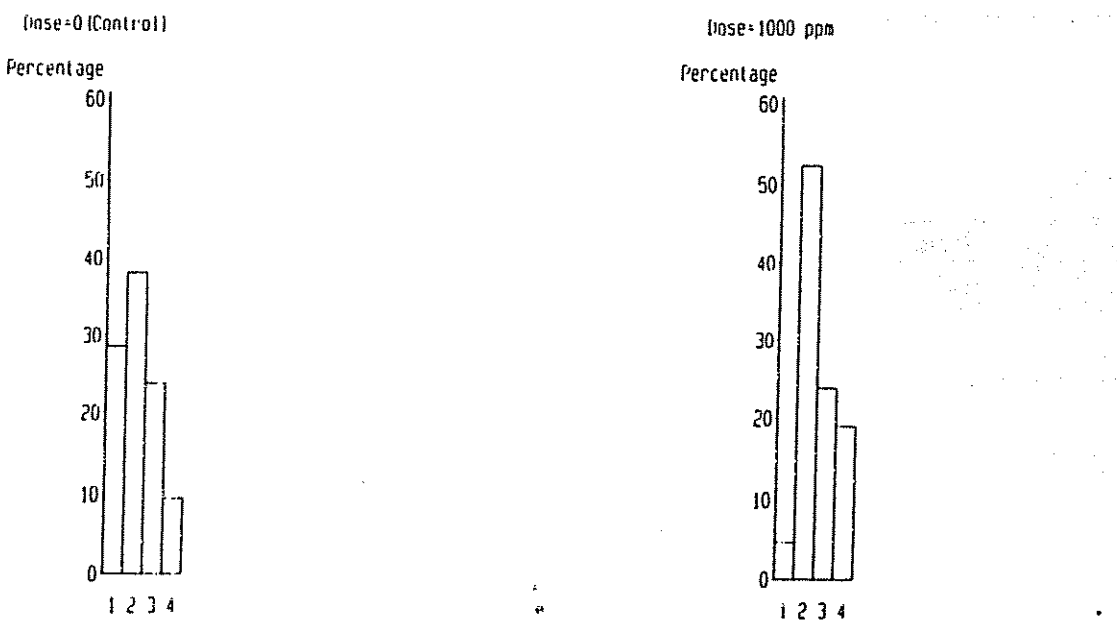
PRE-COITAL INTERVAL (days) - F_1 GENERATION
- F2B LITTER



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 27

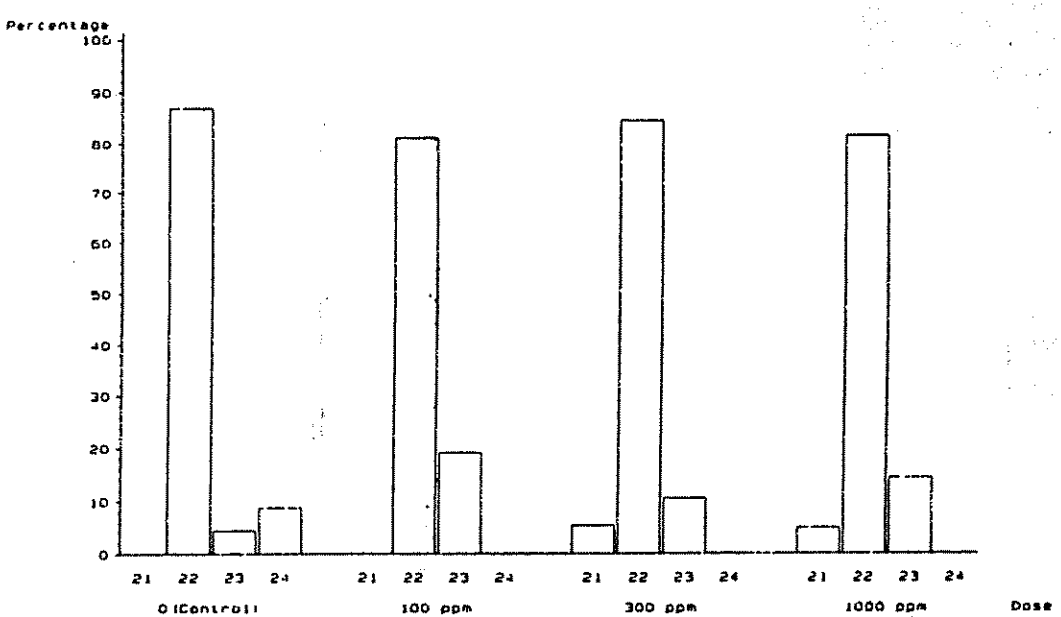
PRE-COITAL INTERVAL (days) - F_1 GENERATION
- F2C LITTER



PERCHLORDETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 28

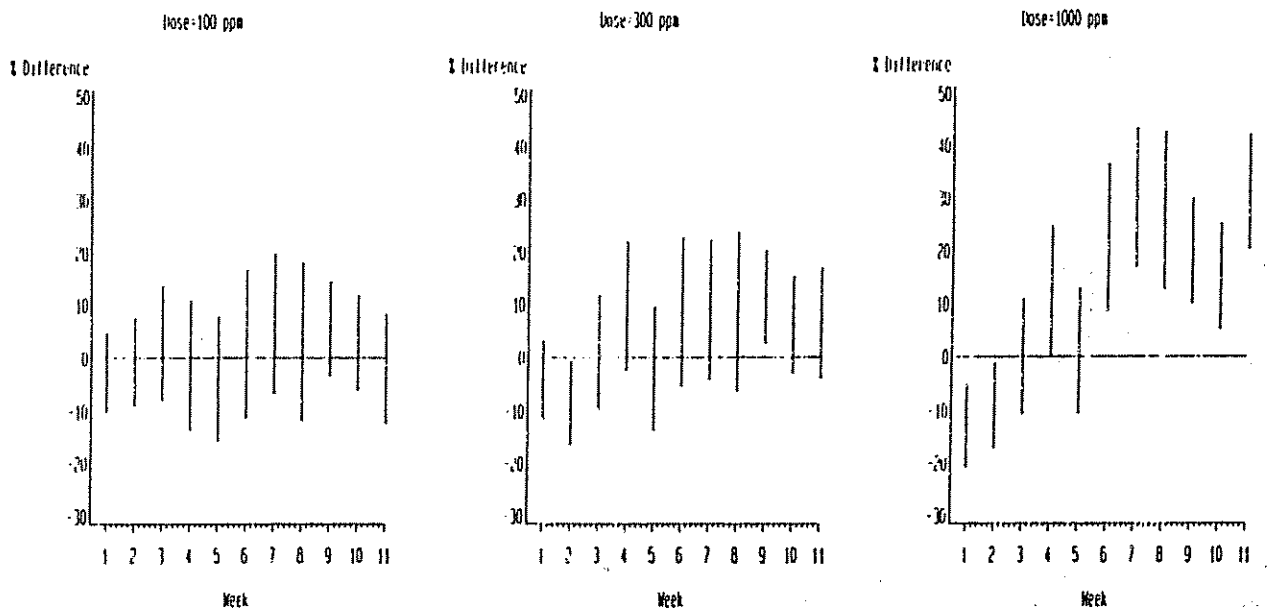
GESTATION PERIOD (days) - F_0 GENERATION
- F1A LITTER



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 23

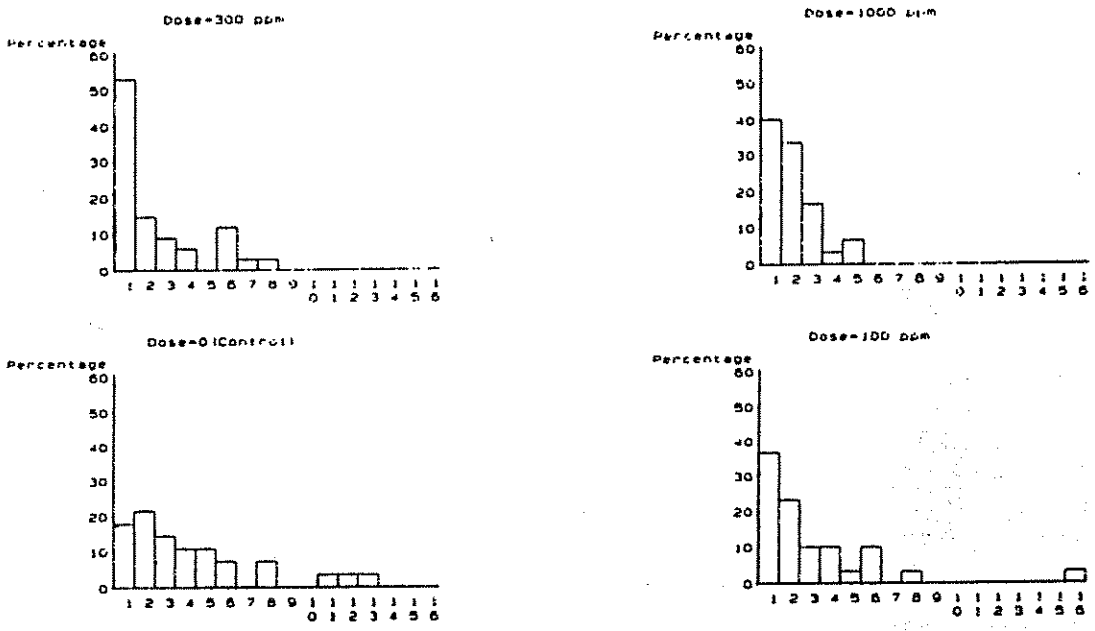
STATISTICAL ANALYSIS PLOTS OF FOOD CONSUMPTION
- F1 FEMALES



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 24

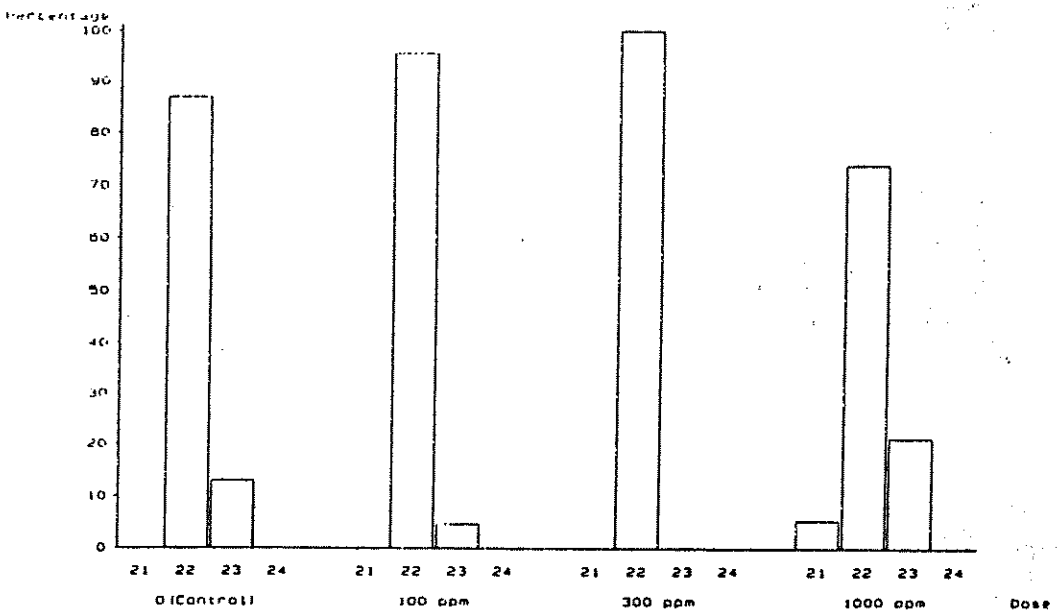
PRE-COITAL INTERVAL (days) - F_0 GENERATION
- F1A LITTER



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 29

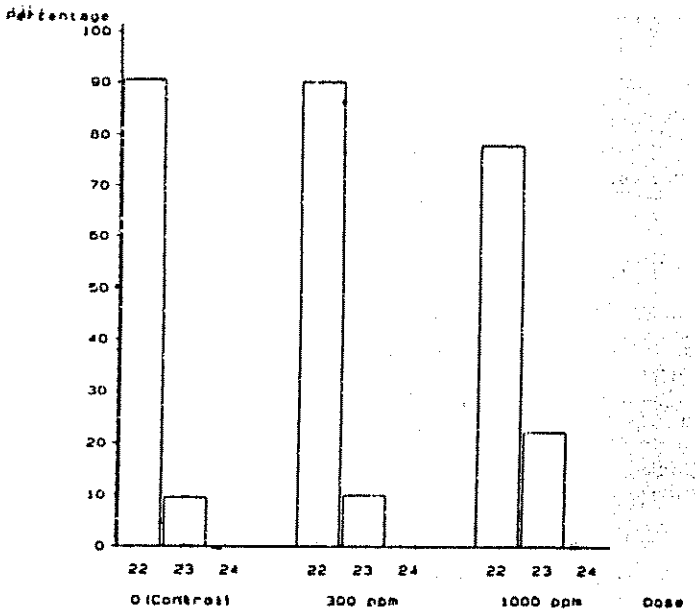
GESTATION PERIOD (days) - F_1 GENERATION
- F2A LITTER



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 30

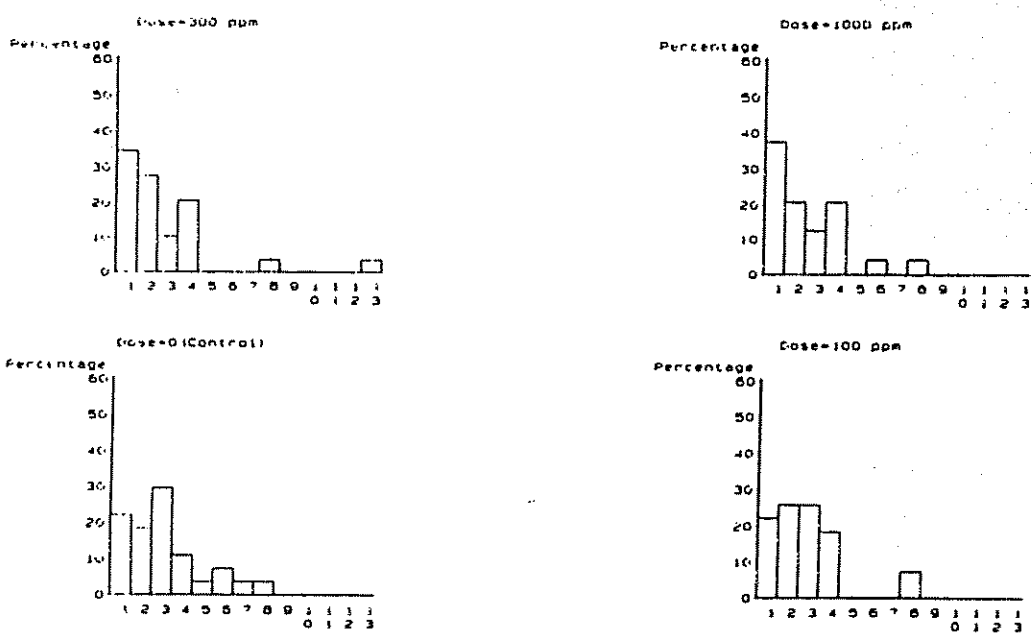
GESTATION PERIOD (days) - F_1 GENERATION
- F2B LITTER



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 25

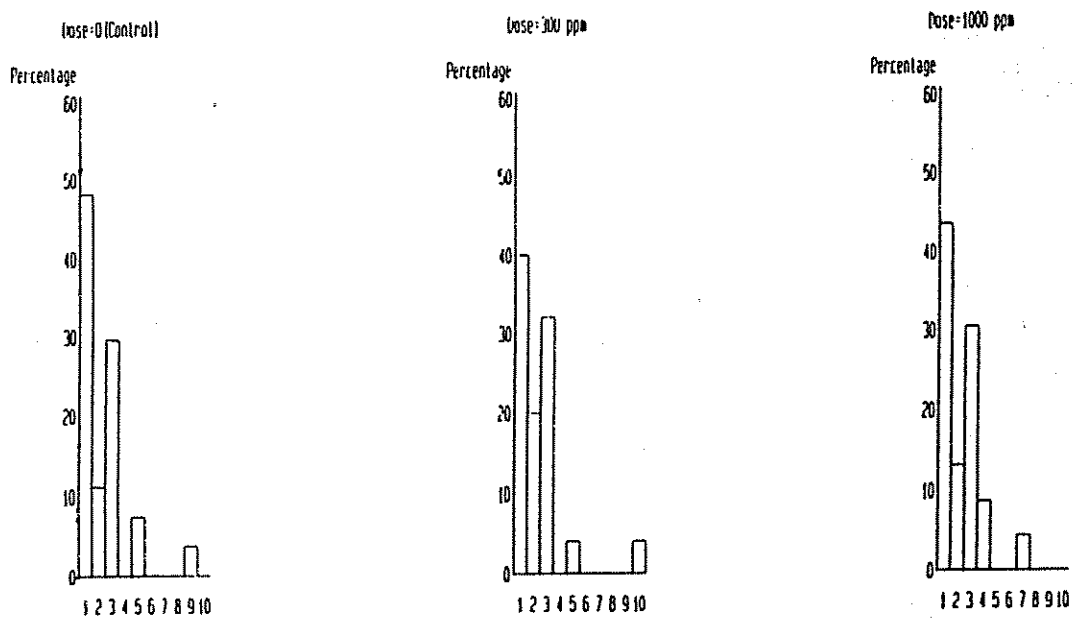
PRE-COITAL INTERVAL (days) - F_1 GENERATION
- F2A LITTER



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 26

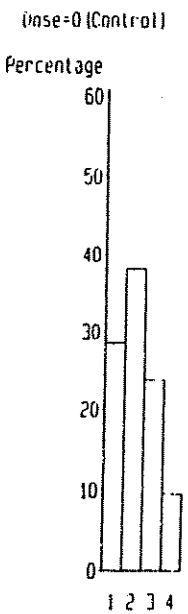
PRE-COITAL INTERVAL (days) - F_1 GENERATION
- F2B LITTER

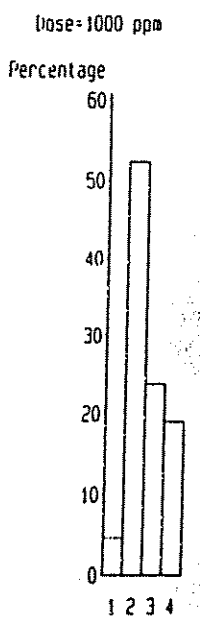


PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 27

PRE-COITAL INTERVAL (days) - F_1 GENERATION
- F2C LITTER

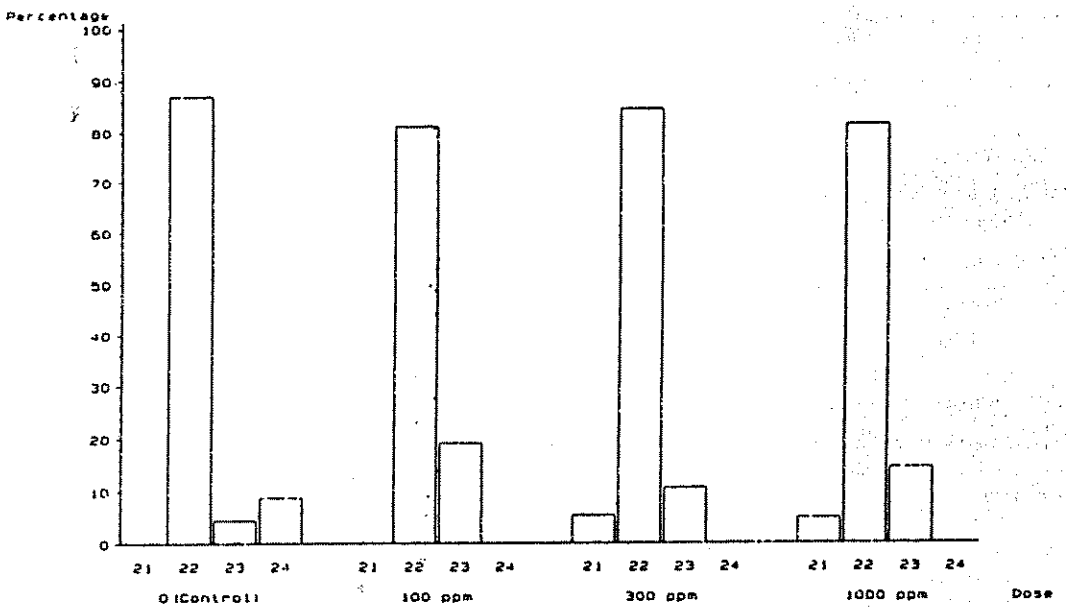




PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 28

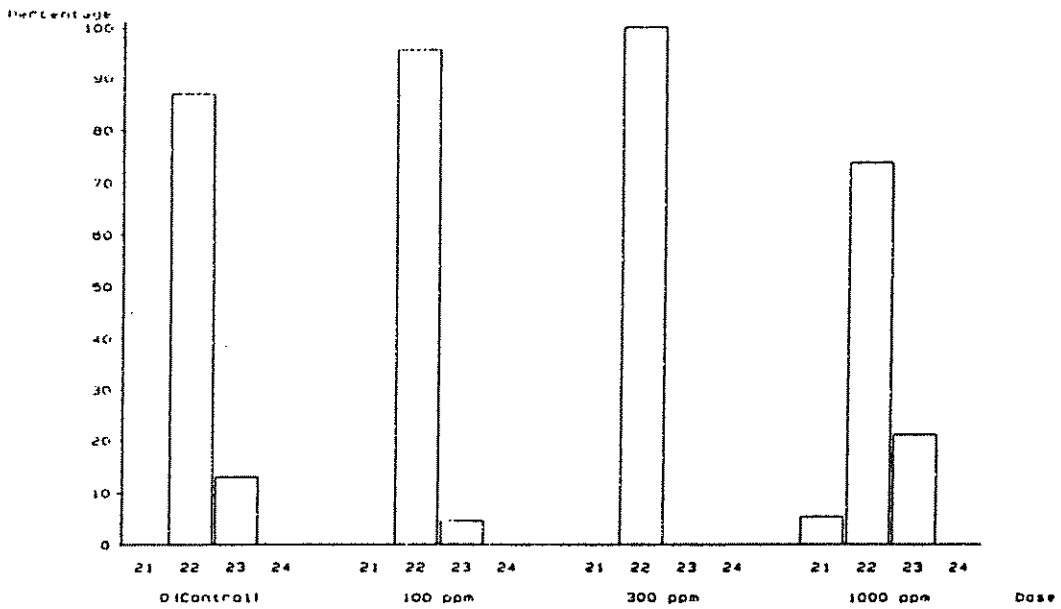
GESTATION PERIOD (days) - F_0 GENERATION
- F1A LITTER



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 29

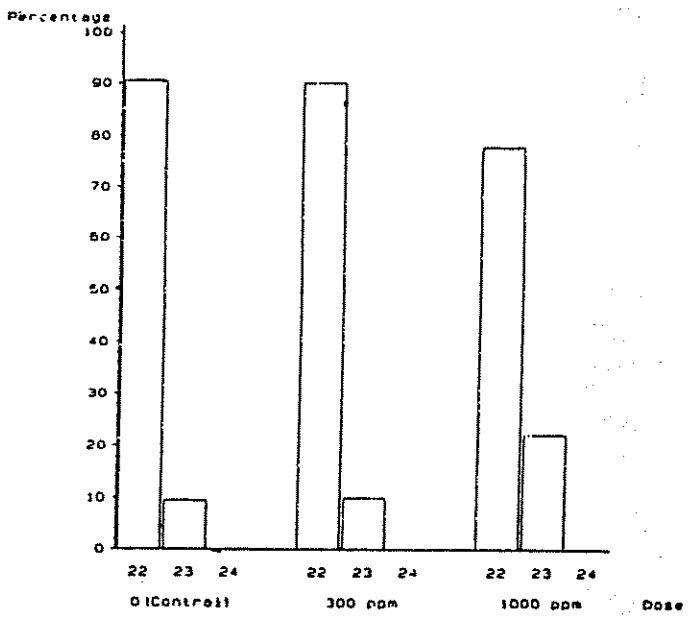
GESTATION PERIOD (days) - F_1 GENERATION
- F2A LITTER



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 30

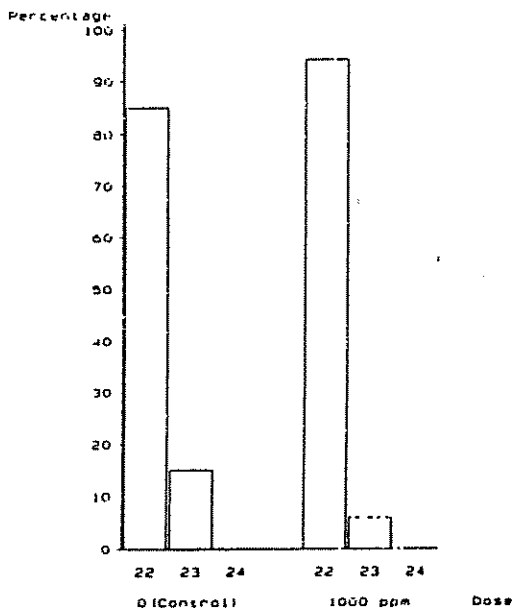
GESTATION PERIOD (days) - F_1 GENERATION
- F2B LITTER



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 31

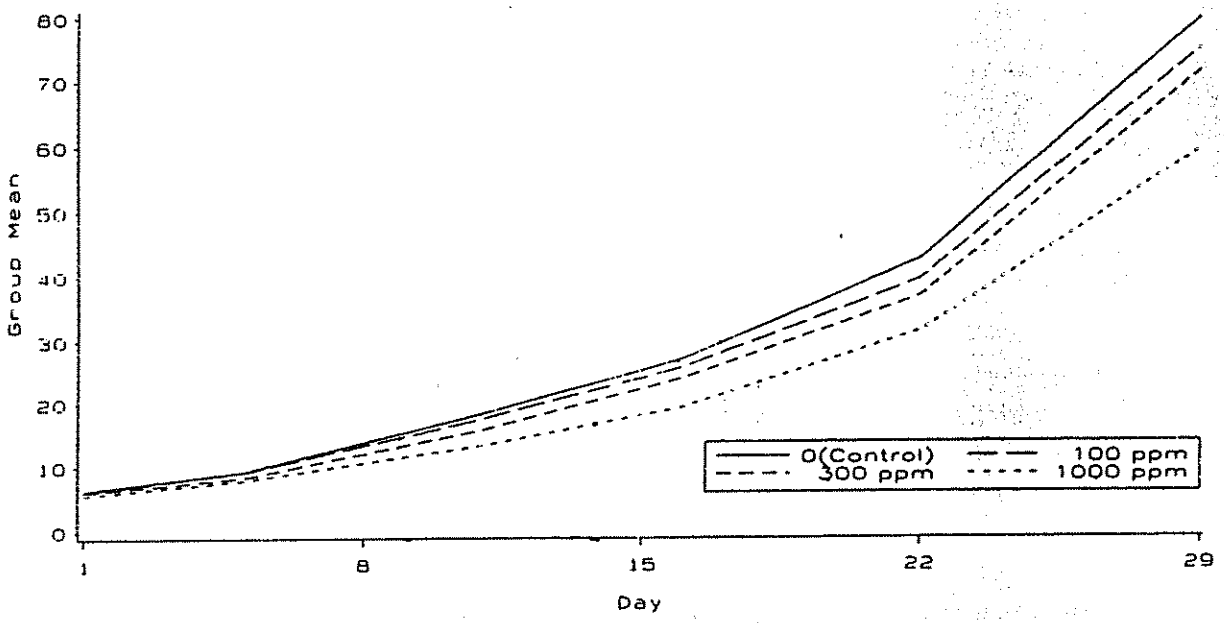
GESTATION PERIOD (days) - F_1 GENERATION
- F2C LITTER



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 32

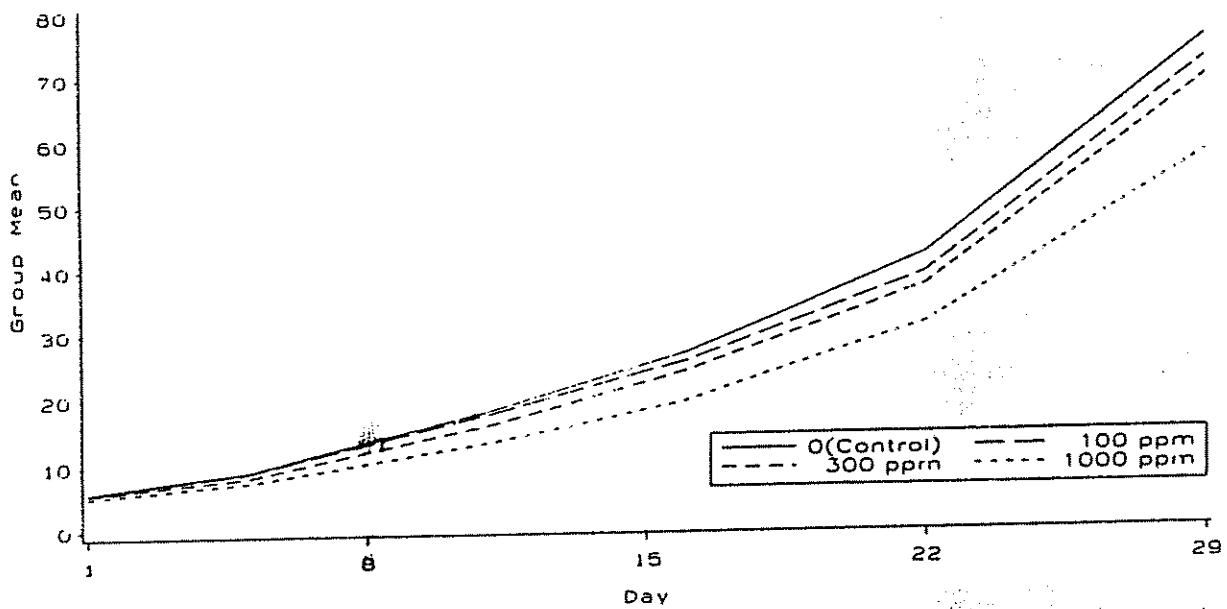
GROUP MEAN PUP WEIGHTS - F_1A LITTER - MALES



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 33

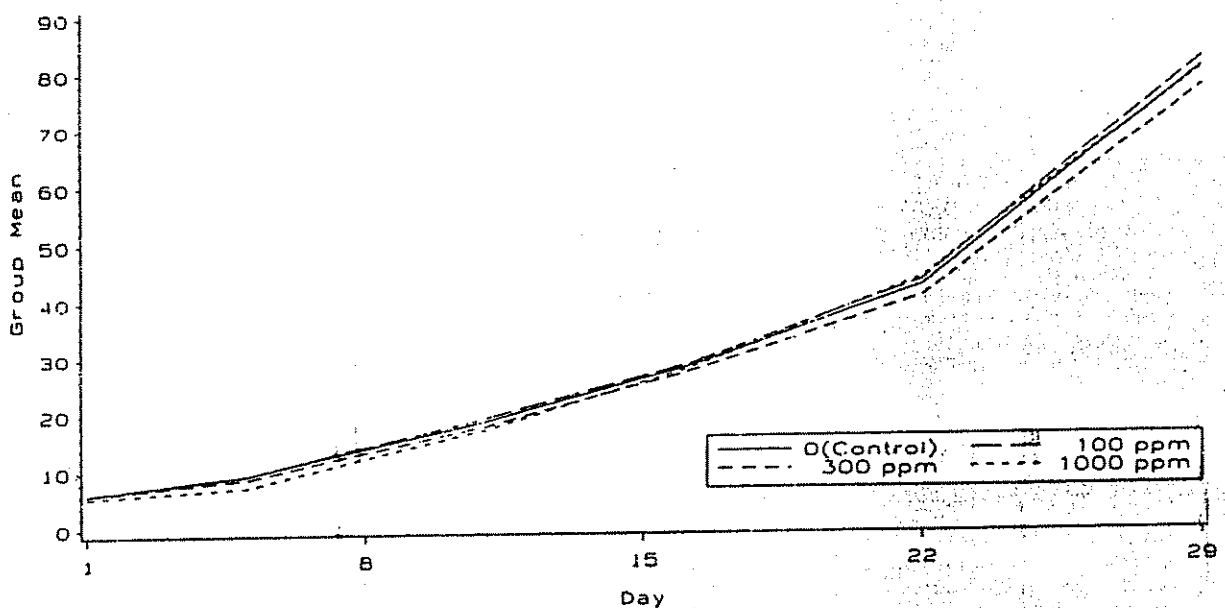
GROUP MEAN PUP WEIGHTS - F_1A LITTER - FEMALES



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 34

GROUP MEAN PUP WEIGHTS - F_2A LITTER - MALES



PERCHLOROETHYLENE: MULTIGENERATION INHALATION STUDY IN THE RAT

FIGURE 35

GROUP MEAN PUP WEIGHTS - F_2A LITTER - FEMALES