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HMX: 14 Day Toxicity Study in Rats by Dietary Administration

Final Report by:

R.J. Greenough
P. McDonald

July, 1985

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U.S. Army Medical Research and Development Command
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Inveresk Research International Limited
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Abstract

Male and female rats were dosed via the diet for 14 days with HMX in order to select dose levels for a 13 wee k study. Concentrations were selected to give doses of 0, 100, 1000, 3000 and 9000 mg HMX/kg/day. Deaths occurred in males at 9000 mg HMX/kg/day and in females at 1000 mg HMX/kg/day and above. There were substantial effects on food intake and upon body weight.

Histopathological examination revealed centrilobular toxic degeneration in the lives of male and female rats. There was also hepatocyte hyperplasia, increased cyto plasmic eosinophilia in the liver and lymphocytic depletion in the spleen and thymus.

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FOREWORD

"I, the undersigned, hereby declare that this work was performed under my supervision, according to the procedures herein described and that this report represents a true and accurate record of the results obtained."

A. B. Wilson

A.B. Wilson, B.V.Sc., M.R.C.V.S., D.A.B.T.

Project No. 415669SR

Report No. 1963

QUALITY ASSURANCE AUTHENTICATION

The conduct of this study has been subjected to periodic inspections by the IRI Quality Assurance Unit. The dates of inspection are given below.

IRI Project No. 415669SR

Report No. 1963

Date of Q.A. Inspection	Date of Report to Management
20 October 1980	21 October 1980
31 October 1980	3 November 1980

This report has been audited by the Quality Assurance Personnel according to the appropriate Standard Operating Procedure. The report is considered to describe accurately the methods and procedures used in the study and the original data generated during the study.

Signed: Andrew Wakelin
(Quality Assurance Manager)

Date: 14th January 1986.

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SUMMARY

The object of this study was to provide information on the subacute toxicity of the test substance and to give an indication of suitable dose levels for subsequent studies in rats.

Groups of 6♂ and 6♀ Fischer 344 rats were fed diets containing Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) for 14 days at nominal dose levels of 0, 333, 1000, 3000 and 9000 mg/kg/day. At the end of this period all the surviving rats were killed and subjected to necropsy and gross pathology. Subsequent histopathological examination of selected target organs was carried out on all control and group 5 animals, together with all the premature decedents from groups 3 and 4.

The results obtained are summarised below:

Mortality

There were 13 premature decedents distributed as follows:

	Males					Females				
Dose Group	1	2	3	4	5	1	2	3	4	5
Nominal Dose Level mg HMX/Kg/ day	0	333	1000	3000	9000	0	333	1000	3000	9000
% Mortality	0	0	0	0	83	0	0	17	17	100

Clinical Signs

Emaciation, hunched posture, subdued appearance, piloerection and frequent incidences of red staining around the eyes and nostrils were observed in group 4 and 5 males and all HMX treated females. One convulsion occurred among the group 5 females.

Body Weight

Males receiving HMX showed a dose related reduction/loss in body weight gain. Groups 4 and 5 showed an actual loss in body weight at start of dosing. This weight loss was not regained by the group 5 animals.

Females receiving HMX all showed a significant body weight loss.

Food Consumption

Marked reductions in food consumption were observed for the HMX treated animals. The female rats consumed 50% or less compared with control animals during the first week of dosing.

Terminal Studies

Gross Pathology and Histology

Histopathological examination revealed centrilobular toxic degeneration in the livers of the group 5 male rats receiving an average achieved dose of 8504 mg HMX/kg/day. Hepatocyte hyperplasia and increased cytoplasmic eosinophilia in the liver, together with lymphocyte depletion in the thymus and spleen were observed for the group 5 females receiving an average achieved dosage of 3055 mg HMX/kg/day. These findings were also observed for the premature decedents from groups 3 and 4 receiving 1280 and 3474 mg HMX/kg/day respectively.

Organ Weights

Absolute liver and kidney weights were reduced for all HMX treated groups when compared to control animals. Relative liver weights were reduced for males whilst relative kidney weights were increased for both males and females. The increases observed in relative kidney weights, for HMX treated animals, can probably be attributed to the reductions observed in body weight gain.

INTRODUCTION

Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) is an explosive and is found in the water effluent from the manufacturing processes for RDX and HMX.

This report describes a 14 day dietary toxicity study in rats conducted to set dose levels for a proposed 90 day study.

The study was undertaken at the Elphinstone Research Centre of Inveresk Research International Limited over the period 9 October - 31 October 1980.

MATERIALS AND METHODS

The HMX (Lot No. HMX-IRI-001) was manufactured by the Royal Ordnance Factory, Somerset. The bulk of the material (5 kg: wetted with 15.25% water and packaged in individual 50 g lots) was stored at Nobel's Explosive Company, Muirside, Dunfermline.

Small amounts of HMX were transported, in an approved container, to the IRI laboratories as required. The samples used in this study were obtained on 4 August, 16 September and 7 October 1980. The test compound was stored in a Ministry of Defence approved container under ambient conditions in the company dispensary. Prior to use the required amount of HMX was removed from this container and dried at approximately 90°C overnight. The dried HMX was then stored in a glass dessicator until used.

Animals

Thirty five male and 35 female Fischer 344 rats (40-60 g body weight) were obtained from Charles River (U.S.A.) Limited, Wilmington, Mass. on 1 October 1980. Sixty rats (30♂ and 3♀) were allowed to acclimatise to their new environment for 8 days before treatment commenced.

Pre-experiment Acceptance Testing

All animals were examined on arrival for signs of disease. Five animals of each sex were selected, firstly on the basis of a clinical examination and secondly in a random choice manner, and subjected to a microbial examination and histopathological evaluation of main organs. The results of these tests showed that the delivery of animals was of an acceptable standard for use on this study.

Animal Management

The rats were housed in an animal room dedicated to this experiment with a light intensity of approximately 200 lux, a 12 h light-dark cycle, temperatures automatically maintained at 20°C + 2°C with extreme limits of 21°C and 24°C, and humidity ca 50% with extreme limits of 34% and 59%.

Caging

The rats were housed one per cage in polypropylene cages (overall dimensions 48 cm x 15 cm x 22 cm) with stainless steel wire grid tops and bottoms. The cages were suspended over trays lined with absorbent paper.

Cages, trays and absorbent paper were changed as necessary.

Diet

Food and tap water were freely available to the rats at all times. The diet was BP Nutrition Rat and Mouse No. 1, a ground diet adequate for all stages of growth in rats.

Typical analyses of both water and diet are presented in Appendix 1.

Allocation of Rats to Cages and Treatment Groups

Empty cages were placed on racks, then upon receipt, starting first with male rats, a transporting box was opened and a rat placed in the first cage at the top left hand corner of the rack. A second rat was removed from the same transporting box and placed in the next cage and so on until 35 cages each contained one male rat.

This process was repeated using new cages and female rats.

Following a 2 day transportation recovery period the animals were allocated to specific treatment groups using a stratified body weight sequenced randomisation procedure.

Four body weight ranges were selected for each sex. Starting first with the males, the animals were removed from their cages, weighed and then segregated into 1 of 4 large stainless steel holding cages according to their body weight. Six rows of cages, each row containing 5 cages, were then arranged on racks. Two sets of computer generated random number permutations were obtained, the first set gave 5 random sets of numbers from 1-6 corresponding to the number of sequences of cages and the second gave 6 random sets of numbers from 1-5 corresponding to the number of treatment groups.

Starting with the lowest weight range of male rats one animal was placed in a cage according to the sequence position indicated by the first number in the first set of random numbers. The cage was then assigned to a treatment group using the second random number set. This process was repeated until all the male rats from the 4 different weight ranges had been assigned to cages and treatment groups.

This complete process was repeated for the female rats.

Animal Identification, Treatment Groups and Dose Levels

Each animal received a unique ear punch which identified it individually within the study and which corresponded to that

animal's number. Each rat was ascribed a cage card which identified that animal by project number, cage number, animal number, sex and treatment group. Each cage card was colour-coded according to the treatment group.

The treatment groups were as follows:

Dose Group	Dose Level HMX (mg/kg/day)	Colour Code	Animal Numbers	
			♂	♀
1	0 (Control)	Green	501-506	531-536
2	333	Blue	507-512	537-542
3	1000	Yellow	513-518	543-548
4	3000	Buff	519-524	549-554
5	9000	Red	525-530	555-560

Animal Room Sanitation

Floors were mopped each morning, before other work in the room had begun. In the afternoon floors were swept and then mopped with a disinfectant solution after work in the room was finished.

Diet Preparation

Diets containing HMX were prepared freshly at the beginning of each study week. The concentration of test compound in the diet was calculated weekly after predicting the mid-week body weight and food consumption for the forthcoming week. The following equations were used in this calculation:

$$\text{Concentration (PPM)} = \frac{\text{Dose level (mg/kg/day) x predicted mid-week body weight (g)}}{\text{predicted daily food consumption (g/day)}}$$

$$\text{Amount of test substance required (g)} = \frac{\text{PPM x weight of diet required}}{1000}$$

After drying to a constant weight HMX was sieved through a plastic 100 μm mesh sieve immediately before use. The requisite amounts of HMX and powdered diet were measured out, transferred

to plastic containers, sealed and mixed for 20 min using a Winkworth change drum tumble mixer.

Dietary Sampling

Ten gram samples were taken from the top, middle and bottom of the container holding the freshly mixed diet to confirm stability and homogeneity of mixing of HMX. These samples, along with a 100 g sample taken for archives, were taken from all diet mixes including controls.

Analysis of HMX in Diets

Analytical Method

HMX was analysed by high performance liquid chromatography (HPLC).

Three samples (of an appropriate size depending on the nominal concentration of HMX in the diet) were weighed into 3 x 8 oz glass jars. To this was added an appropriate volume of internal standard solution (1,3-dinitrobenzene in acetonitrile) and sufficient acetonitrile for complete extraction. The glass jars were then capped and shaken mechanically for 1 h after which the jars were removed and the contents allowed to settle. An aliquot of the liquid fraction was injected into the HPLC and analysed either directly or after suitable dilution using acetonitrile:water (20:30 v/v).

Standard solutions of HMX were prepared by adding known amounts of HMX to samples of untreated diets. These were treated with internal standard solution and extracting solvent as described above for the formulated diet samples. Further details are reported under project DAMD 17-80-C-0053.

Stability and Homogeneity of HMX in Diets

Before commencement of the study experiments were performed to determine homogeneity and stability of HMX treated diets. Accordingly diets containing 1,250, 10,000 and 25,000 PPM (w/w) of HMX were formulated. Ten representative samples from each diet mixture were analysed immediately after preparation. Ten samples from each diet were subjected to accelerated ageing by storage at 40°C in an environmental cabinet and analysed after 14 days. The remainder of each diet was stored at room temperature and analysed after 21 days.

Results of these analyses showed that mixing procedures and stability were satisfactory (see Appendix 2).

HPLC Conditions

HPLC:	Altex pump, Pye Unicam LC3 UV Spectrophotometer
Column:	Hypersil ODS (10 cm x 0.5 cm)
Solvent:	Acetonitrile:water (20:30 v/v)
Flow:	1 ml/min
Wavelength:	228 nm
Recorder:	Servoscribe 1s - 10 mV
Chart Speed:	300 mm/h

Observations on the Animals

Mortality

All animals were inspected for any deaths at the start of each day and again during the afternoon clinical signs check.

Clinical Signs

The rats were observed at intervals throughout the day for any signs of ill health or reaction to treatment.

Physical Examination

Each animal was given a weekly detailed physical examination for external lesions or palpable masses.

Body Weight

The weight of each rat was recorded on the day dosing commenced and twice each week thereafter. Animals were also weighed twice prior to the start of treatment.

Food Consumption

Food consumption was recorded on a weekly basis. The quantity of food eaten by each rat was calculated by measurement of the amount of food given at the beginning of each week and deducting that remaining in the food hopper at the end of each week and any that may have been scattered on the cage floor during that week.

Water Consumption

The quantity of water consumed by each rat was assessed each week by visual assessment of the calibrated water bottle.

Terminal Studies

On day 15 of dosing all the surviving rats were sacrificed by nitrogen asphyxiation. Blood samples of ca 2 ml were taken into heparin via the posterior vena cava from all animals. Plasma was separated by centrifugation and stored deep frozen at -20°C for analysis at a later date.

Gross Necropsy

A full necropsy was performed as detailed below. Each rat was examined externally including the body orifices along with examination of internal organs and tissues.

The following organs were taken at necropsy:

Brain
Heart
*Kidneys
*Liver
Spleen
Thymus

The fresh weights of the organs marked * were recorded before preservation.

All organs were examined in situ, then dissected from the carcass, re-examined, including cut surfaces, and then preserved in 10% neutral buffered formalin.

Tissues were fixed after slicing to a thickness not exceeding 0.5 cm.

Liver lobes were sliced, the kidneys longitudinally bisected and the cut surfaces examined before fixation.

All gross lesions were recorded in narrative, descriptive terms, including size (in mm), number, shape, colour and texture.

Carcasses of animals were discarded immediately following autopsy and the placing of all tissues listed above in fixative.

Processing of Fixed Tissues

The fixation time was 14 days.

Tissues were trimmed to a maximum thickness of 0.3 cm for processing.

Parenchymal organs, e.g. liver, were trimmed to allow the largest surface area possible for examination.

Multi-longitudinal sections through the entire cortex and medulla of each kidney were submitted.

Three cross-sections of brain including (a) frontal cortex and basal ganglia, (b) parietal cortex and thalamus and (c) cerebellum and pons were submitted.

Histological Technique

Tissues were cut to 4-6 μm thickness and stained with haematoxylin and eosin (H & E).

All staining methods used are described in "Histological Laboratory Methods" by Disbrey and Rack (E.S. Livingstone Ltd., Edinburgh, 1970).

Histopathological Examination

Histopathological examination of the tissues listed above was carried out on all control and group 5 (top dose) animals, together with all the premature decedents.

Statistical Analysis

Whenever considered necessary, numerical data were subjected to statistical analysis using Student's 't' test.

The levels of significance as indicated in the report are:

*	Significantly different from controls, $P<0.05$
**	Significantly different from controls, $P<0.01$
***	Signficiantly different from controls, $P<0.001$

Archiving

On completion of all practical work the biological material and data generated during the study was stored, together with samples of the diet formulations and a sample of the test compound used, in the Scientific Archives of Inveresk Research International Limited. All materials relating to this study will be retained for a minimum period of 5 years.

RESULTS

Dosing period: 16-30 October 1980
Necropsy: 31 October 1980

Observations

Mortality

There were 13 premature decedents during the course of this study. The time of death and mortality distribution is presented in Table 1.

All the premature decedents showed a pronounced body weight loss during the period they were dosed. Clinical signs observed prior to death or sacrifice *in extremis* were emaciation, unkempt appearance, hunched posture and piloerection.

Clinical Signs

Males

Group 1 (Control) - No abnormalities were observed.

Group 2 (333 mg/kg/day) - No abnormalities were observed.

Group 3 (1000 mg/kg/day) - 518♂ slight loss of body tone at the start of week 2, no abnormalities were observed for remaining animals in group.

Group 4 (3000 mg/ - Day 4: all animals observed to have a slightly emaciated appearance.

Day 11: mild piloerection in 3/6 animals. Piloerection observed for remainder of dosing period.

Group 5 (9000 mg/ - Day 4: all animals observed to have a slightly emaciated appearance, No. 530♂ had red staining around nostrils and a hunched appearance.

Day 7: all animals emaciated and displaying a hunched posture with piloerection - these signs were observed for the remainder of the dosing period. Nos. 528♂ and 529♂ were observed to have some bald patches.

Day 9: 528♂ found dead in cage, hyperkinesia observed in surviving animals.

Day 11: emaciated, unkempt appearance.

Day 12: 527♂ found dead in cage.

Day 13: 525♂, 526♂ and 529♂ found dead in cage.

Females

Group 1 (Control) - No abnormalities detected.

Group 2 (333 mg/kg/day) - Day 4: all animals observed to have a slightly emaciated appearance.

Day 7: 539♀ had red staining around nostrils, mild piloerection observed in all animals.

Day 11: mild piloerection in 2/6 animals.

Group 3 (1000 mg/kg/day) - Day 4: all animals observed to have an emaciated appearance with a hunched posture and piloerection - these signs persisted for the remainder of the dosing period.

Day 11: 548♀ ataxic, extremely emaciated with a hunched posture and marked piloerection - killed in extremis.

Group 4 (3000 mg/kg/day) - Day 4: all animals observed to have an emaciated appearance and piloerection - these signs persisted for the remainder of the dosing period. Blood stains were observed on the tray papers for Nos. 549♀, 553♀ and 554♀.

Day 7: red staining observed around nose of Nos. 550♀ and 554♀.

Day 8: 553♀ observed to have eaten the tip of its own tail, later observed to be ataxic and vocalising.

Day 9: 550♀ found dead in cage. Surviving animals hyperkinetic.

Day 11: all surviving animals hyperkinetic when aroused. The tail of 553♀ was slightly swollen.

Day 12: 553♀ was observed gnawing at remainder of tail.

Day 13: 553♀ - eyes were observed to be very pale.

Group 5 (9000 mg/kg/day) - Day 4: all animals were emaciated. Subdued appearance, hunched posture, reduced body tone, piloerection and unkempt appearance were also observed - these signs persisted until death. Blood staining observed on tray papers of all animals. Animal 556♀ had red/brown staining around nostrils.

Day 5: animal 556♀ had red staining around nostrils. Animal 557♀ standing on the tip of its digits - not on the whole foot, ataxia, sneezing, red/brown staining on nostrils and fore paws also observed.

Day 6: 555♀ very subdued - resists handling. Red staining around nose, eyes, lower jaw and fore paws - killed in extremis. 556♀ had red staining on eyes, nose and chin, yellow staining observed around perigenital area. Aggressive when handled. No. 557♀ resists handling. No. 558♀ observed having a convulsion during morning observations - eyes dull with red staining, also red staining on nose and jaw. Nos. 559♀ and 560♀ had red staining on eyes, nose and jaw.

Day 7: 556♀ found dead in cage. No. 559♀ hyperkinetic.

Day 8: 557♀, 558♀ and 559♀ found dead in their cages.

Day 9: 560♀ found dead in cage.

Body Weight

Group mean body weights are presented numerically in Tables 2a and 2b and graphically in Figures 1 and 2. Individual values are given in Appendix 3.

Males

Significant dose related reductions in body weight gain were observed. Groups 4 and 5 receiving 3000 or 9000 mg/Kg/ respectively showed an actual body weight loss by Day 4 of dosing. Group 4 animals' body weights were slightly above initial body weights by Day 7.

Females

Animals fed HMX treated diet showed a significant loss in body weight during the first 4 days of dosing. Group 5 animals continued losing weight and eventually died, or were killed in extremis. Groups 2, 3 and 4 showed a marked depression in body weight gain with only group 2 animals exceeding their pre dose body weight values.

Food Consumption

Group mean food consumption values are presented numerically in Table 3.

During the first week of dosing HMX treated animals showed a marked reduction in food intake when compared to controls. Food consumption values were increased for all treated groups during week 2 of dosing although groups 4 and 5 male and 2, 3, 4 and 5 female were still significantly less than controls.

Water Consumption

No differences were detected between control and HMX treated animals.

Achieved Dosage

The actual amounts of HMX consumed by each dose group are presented in Table 4. The achieved dosage for group 5♀ during the second week of dosing has not been calculated due to the premature decedency of the test animals.

Results obtained from analysis of the freshly prepared diets are presented in Appendix 4.

Terminal Studies

Organ Weights

Group mean values for organ weights expressed in absolute terms and as a percentage of body weight are presented in Tables 5a and b. Values for group 5♀ are not presented due to premature decedency of all animals. Individual values for all animals are given in Appendix 5.

Liver weights in all the HMX treated groups showed statistically significant reductions compared with control animals. These reductions were significant in absolute and relative terms for the males but only in absolute terms in the females.

Kidney weights showed a significant dose related reduction in absolute terms, this being reflected in the dose related effects on body weight resulting in elevation of relative kidney weights compared with controls.

Organ weight profiles for most of the premature decedents showed reductions in absolute terms. Relative weights were considered to be slightly elevated, due primarily to the observed body weight loss following dosing with HMX.

Pathological Examination

Gross pathology and histopathological findings for individual animals are presented in Appendix 6.

Histopathological findings are summarised in Table 6.

Gross pathological examination revealed smaller than normal spleens and enlarged adrenals in 4/6 group 5 females. Enlarged adrenals were also observed in the group 4 female premature decedent. It was also noted that the group 3 premature decedent had a smaller than normal liver and small kidneys. The brains from one male and 2 females in group 5 were described as friable. Blood was observed under the skull of 2 group 5 males. Dark red lungs were also described in 2 control females and 4 group 5 males, this high incidence possibly being the result of autolysis.

Histopathological examination revealed centrilobular toxic degeneration in the livers of the group 5 male rats treated with a target dose level of 9000 mg HMX/kg/day (actual achieved dose 8504.3 mg HMX/kg/day). In the case of female rats hepatocyte hyperplasia and increased cytoplasmic eosinophilia was found in the liver together with lymphocyte depletion in the thymus and spleen.

These lesions were found in female rats dosed at a target level of 9000 mg HMX/kg/day (actual achieved dose 3055 mg HMX/kg/day for a maximum of 9 days), and the premature decedents from groups 3 and 4 (target 1000 and 3000 mg HMX/kg/day dose levels).

Two male rats from group 5 also showed haemorrhaging into the cerebral ventricles and meninges.

The 2 control females and 4 males from group 5 which were described as having dark red lungs showed areas of congestion on histological examination, autolytic changes being confirmed.

On the basis of these findings it is recommended that the histopathological evaluation is extended to include

examination of the liver, thymus, spleen and brain of all the HMX treated rats. Such an extension will provide information regarding whether or not there is a 'no effect' level, together with clarification of the sex difference in lesions diagnosed in the animals so far examined.

DISCUSSION

Dose levels selected for the 90 day study were 0, 50, 150, 450, 1350 and 4000 mg HMX/kg/day for males and 0, 50, 115, 270, 620 and 1500 mg HMX/kg/day for females.

Initial reductions in food consumption and body weight loss/reduced body weight gain were observed for all HMX-treated groups. Female rats were shown to be more sensitive than male rats, 1/6 females dying in each of the groups fed 1000 or 3000 mg/HMX/kg, and all the females dying at 9000 mg/kg; 5/6 males treated at 9000 mg/kg died during the study. The emaciated appearance of all premature decendents was consistent with a reduced food intake possibly due to unpalatability of the test diet. Surviving animals tolerated the test diet and by the end of the dosing period, showed a slightly increased level of food consumption and a trend towards body weight gain.

Unpalatability and extreme body weight effects meant that the achieved dosages were unavoidably erratic. Differences observed in liver weights for HMX-treated animals correlate with the histopathological diagnosis of toxic degeneration seen for the males and hepatocyte hyperplasia seen in the females. The organ weight data is difficult to interpret because of the observed body weight changes.

The histopathological findings of toxic degeneration in the livers of male rats and hepatocyte hyperplasia with increased cytoplasmic eosinophilia in the females indicate the liver to be a target organ for HMX treatment related effects. The liver and kidney together with thymus and spleen in which lymphocyte depletion was observed, will need detailed scrutiny in the histopathological evaluation undertaken after the scheduled 3 month dietary study with HMX in rats.

TABLE 1

HMX : 14 Day Toxicity Study in Rats with Dietary Administration
Incidence of Mortality

Day of Dosing	Dose Group/Dose Level (mg HMX/kg/day)									
	1♂ 0	2♂ 333	3♂ 1000	4♂ 3000	5♂ 9000	1♀ 0	2♀ 333	3♀ 1000	4♀ 3000	5♀ 9000
1	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	1B
7	-	-	-	-	-	-	-	-	-	1A
8	-	-	-	-	-	-	-	-	-	3A
9	-	-	-	-	1A	-	-	-	1A	1A
10	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	1B	-	-
12	-	-	-	-	1A	-	-	-	-	-
13	-	-	-	-	3A	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-
Total	0	0	0	0	5	0	0	1	1	6
Number commencing treatment	6	6	6	6	6	6	6	6	6	6

A - Found dead in cage

B - Killed in extremis

TABLE 2a

HMX : 14 Day Toxicity Study in Rats with
Dietary Administration
Group Mean Body Weights (g) - Males

Dose Level (mg/kg/day)	No. of Animals		Treatment Week/Day of Dosing						
			Pre-trial			Week 1		Week 2	
			7	3	0	4	7	11	14
0	6	Mean ± S.D.	95.7 9.0	117.2 10.2	135.8 9.8	155.3 9.8	170.8 11.5	188.2 11.4	203.8 11.2
333	6	Mean ± S.D.	103.2 6.2	125.8 11.3	145.0 12.3	151.8 10.4	167.2 11.3	182.8 12.0	195.2 12.8
1000	6	Mean ± S.D.	99.0 8.8	116.2 8.7	134.8 12.0	* 138.8 10.4	* 152.3 10.9	** 165.2 12.0	** 175.8 11.7
3000	6	Mean ± S.D.	95.3 11.1	116.8 13.2	135.8 14.1	** 126.2 13.4	** 139.0 13.5	*** 149.3 13.1	*** 159.2 14.1
9000	6	Mean ± S.D.	99.5 6.5	120.5 7.4	139.3 8.7	*** 116.5 8.8	*** 122.2 8.2	(a)*** 121.6 9.7	(b) 135.0 0.0

(a) n=5
(b) n=1

* Significantly different from controls, P<0.05
** Significantly different from controls, P<0.01
*** Significantly different from controls, P<0.001

TABLE 2b

HMX : 14 Day Toxicity Study in Rats with
Dietary Administration
Group Mean Body Weights (g) - Females

Dose Level (mg/kg/day)	No. of Animals		Treatment Week/Day of Dosing						
			Pre-trial			Week 1		Week 2	
			7	3	0	4	7	11	14
0	6	Mean ± S.D.	85.3 4.0	99.2 3.9	110.7 4.2	119.5 3.9	125.8 4.2	132.7 4.6	138.7 6.1
333	6	Mean ± S.D.	84.7 4.1	98.3 4.8	108.8 4.2	*** 94.8 4.9	*** 100.8 5.1	*** 108.0 4.3	*** 112.2 3.8
1000	6	Mean ± S.D.	85.3 11.3	101.8 7.4	112.3 7.6	*** 96.0 7.3	*** 101.0 6.2	*** 100.3 10.9	*** (a)109.2 4.5
3000	6	Mean ± S.D.	88.0 2.4	102.8 3.7	113.7 2.6	*** 92.8 4.2	*** 95.0 3.8	*** 98.8 7.2	*** (a)103.4 8.0
9000	6	Mean ± S.D.	86.0 2.8	98.8 2.9	109.7 2.9	*** 84.0 3.2	*** (b)77.8 3.9	†	†

(a) n=5
(b) n=4

*** Significantly different from controls, P<0.001

† = all animals dead

TABLE 3

HMX : 14 Day Toxicity Study in Rats with
Dietary Administration
Group Mean Food Consumption

Treatment Week	Dose Group/Group Mean Food Consumption (g/Animal/Day)									
	1♂ (0)	2♂ (333)	3♂ (1000)	4♂ (3000)	5♂ (9000)	1♀ (0)	2♀ (333)	3♀ (1000)	4♀ (3000)	5♀ (9000)
Pre Trial	16.4	16.9	15.8	17.0	16.7	12.9	12.7	13.3	13.4	12.6
1	17.4	15.1	13.9	11.5	7.6	13.1	6.7	6.6	5.8	3.8
2	18.9	18.3	17.2	15.5	12.2	14.4	11.9	12.2	11.0	5.0

TABLE 4

HMX : 14 Day Toxicity Study in Rats with
Dietary Administration
Achieved Dosage : Group Mean Values (mg/kg/day)

Treatment Week	Dose Group/Achieved Dosage (mg HMX/kg/day)									
	1♂ (0)	2♂ (333)	3♂ (1000)	4♂ (3000)	5♂ (9000)	1♀ (0)	2♀ (333)	3♀ (1000)	4♀ (3000)	5♀ (9000)
1	0	272.6	769.7	2092.9	4430.5	0	191.0	536.7	1533.1	3055.4
2	0	397.8	1145.1	3870.9	12578.1	0	548.3	2023.5	5415.4	-
Average Achieved Dosage	0	335.2	957.4	2981.9	8504.3	0	369.6	1280.1	3474.3	3055.4

Values in parenthesis indicate target concentrations (mg/kg/day)
Achieved dosage based on actual concentration of HMX analysed in diet.

TABLE 5a

HMX : 14 Day Toxicity Study in Rats with Dietary Administration
Absolute Organ Weights (g)
Group Mean Values of Animals Surviving 14 Days Dosing

Dose Level (mg/kg/day)	No. of Animals/ Sex		Kidneys		Liver
			L	R	
0	6 ♂	Mean ± S.D.	0.79 0.03	0.77 0.02	9.42 0.80
333	6 ♂	Mean ± S.D.	0.77 0.06	0.75 0.07	8.45* 0.64
1000	6 ♂	Mean ± S.D.	0.70*** 0.03	0.69*** 0.03	7.53*** 0.58
3000	6 ♂	Mean ± S.D.	0.67** 0.06	0.64*** 0.06	6.34*** 0.75
9000	1 ♂	Mean ± S.D.	0.60 0	0.57 0	5.54 0
0	6 ♀	Mean ± S.D.	0.58 0.02	0.58 0.03	5.54 0.40
333	6 ♀	Mean ± S.D.	0.51*** 0.02	0.50*** 0.02	4.32*** 0.26
1000	5 ♀	Mean ± S.D.	0.50** 0.05	0.49* 0.06	4.32*** 0.16
3000	5 ♀	Mean ± S.D.	0.48*** 0.03	0.48*** 0.03	4.12*** 0.32

* Significantly different from controls, P<0.05
** Significantly different from controls, P<0.01
*** Significantly different from controls, P<0.001

Group mean organ weight values for group 5♀ (9000 mg HMX/kg/day) are not presented due to premature decedency of all animals.

TABLE 5b

HMX : 14 Day Toxicity Study in Rats with Dietary Administration

Organ Weights as a % of Body Weight

Group Mean Values of Animals Surviving 14 Days Dosing

Dose Level (mg/kg/day)	No. of Animals/ Sex		Kidneys		Liver
			L	R	
0	6♂	Mean	0.382	0.376	4.572
		± S.D.	0.016	0.014	0.168
333	6♂	Mean	0.392	0.381	4.308**
		± S.D.	0.009	0.012	0.084
1000	6♂	Mean	0.396	0.394*	4.274*
		± S.D.	0.017	0.012	0.177
3000	6♂	Mean	0.418 **	0.402*	3.986***
		± S.D.	0.012	0.016	0.134
9000	1♂	Mean	0.469	0.445	4.328
		± S.D.	0.0	0.0	0.0
0	6♀	Mean	0.420	0.420	4.030
		± S.D.	0.015	0.015	0.224
333	6♀	Mean	0.462***	0.449*	3.901
		± S.D.	0.013	0.017	0.240
1000	5♀	Mean	0.469*	0.458	4.051
		± S.D.	0.045	0.058	0.125
3000	5♀	Mean	0.474**	0.475**	4.069
		± S.D.	0.024	0.028	0.185

* Significantly different from controls, P<0.05

** Significantly different from controls, P<0.01

*** Significantly different from controls, P<0.001

Group mean organ weight values for group 5♀ (9000 mg HMX/kg/day) are not presented due to premature decedency of all animals.

TABLE 6

HMX : 14 Day Dietary Toxicity Study in Rats
Incidence of Histopathological Findings

Organ	Lesion \ Dose Group/Nominal Dose Level (mg HMX/kg/day)	Group Incidence									
		1 (0)		2 (333)		3a (1000)		4a (3000)		5a (9000)	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Liver	Dense eosinophilic cytoplasm	0/6	0/6	-	-	-	1/1	-	1/1	6/6	4/6
	Cellular degeneration	0/6	0/6	-	-	-	0/1	-	0/1	5/6	0/6
	Cellular hyperplasia	0/6	0/6	-	-	-	0/1	-	1/1	0/6	6/6
	Congestion	0/6	0/6	-	-	-	1/1	-	1/1	0/6	0/6
Thymus	Lymphocyte depletion	0/6	0/6	-	-	-	1/1	-	1/1	1/6	6/6
Spleen	White pulp depletion	0/6	0/6	-	-	-	1/1	-	1/1	0/6	5/6
	Red pulp depletion	0/6	0/6	-	-	-	0/1	-	0/1	0/6	5/6
Kidney	Congestion	0/6	0/6	-	-	-	1/1	-	0/1	1/6	1/6
Lung*	Congestion	-	2/2	-	-	-	0/1	-	-	4/4	-
Brain	Congestion of blood vessels	0/6	0/6	-	-	-	0/1	-	0/1	1/5	0/6
	Haemorrhage	0/6	0/6	-	-	-	0/1	-	0/1	2/5	0/6

(a) Premature decedents observed in groups 3, 4 and 5

\- No histopathology undertaken

* Only lungs found to be abnormal at necropsy were examined histopathologically

FIGURE 1

HMX : 14 Day Toxicity Study in Rats with
Dietary Administration
Group Mean Body Weight (g) - Males

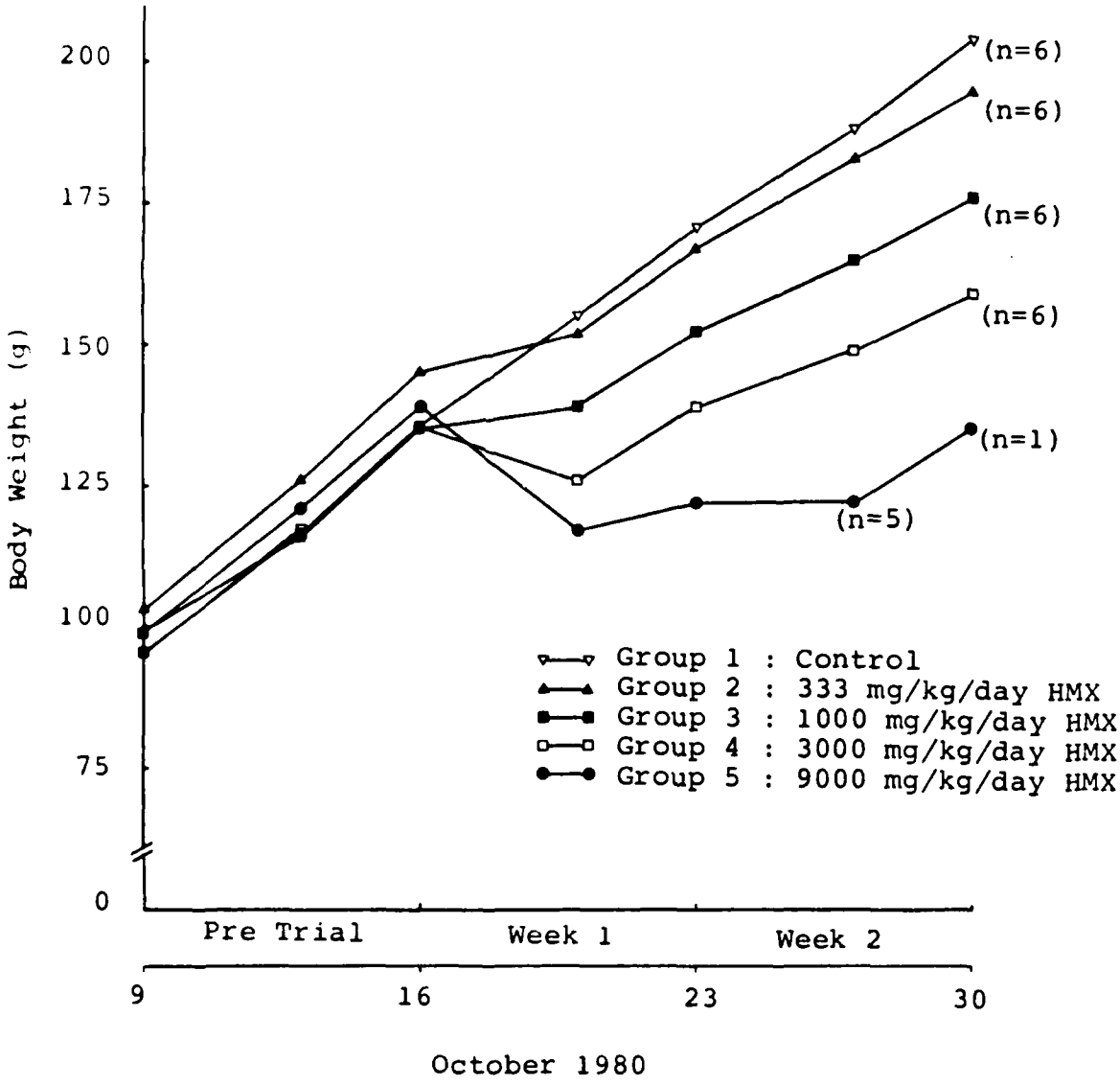
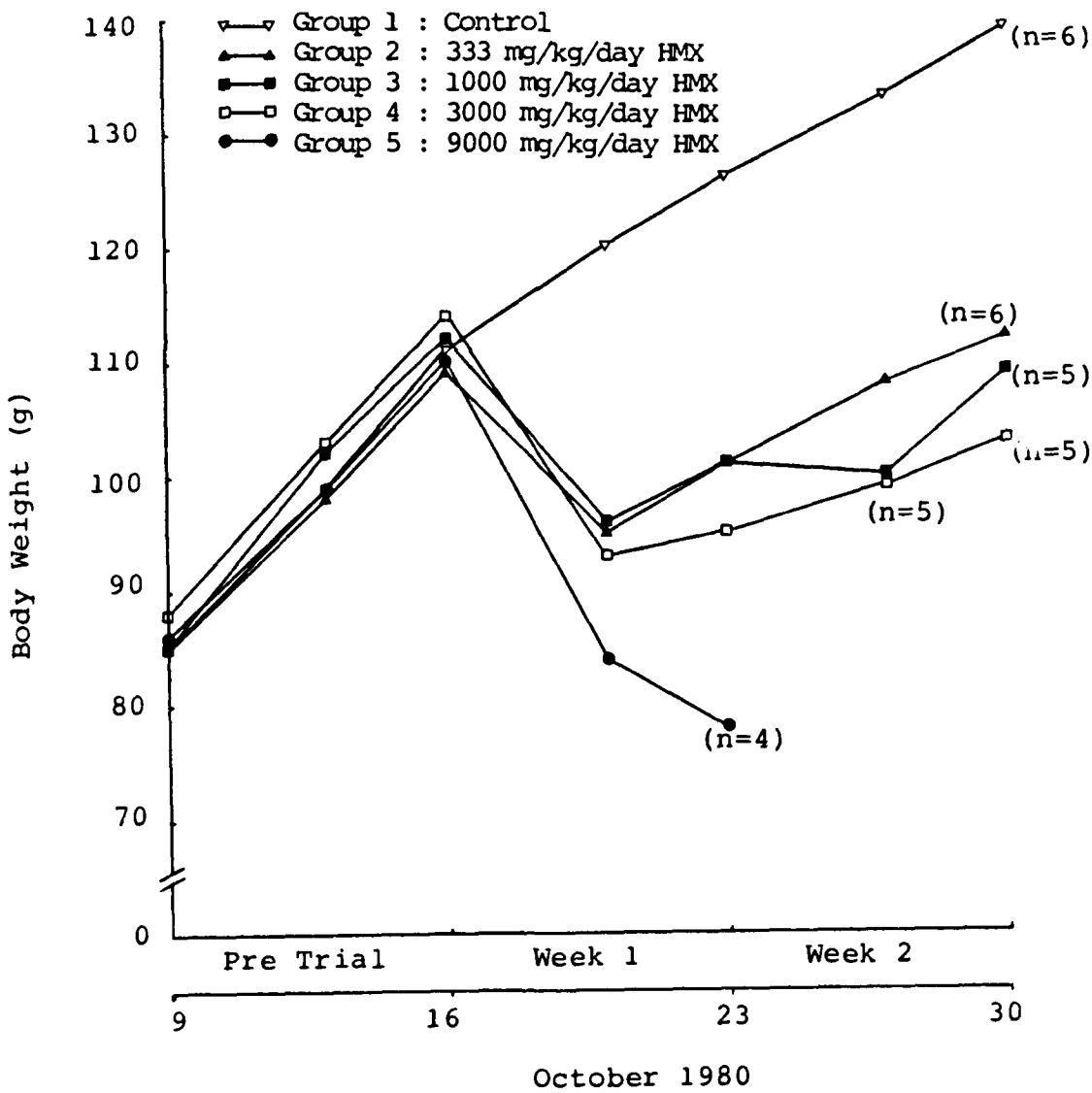


FIGURE 2

HMX : 14 Day Toxicity Study in Rats with
Dietary Administration
Group Mean Body Weight (g) - Females



APPENDIX 1a

HMX: 14 Day Toxicity Study in Rats With Dietary Administration
Analysis of Diet

B.P. NUTRITION (U.K.) LTD.
SPECIAL QUALITY CONTROL OF LABORATORY ANIMAL DIETS

CERTIFICATE OF ANALYSIS

PRODUCT: RAT & MOUSE NO.1 (MODIFIED) EXPANDED FINE GROUND

BATCH NO: 919 PREMIX BATCH NO: P110

DATE OF MANUFACTURE: 15TH AUGUST 1980

Nutrient	Found Analysis		Contaminant	Found Analysis		Limit of Detection
Moisture	7.1	%	Fluorine	7.6	mg/kg	10.0 mg/kg
Crude Fat	3.5*	%	Nitrate as NaNO3	11.0	mg/kg	1.0 mg/kg
Crude Protein	14.9	%	Nitrite as NaNO2	< 1.0	mg/kg	1.0 mg/kg
Crude Fibre	2.2	%	Lead	< 1.0	mg/kg	1.0 mg/kg
Ash	4.8	%	Arsenic	0.23	mg/kg	0.2 mg/kg
Calcium	0.69	%	Cadmium	0.13	mg/kg	0.2 mg/kg
Phosphorus	0.53	%	Mercury	< 0.01	mg/kg	0.01 mg/kg
[redacted]odium	0.22	%	Selenium	0.12	mg/kg	0.02 mg/kg
Chlorine	0.34	%				
Potassium	1.10	%				
Magnesium	0.13	%	Total Aflatoxins	NONE DETECTED	ug/kg	1 ug/kg each of B1,B2,G1,G2
Iron	231	mg/kg				
Copper	7	mg/kg				
Manganese	55	mg/kg				
Zinc	40	mg/kg	Total P.C.B.	NONE DETECTED	mg/kg	0.001 mg/kg
			Total D.D.T.	0.003	mg/kg	0.001 mg/kg
			Dieldrin	0.001	mg/kg	0.001 mg/kg
			Lindane	0.005	mg/kg	0.001 mg/kg
			Heptachlor	NONE DETECTED	mg/kg	0.001 mg/kg
			Malathion	NONE DETECTED	mg/kg	0.02 mg/kg
Vitamin A	7000	iu/kg	Total Viable Organisms	1.13×10^3	per grm	1000/g
[redacted]itamin E	60	mg/kg				
Vitamin C		mg/kg	Mesophilic Spores	17.5×10^2	per grm	100/g
			Salmonellae Species	NONE DETECTED	per grm	Absent in 20 grm
			Presumptive E. Coli	NONE DETECTED	per grm	Absent in 10 grm
			E. Coli Type 1	NONE DETECTED	per grm	Absent in 10 grm
			Fungal Units	NONE DETECTED	per grm	Absent in 10 grm
			Antibiotic Activity			

*repeat 3.4

RECEIVED
17 SEP 1980

Signed C R Popplestone

Dated 10th September 1980

C R POPPLESTONE M Sc., Ph.D., C Chem M.R.I.C.
Quality Control Manager

B.P. Nutrition (U.K.) Limited
Stephield,
Witham,
Essex, CM8 3AB
Telephone: (0376) 513651

ICI

International Consulting and Laboratory Services

Mineral Analysis of a Sample of Water *(after filtration if necessary)* Q/B/3882

Labelled: Maine Water Supply

(milligrammes per litre and milleguivalents per litre)

Cations	mg l	me l	Anions	mg l	me l		mg l	me l
Ca	16	0.80	HCO_3	36	1.20	Calcium Carbonate	40	0.80
Mg	9	0.70	SO_4	20	0.42	Magnesium Carbonate	17	0.40
Na	8	0.35	Cl	9	0.26	Magnesium Sulphate	18	0.30
K	2	0.05	NO_3	0	-	Sodium Sulphate	9	0.12
						Sodium Chloride	12	0.21
						Potassium Chloride	4	0.05
						Silica	4	
Total		1.88	Total		1.88	Total	104	1.88

Assumed present as bicarbonate

Comment

This sample is practically clear and bright in appearance and is free from noticeable colour. The reaction is slightly on the alkaline side of neutrality and the water is soft in character with a low content of dissolved solids. The water is free from metals apart from a minute trace of manganese and is of a satisfactory standard of organic quality.

These results indicate, from the aspect of the chemical and mineral analysis, a wholesome water suitable for drinking and domestic purposes.

PSM/SRL 23rd September, 1980 Signed

ICI

International Consulting and Laboratory Services

Analysis of a Sample of Water:

Received: Your Ref:

Our Ref Q/B/3882

From: Inveresk Research International

Labelled: Maine Water Supply

Date 4.45 pm 15/8/80

Taken by N. Scott Witness D. Brown Signed N. Scott

Results *(in milligrammes per litre)*

Appearance: Bright with a few particles

pH	7.8		Odour		NIL
Electrical Conductivity	150		Colour (Hazen)		3
Chlorine in Chloride	9		Turbidity (Formazin A.P.H.A. units)		0.5
Hardness as $CaCO_3$ Total	75		Nitrogen in Nitrate	LT	0.1
Carbonate	60		Nitrogen in Nitrite	LT	0.01
Non carbonate	15		Ammoniacal Nitrogen		0.10
Alkalinity as $CaCO_3$	60		Albuminoid Nitrogen	LT	.01
			Permanganate value		0.55
Free Carbon Dioxide	2		Residual Chlorine	LT	0.02
Dissolved Solids dried at 180 C	105		Iron(Fe) LT 0.03	Zinc(Zn)	LT 0.03

Copper(Cu) LT 0.03 Lead(Pb) LT 0.03 Manganese(Mn) 0.04 Aluminium(Al) 0.03

Cadmium (Cd) LT 0.001 Fluorine in Fluoride LT 0.1 Silica(SiO_2) 4

(Absence refers to a detection limit of 0.03mg l of each metal unless otherwise stated)

Comment

Volatile Total Dissolved Solids	10
Mercury expressed as Hg	LT 0.0005
Selenium expressed as Se	LT 0.001
Dissolved Oxygen	8.8

PSM/SL 23rd September, 1980 Signed

APPENDIX 1b (continued)

ICLS

G/B/3882

Polynuclear Aromatic Hydrocarbons

Fluoranthene	NDLT 20 ng/litre
Benzo (ghi) perylene	NDLT 4 ng/litre
Benzo (k) fluoranthene	NDLT 4 ng/litre
2, 3 O - phenylene pyrene	NDLT 4 ng/litre
Benzo (b) fluoranthene	NDLT 4 ng/litre
Benzo (a) pyrene	NDLT 4 ng/litre

Total P.A.H. NDLT 40 ng/litre

Organochlorine Pesticides

alpha B.H.C.	NDLT 10 ng/litre
gamma B.H.C.	NDLT 10 ng/litre
Heptachlor	NDLT 20 ng/litre
Aldrin	NDLT 20 ng/litre
Dieldrin	NDLT 40 ng/litre
p.p. D.D.T.	NDLT 20 ng/litre

Polychlorinated biphenyls

NDLT 400 ng/litre (expressed as AROCHLOR 1248)

NDLT = Not Detected, Less Than

APPENDIX 2

HMX : 14 Day Dietary Toxicity Study in Rats
Summary Data for Pre-experimental Homogeneity and Stability
Testing of HMX Treated Diet

Time Analysed	Nominal Concentration (ppm)					
	1,250 ppm		10,000 ppm		25,000 ppm	
	Mean	*Coeff Var %	Mean	*Coeff Var %	Mean	*Coeff Var %
Immediately after Preparation	1177	5.3	10198	5.4	24817	2.5
After 14 d at 40°C	1234	3.8	10130	3.6	25004	4.4
After 21 d at Ambient	1291	3.9	10311	3.0	25598	3.5

*Coefficient of Variation (%)

APPENDIX 3a

HMX : 14 Day Toxicity Study in Rats with
Dietary Administration
Individual Body Weights (g) - Males

Dose Level (mg/kg/day)	Animal No./ Sex \ Treatment Week/Day of Dosing	Pre-trial			Week 1		Week 2	
		7	3	0	4	7	11	14
0	501 ♂	99	123	142	161	179	198	212
	502	85	105	125	144	161	179	198
	503	99	117	135	155	169	187	197
	504	109	133	151	171	190	206	223
	505	86	108	126	147	163	181	198
	506	96	117	136	154	163	178	195
Mean		95.7	117.2	135.8	155.3	170.8	188.2	203.8
± S.D.		9.0	10.2	9.8	9.8	11.5	11.4	11.2

APPENDIX 3a (continued)

Dose Level (mg/kg/day)	Animal No./ Sex \ Treatment Week/Day of Dosing	Pre-trial			Week 1		Week 2	
		7	3	0	4	7	11	14
333	507 ♂	105	124	142	150	165	175	187
	508	109	133	153	158	176	194	205
	509	95	105	123	134	150	167	179
	510	111	137	158	165	183	199	214
	511	99	125	144	150	164	184	197
	512	100	131	150	154	165	178	187
Mean		103.2	125.8	145.0	151.8	167.2	182.8	195.2
± S.D.		6.2	11.3	12.3	10.4	11.3	12.0	12.8

APPENDIX 3a (continued)

Dose Level (mg/kg/day)	Treatment Week/Day of Dosing / Animal No./ Sex	Pre-trial			Week 1		Week 2	
		7	3	0	4	7	11	14
1000	513 ♂	89	109	125	127	141	150	160
	514	98	121	139	141	155	165	177
	515	108	130	155	157	172	187	196
	516	90	110	130	135	148	163	175
	517	99	119	138	141	153	163	171
	518	110	108	122	132	145	163	176
Mean		99.0	116.2	134.8	138.8	152.3	165.2	175.8
± S.D.		8.8	8.7	12.0	10.4	10.9	12.0	11.7

APPENDIX 3a (continued)

Dose Level (mg/kg/day)	Animal No./ Sex \ Treatment Week/Day of Dosing	Pre-trial			Week 1		Week 2	
		7	3	0	4	7	11	14
3000	519 ♂	100	118	138	130	142	153	160
	520	87	107	127	113	125	136	145
	521	95	120	137	127	143	156	169
	522	90	109	128	122	135	145	155
	523	115	141	162	150	162	170	181
	524	85	106	123	115	127	136	145
Mean		95.3	116.8	135.8	126.2	139.0	149.3	159.2
± S.D.		11.1	13.2	14.1	13.4	13.5	13.1	14.1

APPENDIX 3a (continued)

Dose Level (mg/kg/day)	Treatment Week/Day of Dosing / Animal No./ Sex	Pre-trial			Week 1		Week 2	
		7	3	0	4	7	11	14
9000	525 ♂	92	112	130	103	109	110	†
	526	107	128	150	128	134	134	†
	527	92	112	131	110	119	114	†
	528	100	121	140	118	122	†	-
	529	106	129	149	122	126	127	†
	530	100	121	136	118	123	123	135
Mean		99.5	120.5	139.3	116.5	122.2	121.6	135.0
± S.D.		6.5	7.4	8.7	8.8	8.2	9.7	0.0

† = animal dead

APPENDIX 3b

HMX : 14 Day Toxicity Study in Rats
with Dietary Administration
Individual Body Weights (g) - Females

Dose Level (mg/kg/day)	Treatment Week/Day of Dosing / Animal No./ Sex	Pre-trial			Week 1		Week 2	
		7	3	0	4	7	11	14
0	531 ♀	87	103	115	123	130	137	143
	532	82	96	107	115	122	128	130
	533	84	95	105	116	120	127	132
	534	80	96	109	117	125	131	140
	535	90	103	114	122	128	136	143
	536	89	102	114	124	130	137	144
Mean		85.3	99.2	110.7	119.5	125.8	132.7	138.7
± S.D.		4.0	3.9	4.2	3.9	4.2	4.6	6.1

APPENDIX 3b (continued)

Dose Level (mg/kg/day)	Treatment Week/Day of Dosing / Animal No./ Sex	Pre-trial			Week 1		Week 2	
		7	3	0	4	7	11	14
333	537 ♀	85	98	107	91	97	106	110
	538	80	93	105	94	100	107	112
	539	85	98	109	97	103	110	114
	540	84	99	108	95	102	108	114
	541	92	107	117	103	109	115	117
	542	82	95	107	89	94	102	106
Mean		84.7	98.3	108.8	94.8	100.8	108.0	112.2
± S.D.		4.1	4.8	4.2	4.9	5.1	4.3	3.8

APPENDIX 3b (continued)

Dose Level (mg/kg/day)	Treatment Week/Day of Dosing / Animal No./ Sex	Pre-trial			Week 1		Week 2	
		7	3	0	4	7	11	14
1000	543 ♀	80	95	102	86	93	102	107
	544	92	106	117	97	97	102	105
	545	87	102	113	97	102	106	113
	546	95	107	118	103	109	110	115
	547	65	91	104	89	98	103	106
	548	93	110	120	104	107	79	†
Mean		85.3	101.8	112.3	96.0	101.0	100.3	109.2
± S.D.		11.3	7.4	7.6	7.3	6.2	10.9	4.5

† = animal dead

APPENDIX 3b (continued)

Dose Level (mg/kg/day)	Treatment Week/Day of Dosing / Animal No./ Sex	Pre-trial			Week 1		Week 2	
		7	3	0	4	7	11	14
3000	549 ♀	88	104	113	89	93	93	99
	550	85	98	110	89	90	†	-
	551	86	101	114	93	93	101	107
	552	92	109	118	100	101	109	114
	553	88	103	113	91	97	91	93
	554	89	102	114	95	96	100	104
Mean ± S.D.		88.0 2.4	102.8 3.7	113.7 2.6	92.8 4.2	95.0 3.8	98.8 7.2	103.4 8.0

† = animal dead

APPENDIX 3b (continued)

Dose Level (mg/kg/day)	Treatment Week/Day of Dosing / Animal No./ Sex	Pre-trial			Week 1		Week 2	
		7	3	0	4	7	11	14
9000	55 ♀	87	100	110	84	†	-	-
	56	90	103	114	88	†	-	-
	57	84	96	107	81	73	†	-
	58	83	96	108	80	81	†	-
	59	88	101	112	84	76	†	-
	60	84	97	107	87	81	†	-
Mean		86.0	98.8	109.7	84.0	77.8	-	-
± S.D.		2.8	2.9	2.9	3.2	3.9		

† = animal dead

APPENDIX 4

HMX : 14 Day Toxicity Study in Rats with
Dietary Administration
Formulated Diet Analysis

Treatment Week	Dose Group/Sex	Dose Level (mg/kg/day)	Theoretical Concentration (PPM)	Observed Concentration (PPM)			Mean Concentration (PPM)	Standard Deviation (PPM)	Deviation (%) from Mean Concentration
				Sample 1	Sample 2	Sample 3			
1	1 ♂	0	0	0	0	0	0	0	0
	2	333	2714	2723	2842	2667	2744	± 89	+ 1.1
	3	1000	7650	7366	7558	8002	7642	± 326	- 0.1
	4	3000	23550	23322	22527	23489	23113	± 514	- 1.9
	5	9000	72000	67087	70922	71856	69955	± 2527	- 2.8
	1 ♀	0	0	0	0	0	0	0	0
	2	333	2642	2744	2684	2903	2765	± 129	+ 4.7
	3	1000	8133	8430	7855	7867	8051	± 329	- 1.0
	4	3000	24800	25327	25647	25155	25376	± 250	+ 2.3
	5	9000	72000	72017	69442	70809	70756	± 1288	- 1.7

Theoretical concentration (PPM) calculated from predicted group mean mid-week body weight and predicted group mean food consumption.

APPENDIX 4 (continued)

Treatment Week	Dose Group/Sex	Dose Level (mg/kg/day)	Theoretical Concentration (PPM)	Observed Concentration (PPM)			Mean Concentration (PPM)	Standard Deviation (PPM)	Deviation (%) from Mean Concentration
				Sample 1	Sample 2	Sample 3			
2	1 ♂	0	0	0	0	0	0	0	0
	2	333	3869	4055	3876	3873	3935	± 104	+ 1.7
	3	1000	11448	10752	10933	10671	10785	± 134	- 5.8
	4	3000	36720	37858	36512	36514	36961	± 777	- 0.7
	5	9000	120600	129395	125597	122352	125781	± 3525	+ 4.3
	1 ♀	0	0	0	0	0	0	0	0
	2	333	4995	5107	4893	4791	4930	± 161	- 1.3
	3	1000	15000	16969	16941	15847	16586	± 640	+10.6
	4	3000	49000	48703	48370	49143	48739	± 388	- 0.5
	5	9000	175000	175287	181643	175516	177482	± 3605	+ 1.1

APPENDIX 5a

HMX : 14 Day Toxicity Study in Rats with Dietary Administration
Absolute Organ Weights (g)
Individual Values - Males Surviving 14 Days Dosing

Dose Level (mg/kg/day)	Animal No.	Kidneys		Liver
		L	R	
0	501	0.83	0.80	9.92
	502	0.77	0.75	9.19
	503	0.74	0.76	8.61
	504	0.80	0.79	10.78
	505	0.79	0.79	9.18
	506	0.78	0.75	8.84
	Mean	0.79	0.77	9.42
	± S.D.	0.03	0.02	0.80
333	507	0.74	0.72	8.19
	508	0.81	0.82	9.07
	509	0.71	0.65	7.92
	510	0.87	0.85	9.42
	511	0.77	0.73	8.19
	512	0.71	0.72	7.92
	Mean	0.77	0.75	8.45
	± S.D.	0.06	0.07	0.64
1000	513	0.68	0.65	7.13
	514	0.72	0.70	7.88
	515	0.74	0.74	8.44
	516	0.68	0.69	6.97
	517	0.68	0.67	7.08
	518	0.68	0.71	7.70
	Mean	0.70	0.69	7.53
	± S.D.	0.03	0.03	0.58
3000	519	0.69	0.65	6.15
	520	0.64	0.59	5.77
	521	0.70	0.67	6.86
	522	0.61	0.58	5.94
	523	0.76	0.73	7.62
	524	0.59	0.61	5.70
	Mean	0.67	0.64	6.34
	± S.D.	0.06	0.06	0.75
9000	530	0.60	0.57	5.54

APPENDIX 5a (continued)

Individual Values - Females Surviving 14 Days Dosing

Dose Level (mg/kg/day)	Animal No.	Kidneys		Liver
		L	R	
0	531	0.61	0.60	6.10
	532	0.54	0.56	5.11
	533	0.58	0.54	5.24
	534	0.56	0.58	5.94
	535	0.58	0.56	5.44
	536	0.59	0.62	5.38
	Mean	0.58	0.58	5.54
	± S.D.	0.02	0.03	0.40
333	537	0.52	0.49	4.80
	538	0.50	0.49	4.19
	539	0.53	0.52	4.13
	540	0.49	0.47	4.16
	541	0.53	0.52	4.21
	542	0.50	0.49	4.40
	Mean	0.51	0.50	4.32
	± S.D.	0.02	0.02	0.26
1000	543	0.43	0.39	4.22
	544	0.54	0.54	4.28
	545	0.50	0.47	4.22
	546	0.54	0.55	4.59
	547	0.51	0.49	4.27
	Mean	0.50	0.49	4.32
	± S.D.	0.05	0.06	0.16
3000	549	0.45	0.46	3.84
	551	0.52	0.50	4.49
	552	0.49	0.48	4.27
	553	0.45	0.45	3.73
	554	0.49	0.51	4.28
	Mean	0.48	0.48	4.12
	± S.D.	0.03	0.03	0.32

APPENDIX 5b

HMS : 14 Day Toxicity Study in Rats with Dietary Administration
Organ Weights as a % of Body Weight
Individual Values - Males Surviving 14 Days Dosing

Dose Level (mg/kg/day)	Animal Number	Body Weight (g)	Kidneys		Liver
			L	R	
0	501	216	0.384	0.370	4.593
	502	202	0.381	0.371	4.550
	503	200	0.370	0.380	4.305
	504	223	0.359	0.354	4.834
	505	200	0.395	0.395	4.590
	506	194	0.402	0.387	4.557
	Mean		0.382	0.376	4.572
	± S.D.		0.016	0.014	0.168
333	507	192	0.385	0.375	4.266
	508	210	0.386	0.390	4.319
	509	179	0.397	0.363	4.425
	510	215	0.405	0.395	4.381
	511	195	0.395	0.374	4.200
	512	186	0.382	0.387	4.258
	Mean		0.392	0.381	4.308
	+ S.D.		0.009	0.012	0.084
1000	513	160*	0.425	0.406	4.456
	514	180	0.400	0.389	4.378
	515	198	0.374	0.374	4.263
	516	175	0.389	0.394	3.983
	517	170	0.400	0.394	4.165
	518	175	0.389	0.406	4.400
	Mean		0.396	0.394	4.274
	± S.D.		0.017	0.012	0.177
3000	519	162	0.426	0.401	3.796
	520	147	0.435	0.401	3.925
	521	169	0.414	0.396	4.059
	522	152	0.401	0.382	3.908
	523	182	0.418	0.401	4.187
	524	142	0.415	0.430	4.014
	Mean		0.418	0.402	3.986
	± S.D.		0.012	0.016	0.134
9000	530	128	0.469	0.445	4.328

* = Body weight at day 14 of dosing - not weighed at necropsy.

APPENDIX 5b (continued)

Individual Values - Females Surviving 14 Days Dosing

Dose Level (mg/kg/day)	Animal Number	Body Weight (g)	Kidneys		Liver
			L	R	
0	531	143	0.427	0.420	4.266
	532	131	0.412	0.427	3.901
	533	130	0.446	0.415	4.031
	534	137	0.409	0.423	4.336
	535	142	0.408	0.394	3.831
	536	141	0.418	0.440	3.816
	Mean		0.420	0.420	4.030
	± S.D.		0.015	0.015	0.224
333	537	112	0.464	0.438	4.286
	538	107	0.467	0.458	3.916
	539	111	0.477	0.468	3.721
	540	112	0.438	0.420	3.714
	541	114	0.465	0.456	3.693
	542	108	0.463	0.454	4.074
	Mean		0.462	0.449	3.901
	± S.D.		0.013	0.017	0.240
1000	543	104	0.413	0.375	4.058
	544	103	0.524	0.524	4.155
	545	110	0.435	0.427	3.836
	546	112	9.482	0.491	4.098
	547	104	0.490	0.471	4.106
	Mean		0.469	0.458	4.051
	± S.D.		0.045	0.058	0.125
3000	549	97	0.464	0.474	3.959
	551	105	0.495	0.476	4.276
	552	112	0.438	0.429	3.813
	553	91	0.495	0.495	4.099
	554	102	0.480	0.500	4.196
	Mean		0.474	0.475	4.069
	± S.D.		0.024	0.028	0.185

APPENDIX 5c

HMX : 14 Day Toxicity Study in Rats
with Dietary Administration
Premature Decedents
Absolute Organ Weights (g) - Individual Values

Dose Level (mg/kg/day)	Animal Number / Sex	Kidneys		Liver
		L	R	
9000	525 ♂	0.58	0.54	6.15
	526	0.55	0.60	7.04
	527*	NDA	NDA	NDA
	528	0.65	0.63	5.79
	529	0.59	0.64	6.33
1000	548 ♀	0.46	0.44	2.46
3000	550 ♀	0.44	0.44	4.63
9000	555 ♀	0.41	0.42	2.42
	556	0.47	0.49	4.99
	557	0.42	0.44	2.73
	558	0.56	0.53	3.15
	559	0.52	0.50	3.53
	560	0.42	0.44	2.41

* No organ weights recorded

APPENDIX 5d

HMX : 14 Day Toxicity Study in Rats
with Dietary Administration
Premature Decedents
Organ Weights as a % of Body Weight
Individual Values

Dose Level (mg/kg/day)	Animal Number	Body Weight (g)	Kidneys		Liver
			L	R	
9000	525 ♂	113	0.513	0.478	5.442
	526	140	0.393	0.429	5.029
	527*	113	NDA	NDA	NDA
	528	121	0.537	0.521	4.785
	529	130	0.454	0.492	4.869
1000	548 ♀	79	0.582	0.557	3.114
3000	550 ♀	85	0.518	0.518	5.447
9000	555 ♀	68	0.603	0.618	3.559
	556	82	0.573	0.598	6.085
	557	63	0.667	0.698	4.333
	558	72	0.778	0.736	4.375
	559	73	0.712	0.685	4.836
	560	66	0.636	0.667	3.652

* No organ weights recorded

APPENDIX 6

HMX: 14 Day Toxicity Study in Rats with Dietary Administration Gross Pathology and Histopathological Findings in Individual Animals

Abbreviations used:

TK	Terminal kill
KIE	Killed *in extremis*
FD	Found dead
NAD	No abnormality detected
HE	Haematoxylin and Eosin
SS	Special stain

APPENDIX 6 (continued)

Project No: 415669SR Group No: 1
Animal No: 501 Sex: ♂

Time on Study	Death
2 weeks	TK

Internal and External Necropsy Findings	Organ	Histopathology	Number of Sections Examined HE		SS
NAD		NAD.	1	Liver	
			2	Kidneys	
			1	Heart	
			3	Brain	
			1	Spleen	
			1	Thymus	

APPENDIX 6 (continued)

Project No: 415669SR Group No: 1
Animal No: 502 Sex: ♂

Time on Study	Death
2 weeks	TK

Internal and External Necropsy Findings	Organ	Histopathology	Number of Sections Examined HE		SS
NAD		NAD.	1	Liver	
			2	Kidneys	
			1	Heart	
			1	Brain	
			1	Spleen	
			1	Thymus	

APPENDIX 6 (continued)

Project No: 415669SR Group No: 1
Animal No: 503 Sex: ♂

Time on Study	Death
2 weeks	TK

Internal and External Necropsy Findings	Organ	Histopathology	Number of Sections Examined HE		SS
NAD		NAD.	1	Liver	
			2	Kidneys	
			1	Heart	
			3	Brain	
			1	Spleen	
			1	Thymus	

APPENDIX 6 (continued)

Project No: 415669SR Group No: 1
Animal No: 504 Sex: ♂

Time on Study	Death
2 weeks	TK

Internal and External Necropsy Findings	Organ	Histopathology	Number of Sections Examined HE		SS
NAD		NAD.	1	Liver	
			2	Kidneys	
			1	Heart	
			3	Brain	
			1	Spleen	
			1	Thymus	

APPENDIX 6 (continued)

Project No: 415669SR Group No: 1
Animal No: 505 Sex: ♂

Time on Study	Death
2 weeks	TK

Internal and External Necropsy Findings	Organ	Histopathology
NAD		NAD.

Number of Sections Examined		
HE		SS
1	Liver	
2	Kidneys	
1	Heart	
3	Brain	
1	Spleen	
1	Thymus	

APPENDIX 6 (continued)

Project No: 415669SR **Group No:** 1
Animal No: 506 **Sex:** ♂

Time on Study	Death
2 weeks	TK

Internal and External Necropsy Findings	Organ	Histopathology
NAD		NAD.

Number of Sections Examined		
HE		SS
1	Liver	
2	Kidneys	
1	Heart	
3	Brain	
1	Spleen	
1	Thymus	

APPENDIX 6 (continued)

Project No: 415669SR Group No: 5
Animal No: 525 Sex: ♂

Time on Study	Death
13 days	FD

Internal and External Necropsy Findings	Organ	Histopathology	Number of Sections Examined HE		SS
NAD	Liver	Areas hepatocytes with dense staining cytoplasm. Mild cellular degeneration - mainly centrilobular.	1	Liver	
NAD	Kidney	Tubular structure indistinct - probably due to autolysis.	2	Kidneys	
Lungs dark red in all lobes.	Lungs	Congested alveoli possibly contain fluid, or blood, but autolysis advanced.	1	Heart	
Brain friable.	Brain	Not present.	0	Brain	
Blood under skull.			1	Spleen	
			1	Thymus	
			2	Lung	

APPENDIX 6 (continued)

Project No: 415669SR **Group No:** 5
Animal No: 526 **Sex:** ♂

Time on Study	Death
13 days	FD

Internal and External Necropsy Findings	Organ	Histopathology
NAD	Liver	Areas hepatocytes with dense staining cytoplasm. Mild cellular degeneration - mainly centrilobular.
Lungs dark red.	Lungs	Very congested. Some blood in alveoli.
Red staining round mouth.		

Number of Sections Examined		
HE		SS
1	Liver	
2	Kidneys	
1	Heart	
1	Brain	
1	Spleen	
1	Thymus	
1	Lung	

APPENDIX 6 (continued)

Project No: 415669SR Group No: 5
Animal No: 527 Sex: ♂

Time on Study	Death
12 days	FD

Internal and External Necropsy Findings	Organ	Histopathology
NAD	Liver	Areas hepatocytes with dense staining cytoplasm. Mild cellular degeneration - mainly centrilobular. Fat stain negative.
Lungs - dark red patches on all lobes.	Lungs	Congested but autolysis advanced.
NAD	Brain	Ventricles filled with blood.
Yellow staining round mouth and fore-feet.		

Number of Sections Examined		
HE		SS
1	Liver	F1
2	Kidneys	
1	Heart	
3	Brain	
1	Spleen	
1	Thymus	
2	Lung	

APPENDIX 6 (continued)

Project No: 415669SR **Group No:** 5
Animal No: 528 **Sex:** ♂

Time on Study	Death
9 days	FD

Internal and External Necropsy Findings	Organ	Histopathology
NAD	Liver	Large areas of hepatocytes with dense staining cytoplasm. Mild cellular degeneration. Fat stain negative.
NAD	Brain	Blood in ventricles and sub arachnoid space.
Red staining around the mouth.		

Number of Sections Examined		
HE		SS
1	Liver	F1
2	Kidneys	
1	Heart	
3	Brain	
1	Spleen	
1	Thymus	

APPENDIX 6 (continued)

Project No: 415669SR **Group No:** 5
Animal No: 529 **Sex:** ♂

Time on Study	Death
13 days	FD

Internal and External Necropsy Findings	Organ	Histopathology
NAD	Liver	Areas with dense staining hepatocytes. Mild cellular degeneration.
NAD	Thymus	Some lymphocyte depletion.
Lungs dark red. Froth in trachea.	Lung	Congested. Some alveoli may contain blood or fluid but autolysis caused loss of structure.
Blood under skull.	Brain	Blood vessels congested.

Number of Sections Examined		
HE		SS
1	Liver	
2	Kidneys	
1	Heart	
3	Brain	
1	Spleen	
1	Thymus	
1	Lung	

APPENDIX 6 (continued)

Project No: 415669SR Group No: 5
Animal No: 530 Sex: ♂

Time on Study	Death
2 weeks	TK

Internal and External Necropsy Findings	Organ	Histopathology	Number of Sections Examined HE		SS
NAD	Liver	Cytoplasm of hepatocytes very dense staining. Loss of some nuclei. Some nuclei enlarged.	1	Liver	
NAD	Kidney	Slight congestion in glomeruli.	2	Kidneys	
			1	Heart	
			3	Brain	
			1	Spleen	
			1	Thymus	

APPENDIX 6 (continued)

Project No: 415669SR Group No: 1
Animal No: 531 Sex: ♀

Time on Study	Death
2 weeks	TK

Internal and External Necropsy Findings	Organ	Histopathology	Number of Sections Examined HE		SS
Lungs - irregular dark areas in all lobes.	Lung	Slight congestion.	1	Liver	
			2	Kidneys	
			1	Heart	
			3	Brain	
			1	Spleen	
			1	Thymus	
			1	Lung	

APPENDIX 6 (continued)

Project No: 415669SR Group No: 1
Animal No: 532 Sex: ♀

Time on Study	Death
2 weeks	TK

Internal and External Necropsy Findings	Organ	Histopathology	Number of Sections Examined HE		SS
NAD		NAD.	1	Liver	
			2	Kidneys	
			1	Heart	
			1	Brain	
			1	Spleen	
			1	Thymus	

APPENDIX 6 (continued)

Project No: 415669SR Group No: 1
Animal No: 533 Sex: ♀

Time on Study	Death
2 weeks	TK

Internal and External Necropsy Findings	Organ	Histopathology
Lungs - irregular reddening on all lobes.	Lungs	Slight congestion.

Number of Sections Examined		
HE		SS
1	Liver	
2	Kidneys	
1	Heart	
3	Brain	
1	Spleen	
1	Thymus	
2	Lung	

APPENDIX 6 (continued)

Project No: 415669SR Group No: 1
Animal No: 534 Sex: ♀

Time on Study	Death
2 weeks	TK

Internal and External Necropsy Findings	Organ	Histopathology	Number of Sections Examined HE		SS
NAD		NAD.	1	Liver	
			2	Kidneys	
			1	Heart	
			1	Brain	
			1	Spleen	
			1	Thymus	

APPENDIX 6 (continued)

Project No: 415669SR Group No: 1
Animal No: 535 Sex: ♀

Time on Study	Death
2 weeks	TK

Internal and External Necropsy Findings	Organ	Histopathology
NAD		NAD.

Number of Sections Examined		
HE		SS
1	Liver	
2	Kidneys	
1	Heart	
1	Brain	
1	Spleen	
1	Thymus	

APPENDIX 6 (continued)

Project No: 415669SR Group No: 1
Animal No: 536 Sex: ♀

Time on Study	Death
2 weeks	TK

Internal and External Necropsy Findings	Organ	Histopathology	Number of Sections Examined HE		SS
NAD		NAD.	1	Liver	
			2	Kidneys	
			1	Heart	
			3	Brain	
			1	Spleen	
			1	Thymus	

APPENDIX 6 (continued)

Project No: 415669SR **Group No:** 3
Animal No: 548 **Sex:** ♀

Time on Study	Death
11 days	KIE

Internal and External Necropsy Findings	Organ	Histopathology
Liver small.	Liver	Blood vessels and sinusoids congested. Cytoplasm of hepatocytes very dense staining.
Kidneys small.	Kidneys	Blood vessels congested, especially in glomeruli.
NAD	Spleen	White pulp depleted of mature lymphocytes.
NAD	Thymus	Lymphocyte depletion.

Number of Sections Examined		
HE		SS
1	Liver	
2	Kidneys	
1	Heart	
3	Brain	
1	Spleen	
1	Thymus	

APPENDIX 6 (continued)

Project No: 415669SR **Group No:** 4
Animal No: 550 **Sex:** ♀

Time on Study	Death
9 days	FD

Internal and External Necropsy Findings	Organ	Histopathology
		Sections not reliable for interpretation owing to autolysis.
NAD	Liver	Very dense staining cytoplasm. Blood vessels and sinusoids congested. Mild degree cellular hyperplasia.
NAD	Spleen	White pulp depletion.
NAD	Thymus	Lymphocyte depletion.
Adrenals slightly enlarged.	Adrenals	NAD but sections autolytic.
Red staining round mouth and nose.		

Number of Sections Examined		
HE		SS
1	Liver	
2	Kidneys	
1	Heart	
3	Brain	
1	Spleen	
1	Thymus	
2	Adrenals	

APPENDIX 6 (continued)

Project No: 415669SR Group No: 5
Animal No: 555 Sex: ♀

Time on Study	Death
6 days	KIE

Internal and External Necropsy Findings	Organ	Histopathology
NAD	Liver	Very cellular. Many nuclei, less cytoplasm. Hepatocyte hyperplasia.
NAD	Kidney	Blood vessels in glomeruli congested.
Spleen small and pale.	Spleen	Red and white pulp depletion.
NAD	Thymus	Lymphocyte depletion.
Adrenals both enlarged.	Adrenals	NAD.
Animal extremely lean.		

Number of Sections Examined		
HE		SS
1	Liver	
2	Kidneys	
1	Heart	
3	Brain	
1	Spleen	
1	Thymus	
2	Adrenals	

APPENDIX 6 (continued)

Project No: 415669SR **Group No:** 5
Animal No: 556 **Sex:** ♀

Time on Study	Death
1 week	FD

Internal and External Necropsy Findings	Organ	Histopathology	Number of Sections Examined HE		SS
NAD	Liver	Cytoplasm of hepatocytes fairly dense staining. Some double nuclei. Slight degree cellular hyperplasia.	1	Liver	
			2	Kidneys	
			1	Heart	
			1	Brain	
			1	Spleen	
NAD	Thymus	Lymphocyte depletion.	1	Thymus	
Red staining round nose.					
Yellow staining in genital area.					

APPENDIX 6 (continued)

Project No: 415669SR Group No: 5
Animal No: 557 Sex: ♀

Time on Study	Death
8 days	FD

Internal and External Necropsy Findings	Organ	Histopathology
NAD	Liver	Cytoplasm of hepatocytes fairly dense staining. Cellular hyperplasia.
Spleen small.	Spleen	White and red pulp depletion.
NAD	Thymus	Lymphocyte depletion.
Adrenals enlarged.	Adrenals	NAD but some autolysis.
Brain friable.	Brain	NAD.
Absence of body fat.		
Red/brown staining round nose and mouth.		

Number of Sections Examined		
HE		SS
1	Liver	
2	Kidneys	
1	Heart	
3	Brain	
1	Spleen	
1	Thymus	
2	Adrenals	

Project No: 415669SR **Group No:** 5
Animal No: 558 **Sex:** ♀

Time on Study	Death
8 days	FD

Internal and External Necropsy Findings	Organ	Histopathology
NAD	Liver	Cytoplasm of hepatocytes - dense staining. Cellular hyperplasia.
NAD	Kidney	Autolysis.
Spleen small.	Spleen	Red and white pulp depletion.
NAD	Thymus	Lymphocyte depletion.
Brain friable.	Brain	NAD.
Adrenals enlarged.	Adrenals	NAD but autolysis.
Red staining round nose and mouth.		
Absence of body fat.		

Number of Sections Examined		
HE		SS
1	Liver	
2	Kidneys	
1	Heart	
1	Brain	
1	Spleen	
1	Thymus	
2	Adrenals	

Project No: 415669SR **Group No:** 5
Animal No: 559 **Sex:** ♀

Time on Study	Death
8 days	FD

Internal and External Necropsy Findings	Organ	Histopathology
NAD	Liver	Dense staining cytoplasm in hepatocytes, cellular hyperplasia.
Spleen small.	Spleen	White and red pulp depletion.
NAD	Thymus	Lymphocyte depletion.
Adrenals both enlarged.	Adrenals	NAD but autolysis.
Absence of body fat.		
Red staining round eyes, mouth and nose.		

Number of Sections Examined		
HE		SS
1	Liver	
2	Kidneys	
1	Heart	
1	Brain	
1	Spleen	
1	Thymus	
2	Adrenals	

APPENDIX 6 (continued)

Project No: 415669SR Group No: 5
Animal No: 560 Sex: ♀

Time on Study	Death
9 days	FD

Internal and External Necropsy Findings	Organ	Histopathology
NAD	Liver	Hepatocellular hyperplasia.
Spleen small.	Spleen	White and red pulp depletion.
Thymus small.	Thymus	Lymphocyte depletion.
Adrenals slightly enlarged.	Adrenals	NAD but autolysis.
Red staining on nose.		

Number of Sections Examined		
HE		SS
1	Liver	
2	Kidneys	
1	Heart	
1	Brain	
1	Spleen	
1	Thymus	
2	Adrenals	

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