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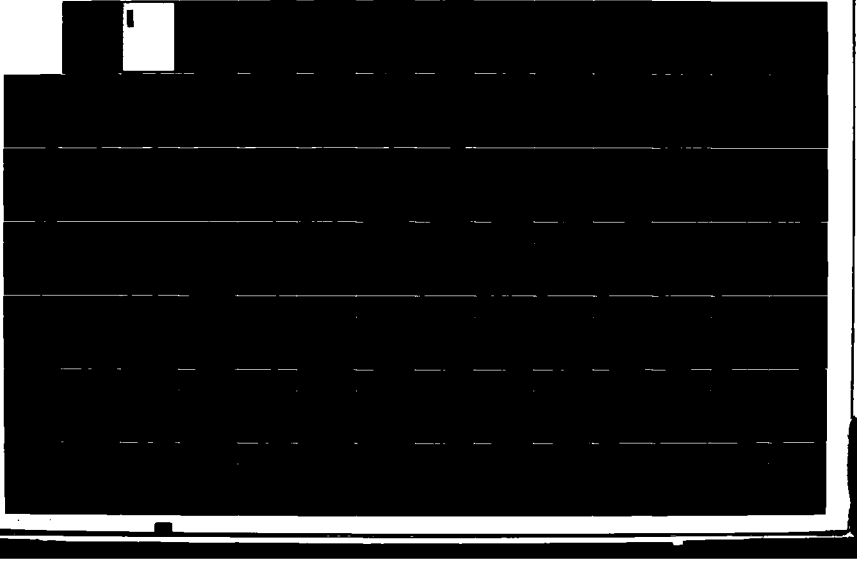

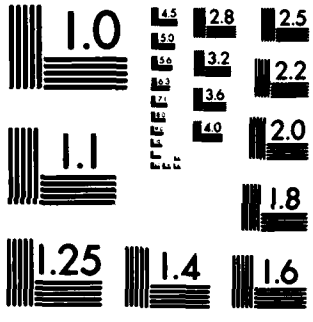

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Abstract

A range of acute toxicity tests with HMX was carried out as follows:

Rat Oral LD50	♂	6.5 g/kg
	♀	7.6 g/kg
Mouse Oral LD50	♂	2.0 g/kg
	♀	3.8 g/kg

Rabbit Oral LD50 using only small numbers of animals:

♂	100-250 mg/kg
♀	50 mg/kg

Deaths were delayed and convulsions occurred.

Rat Dermal LD50	♂ and ♀	>5 g/kg
Rabbit Dermal LD50	♂ Abraded	630 mg/kg
	♂ Non-abraded	670 mg/kg
	♀ Abraded	720 mg/kg
	♀ Non-abraded	1340 mg/kg

Deaths were delayed and convulsions occurred.

Rat Intravenous LD50 (in DMSO)	♂	25 mg/kg
	♀	38 mg/kg

Rabbit Intravenous LD50 using only small numbers of animals:

♂ and ♀ 10-15 mg/kg

Rabbit Primary Skin Irritation - mild skin irritant at 60% w/w in physiological saline.

Rabbit Eye Irritation - practically non-irritant as either dried powder or at 60% w/w in distilled water.

Guinea Pig Sensitisation - no evidence of sensitisation in Magnusson-Kligman test.

AD

IRI Report No. 2051

HMX: ACUTE TOXICITY TESTS IN LABORATORY ANIMALS

Final Report by:

J.A. Cuthbert
K.J. D'Arcy-Burt
S.M.A. Carr

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Contracting Officer's Technical Representative:

Jesse J. Barkley, Jr.
U.S. Army Medical Bioengineering Research
and Development Laboratory
Fort Detrick, Frederick, Maryland 21701-5010

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

Project No. 415669AC

Report No. 2051

QUALITY ASSURANCE AUTHENTICATION

The execution of this type of short-term study is not individually inspected. The processes involved are inspected at intervals according to a pre-determined schedule.

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

IRI Project No. 415669AC

Report No. 2051

Signed: Andrew Waddell
(Quality Assurance Manager)

Date: 3rd March 1986

CONTENTS

SUMMARY

1. ACUTE ORAL TOXICITY (LD50) IN RATS

The Median Oral Lethal Doses (LD50s) with 95% confidence limits were calculated to be as follows:-

♂	6.49 (5.98-6.99) g/kg
♀	7.59 (6.88-8.31) g/kg
♂+♀	7.36 (6.89-7.83) g/kg

As such this material may be considered practically non-toxic in rats.

2. ACUTE ORAL TOXICITY (LD50) IN MICE

The Median Oral Lethal Doses (LD50s) with 95% confidence limits were calculated to be as follows:

♂	1.96 (1.69-2.22) g/kg
♀	3.81 (3.43-4.20) g/kg
♂+♀	2.71 (2.48-2.94) g/kg

As such this material may be considered slightly toxic in mice.

3. ACUTE ORAL TOXICITY IN RABBITS (DOSE RANGING)

In males the oral LD50 might be between 100 and 250 mg/kg, while in females it could be less than 50 mg/kg.

4. ACUTE DERMAL TOXICITY (LD50) STUDY IN RATS

The Percutaneous Median Lethal Dose (LD50) is greater than 5.0 g/kg body weight.

5. ACUTE DERMAL TOXICITY (LD50) IN RABBITS

The Percutaneous Median Lethal Doses (with 95% confidence limits) were calculated to be:-

♂ non-abraded	-	634.12 (532.49- 735.75) mg/kg
♂ abraded	-	673.81 (562.34- 785.28) mg/kg
♀ non-abraded	-	718.56 (595.59- 841.53) mg/kg
♀ abraded	-	1336.70 (414.56-1758.84) mg/kg
♂/♀ non-abraded/abraded	-	982.03 (861.46-1102.60) mg/kg

6. INTRAVENOUS TOXICITY (LD50) IN RATS

The Intravenous Median Lethal Dose (LD50s) were calculated to be:-

♂	25.13	(23.04-27.41) mg/kg
♀	38.08	(32.25-43.91) mg/kg

7. INTRAVENOUS TOXICITY IN RABBITS (DOSE RANGING)

At a concentration of 250 mg/ml (HMX in DMSO) the Median Intravenous Lethal Dose may lie between 10 and 15 mg/kg.

No deaths or clinical signs were recorded in the control group.

8. PRIMARY SKIN IRRITATION IN RABBITS

HMX would be classified as a mild skin irritant on the basis of the calculated primary skin irritation scores:-

HMX 60% w/w in physiological saline	0.67
10% aqueous sodium lauryl sulphate (positive control)	5.59

9. EYE IRRITATION IN RABBITS (HMX 60% W/W IN DISTILLED WATER)

HMX (in distilled water) would be classified as practically non-irritating to eyes.

10. EYE IRRITATION IN RABBITS (DRIED HMX)

Dried HMX would be classified as practically non-irritating to eyes.

11. ALLERGENIC POTENTIAL IN GUINEA PIGS (MAGNUSSON-KLIGMAN MAXIMISATION TEST)

There was no evidence from the Magnusson-Kligman Maximisation test to suggest that HMX is a sensitiser in guinea pigs.

INTRODUCTION

The US Army requires information on the acute toxicity potential of Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) ~~under DAMD 17-80-C-0053~~ by oral, dermal and intravenous routes. In addition data on the acute skin and eye irritancy and skin sensitisation potentials of HMX are required. This report gives details of the following tests performed on HMX to meet these requirements.

1. Acute Oral Toxicity (LD50) Test in Rats.
2. Acute Oral Toxicity (LD50) Test in Mice.
3. Acute Oral Toxicity in Rabbits (Dose Ranging).
4. Acute Dermal Toxicity (LD50) in Rats.
5. Acute Dermal Toxicity (LD50) in Rabbits.
6. Intravenous Toxicity (LD50) in Rats.
7. Intravenous Toxicity in Rabbits (Dose Ranging).
8. Primary Skin Irritation Test in Rabbits.
9. Eye Irritation (HMX 60% w/w in distilled water) in Rabbits.
10. Eye Irritation (dried HMX) in Rabbits.
11. Allergenic Potential in Guinea Pigs (Magnusson-Kligman Maximisation Test).

The tests were performed at Elphinstone Research Centre, Field Station of Inveresk Research International Limited.

Archives

All data produced in these studies are located in the Archives of Inveresk Research International Limited.

TEST MATERIALS AND ANIMALS

TEST MATERIALS

HMX, Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine was supplied by the Royal Ordnance Factory, Bridgewater, Somerset, England. A stock of HMX was held at I.C.I. Nobel Explosives Company Limited, Blairhill, Muirside, Fife and pots containing approximately 50 g dry weight HMX were transported to Inveresk Research International Limited when required.

TEST ANIMALS

Rats and mice were supplied by Charles River UK Limited. Rats arrived at Elphinstone Research Centre on 17 June 1980 for the acute oral and acute dermal tests and on 5 December 1980 for the intravenous test. Mice arrived at Elphinstone Research Centre on 17 June 1980.

Guinea pigs were supplied by Porcellus Animals Limited and arrived at Elphinstone Research Centre on 1 July 1980.

Rabbits were supplied by Cheshire Rabbits Farms and arrived at Elphinstone Research Centre on 24 June 1980 for the skin and eye irritation test, on 29 October 1980 for the acute oral test, on 18 November 1980 for the intravenous toxicity test and on 18 July 1980, 27 August 1980 and 30 September 1980 for the acute dermal toxicity tests.

EXPERIMENTAL PROCEDURES

1. ACUTE ORAL TOXICITY (LD50) IN RATS

a) Materials

HMX, as supplied, from pots Nos. 2 (14.90% water), 4 (19.30% water) and 8 (19.30% water).

Vehicle - 0.5% low viscosity carboxymethylcellulose (CMC).

b) Animals

Male and female rats of the Fischer 344 strain were used. The mean body weight on being dosed was 150 g (range 141-155 g) in the dose ranging study for males and 126 g (range 120-130 g) in the dose ranging study for females. The mean body weight in the main study was 183 g (range 170-204 g) for males and 131 g (range 119-144 g) for females.

They were housed in groups of 5, by sex, in polypropylene cages with stainless steel grid tops and sterilised wood shavings. Mean environmental temperature was 23°C (extremes of 20°C-26°C) and mean humidity was 58% (extremes of 42%-70%).

The animals were fed on BP Nutrition expanded Rat and Mouse Maintenance Diet No. 1 (Appendix 1) but were deprived of food for a 16 h period prior to dosing. Water was available ad libitum throughout the study.

c) Method

The rats were dosed once by gavage with a freshly prepared suspension of HMX in 0.5% low viscosity CMC at a constant dose volume of 20 ml/kg.

Dose Ranging Study (11 July-25 July 1980)

In the dose ranging study, HMX in suspension, was administered to one male and one female rat for each of 5 dose levels. The dose levels investigated were 300, 700, 1,500, 5,000 and 15,000 mg wet HMX/kg. These were equivalent to approximately 255, 596, 1277, 4255 and 12,765 mg dry HMX/kg. Aliquots of these suspensions were taken at intervals for analysis.

The rats were observed in the morning and afternoon for 14 days following administration of the test substance. At death, or at the end of the observation period and sacrifice, each animal was subjected to a gross post mortem examination.

Main Study (18 July-1 August 1980)

From the results of the dose ranging study, five dose levels were selected for the main study in which 5 groups of 5 males and 5 female rats were dosed once. The dose levels selected were 3,000, 4,500, 6,750, 10,125 and 15,187.5 mg wet HMX/kg. These were equivalent to approximately 2421, 3632, 5447, 8171 and 12,256 mg dry HMX/kg. Aliquots of these suspensions were taken at intervals for analysis. An additional group of 5 male and 5 female rats was dosed with vehicle only at a constant dose volume of 20 ml/kg.

The rats were observed in the morning and afternoon for 14 days following administration of the test substance. At death, or at the end of the observation period and at termination, each animal was subjected to a gross post mortem examination.

Statistical Analysis

The LD50 was calculated using a method based on the following:-

Finney (1971) "Probit Analysis", Cambridge University Press.

The toxicity of the compound was rated according to the following table:-

Toxicity Rating	Commonly used term	LD50 single oral dose in rats/kg
1	Extremely toxic	1 mg or less
2	Highly toxic	1-50 mg
3	Moderately toxic	50-500 mg
4	Slightly toxic	0.5-5 g
5	Practically non-toxic	5-15 g
6	Relatively harmless	15 g or more

d) Results

Dose Ranging (Table 1)

In the dose ranging study, mortality was only observed for the female dosed at 12,765 mg dry HMX/kg.

Abnormal clinical signs were noted only in this animal and included hypokinesia and ataxia from 1 h-2 h after dosing.

Post mortem observations included white fluid in the stomach and gastro-intestinal tract and lungs red and patchy.

Main Study (Table 2)

In the main study, mortality was 0/10, 0/10, 4/10, 8/10 and 10/10 in the 2421, 3632, 5447, 8172 and 12,256 mg dry HMX/kg dose groups, respectively.

Clinical signs included piloerection, hyperkinesia, hypokinesia and ataxia from ½ h 5 days after dosing.

Post mortem observations included white fluid in stomach and gastro-intestinal tract, kidneys pale and abnormally pink lungs.

There were no deaths in the control group and no clinical signs were recorded.

No abnormalities were detected at post mortem examination.

Statistical Analysis

The Oral Median Lethal Doses (LD50s) with 95% confidence limits of dry HMX were calculated to be:

♂	5.51 (5.02-5.93) g/kg
♀	6.44 (5.84-7.05) g/kg
♂ + ♀	6.25 (5.85-6.65) g/kg

Chemical Analysis

The analysis of HMX from the sampled aliquots is presented in Tables 3 and 4. The method of analysis is detailed in Appendix 8.

2. ACUTE ORAL TOXICITY (LD50) IN MICE

a) Materials

HMX, as supplied, from pots Nos. 2 (14.90% water) and 54 (20.30% water).

Vehicle - 0.5% low viscosity CMC.

b) Animals

Male and female mice of the B6C3F1 strain were used. The mean body weight on being dosed was 22 g (range 20-22 g) in the dose ranging study for males and 21 g (range 19-22 g) in the dose ranging study for females. The mean body weight on being dosed in the main study was 22 g (range 18-24 g) for the males and 18.5 g (range 16-21 g) for the females.

They were housed individually in polypropylene cages with stainless steel grid tops and sterilised wood shavings. Mean environmental temperature was 22°C (extremes of 19°C-25°C) and mean humidity was 57% (extremes of 52%-74%).

The animals were fed on BP Nutrition expanded Rat and Mouse Maintenance Diet No. 1 but were deprived of food for a 4 h period prior to dosing. Water was available ad libitum throughout the study.

c) Method

The mice were dosed once by gavage with a freshly prepared suspension of HMX in 0.5% low viscosity CMC at a constant dose volume of 20 ml/kg.

Dose Ranging Study (10 July-24 July 1980)

In the dose ranging study, HMX was administered to one male and one female mouse of each of 5 dose levels. The dose levels investigated were 300, 700, 1,500, 5,000 and 15,000 mg wet HMX/kg. These were equivalent to approximately 247, 577, 1236, 4120 and 12,360 mg dry HMX/kg. Aliquots of these suspensions were taken at intervals for analysis.

The mice were observed in the morning and afternoon for 14 days following administration of the test substance. At death, or at the end of the observation period and sacrifice, each animal was subjected to a gross post mortem examination.

Main Study (17 July-31 July 1980)

From the results of the dose ranging study, 5 dose levels were selected for the main study in which 5 groups of 5 males and 5 female mice were dosed with HMX in carboxymethylcellulose at a constant dose volume of 20 mg/kg. The dose levels selected were 1,200, 2,040, 3,468, 5,895.6 and 10,022.5 mg wet HMX/kg. These were equivalent to approximately 956, 1626, 2764, 4699 and 7988 mg dry HMX kg. Aliquots of these suspensions were taken at intervals for analysis. An additional group were taken at intervals for analysis. An additional group of 5 male and 5 female mice was dosed with vehicle only (0.5% low viscosity CMC) at a constant dose volume of 20 ml/kg.

The mice were observed on the morning and afternoon for 14 days following administration of the test substance. At death, or at the end of the observation period and sacrifice, each animal was subjected to a gross post mortem examination.

Statistical Analysis

The LD50 was calculated using a method based on the following:-

Finney (1971), "Probit Analysis", Cambridge University Press.

d) Results

Dose Ranging (Table 5)

In the dose ranging study in pairs of mice, mortality was 0/2, 0/2, 1/2, 1/2 and 1/2 in the dose groups 247, 577, 1236, 4120 and 12,360 mg dry HMX/day dose groups, respectively.

Clinical signs included piloerection, hypokinesia and ataxia, lasting for up to 3 days after dosing.

Post mortem observations included white fluid in stomach and upper gastro-intestinal tract with kidneys pale and mottled.

Main Study (Table 6)

In the main study, mortality was as follows:-

Dose Level mg dry HMX/kg/ day	♂	♀
956	0/5	0/5
1626	4/5	0/5
2764	5/5	0/5
4699	5/5	5/5
7988	5/5	5/5

Clinical signs included piloerection, soiled coat, hyperkinesia, hypokinesia, ataxia, sedation, eyes half shut and penis protruded. In one animal the penis was very swollen and stained with blood with a constant stream of urine.

Post mortem observations included stomach and gastro-intestinal tract filled with white fluid, gut contents fluid, blood filled gut, stomach wall white, penis extended and dark red and lungs red.

There were no deaths or clinical signs recorded in the control group.

No abnormalities were detected at post mortem observations in the control group.

Statistical Analysis

The Oral Median Lethal Doses (LD50s) with 95% confidence limits of dry HMX were calculated to be:-

♂	1.67 (1.44-1.89) g/kg/day
♀	3.24 (2.92-3.57) g/kg/day
♂ + ♀	2.30 (2.11-2.50) g/kg/day

Chemical Analysis

The analysis of HMX from the sampled aliquots is presented in Tables 7 and 8. The method of analysis is detailed in Appendix 8.

3. ACUTE ORAL TOXICITY IN RABBITS (5 November-2 December 1980)

a) Materials

HMX (dried) from pots Nos. 19-23 and 58.

Vehicle - 0.5% low viscosity CMC.

b) Animals

Male and female rabbits of the New Zealand White strain were used. The mean body weight on being dosed was 2.95 kg (range 2.49-3.80 g) for males and 2.82 kg (range 2.60-3.20 g) for females.

They were housed individually in cages with a grid floor, beneath which was a peat moss filled tray. Mean environmental temperature was 18°C (extremes of 15°C-22°C) and mean humidity was 58% (extremes of 47%-69%).

The animals were fed on Spratt's Rabbit Diet supplied by Spillers but were deprived of food for a 16 h period prior to dosing. Water was available ad libitum throughout the study.

c) Method

The rabbits were dosed once by means of a rubber catheter with a freshly prepared suspension of HMX in CMC at a constant dose volume of 10 ml/kg.

HMX in suspension was administered to one male and one female rabbit for each of 6 dose levels. The dose levels investigated were 50, 100, 250, 428.5, 1000 and 2000 mg dry HMX/kg body weight and dosing was carried out at various levels of successive days.

The rabbits were observed in the morning and afternoon for 14 days following administration of the test substance. At death, or at the end of the observation period and at termination, each animal was subjected to a gross post mortem examination when selected tissues (liver, kidneys, spleen, heart, lungs, brain and spinal cord) were taken for future histological examination if required.

d) Results

Details are given in Table 9. Mortality was 1/2, 1/2, 2/2, 2/2, 2/2 and 2/2 in the 50, 100, 250, 428.5, 1,000 and 2,000 mg/kg dose groups, respectively. The female in the 50 mg/kg dose group, the male in the 428.5 mg/kg dose group and both the male and female in the 1,000 mg/kg dose group were sacrificed before the end of the 14 day observation period to prevent further distress following convulsive episodes.

Major clinical signs which were present at all dose levels included hypokinesia, hyperkinesia, clonic convulsions, miosis and mydriasis. Details of these and other clinical signs are given in Table 9.

Post mortem observations included irregular reddening on all lung lobes, kidneys mottled or pale with surface blood vessls prominent and brown fluid with white particulate material in thoracic cavity. Details of these and other post mortem observations are given in Table 9.

4. ACUTE DERMAL TOXICITY STUDY IN RATS

a) Materials

HMX from pots Nos. 29 (18.7% water) and 76 (17.5% water).

Vehicle - physiological saline

b) Animals

Male and female rats of the Fischer 344 strain were used. The mean body weight on being dosed was 193 g (range 140-242 g) in the dose ranging study, 196 g (range 138-250 g) in the main study and 193 g (range 143-256 g) in the control group.

They were housed in suspended plastic cages with a maximum of 5 animals per cage with wood shavings for bedding. Mean environmental temperature was 23°C (extremes of 20°C-16°C) and mean humidity was 59% (extremes of 42%-70%).

The animals were fed on BP Nutrition expanded Rat and Mouse Maintenance Diet No. 1. Food and water were available ad libitum throughout the study.

c) Method

The application site, which was the entire dorsal surface of the trunk of the rat between the fore and hind limbs, was shaved and the skin abraded in such a way as to penetrate the stratum corneum but not the dermis by making abrasions longitudinally over the entire area of exposure, by means of a 'sterilin' blood lancet.

The test material was applied to the prepared skin on a piece of gauze of a length to cover approximately 10% of the body surface. The gauze was covered with an impervious covering of Sleek occlusive tape for a period of 24 h after which time the covering was removed and the skin was wiped to remove any remaining test material. Animals were housed individually for the 24 h contact period.

Dose Ranging Study (22 July-5 August 1980)

In the dose ranging study, freshly prepared HMX at a concentration of 600 mg/ml in physiological saline was administered to one male and one female rat for each of 4 dose levels. The dose levels investigated were 0.5, 1.0, 2.0 and 5.0 g wet HMX/kg body weight. These were

equivalent to approximately 0.42, 0.85, 1.69 and 4.23 g dry HMX/kg. These dose levels were achieved by varying the dose volume.

The rats were observed for 14 days following dosing after which they were sacrificed and subjected to a gross post mortem examination.

All rats were weighed on dosing, at 7 days post dosing and at sacrifice. Individual body weights are detailed in Table 10.

Main Study (29 July-12 August 1980)

From the results of the dose ranging study, one dose level was selected for the main study, in which a group, comprising 8 male and 8 female rats, was dosed once at a level of 5.0 g of wet HMX/kg. This was equivalent to approximately 4.23 g dry HMX/kg. A dose volume of 8.33 ml/kg/day was used in the main study.

The rats were observed for 14 days following dosing after which time they were sacrificed and subjected to a gross post mortem examination.

All rats were weighed on dosing, at 7 days post dosing and at sacrifice. Individual body weights are detailed in Table 11.

A control group of 5 male and 5 female rats was dosed with physiological saline at a constant dose volume of 8.33 ml/kg.

The control group rats were observed for 14 days following dosing after which time they were sacrificed and subjected to a gross post mortem examination.

All control group rats were weighed on dosing, at 7 days post dosing and at sacrifice. Individual and mean body weights are detailed in Table 12.

Histopathology

Two portions of treated skin were taken at post mortem and fixed for 3 weeks in 10% neutral buffered formalin. Tissues were then processed and 4 µm thick haematoxylin and eosin stained sections prepared. These were examined by a pathologist in random order.

d) Results

Dose Ranging Study

No deaths were recorded in the dose ranging study.

Reduced activity was noted in all animals in the 24 h following dosing this probably being due to the restrictive nature of the occlusive covering.

No abnormalities were noted at post mortem.

Main Study

i. In the main study, no deaths occurred in the 4.23 g dry HMX/kg dose group.

Reduced activity was noted in all control animals in the 24 h following dosing, this probably being due to the restrictive nature of the occlusive covering.

No abnormalities were noted at post mortem.

ii. No deaths were noted in the control group.

Reduced activity was noted in all control animals in 24 h following dosing, this probably being due to the restrictive nature of the occlusive covering.

No abnormalities were noted at post mortem.

Histopathology

No abnormalities were noted on histopathological examination of skin samples in control or HMX treated animals.

Analysis of Results

The Percutaneous Median Lethal Dose (LD50) in rats of HMX in physiological saline (60% w/v) is greater than 4.23 g dry HMX/kg.

5. ACUTE DERMAL TOXICITY (LD50) IN RABBITS

a) Materials

HMX (dried) from pots Nos. 1, 26, 27, 29, 33, 51, 52, 53, 59, 76, 77, 78 and 79.

Vehicle - 1% high viscosity CMC (Phases 2 and 3).

Physiological saline (Phases 1 and 2).

b) Animals

Male and female rabbits of the New Zealand White strain were used. The mean body weight on being dosed for Phases 1, 2 and 3 was 3.03 kg (range 2.56-3.66 kg) for males and 3.11 kg (range 2.47-3.84 kg) for females. The average body weight on being dosed in the main study was 3.13 kg (range 2.35-3.63 kg) for males and 3.08 kg (range 2.45-3.73 kg) for females.

They were housed individually in cages with a grid floor, beneath which was a peat moss filled tray. Mean environmental temperature was 18.5°C (extremes of 15°C-23°C) for the dose ranging studies and 17°C (extremes of 14°C-20°C) for the main study. Mean humidity was 68% (extremes of 58%-78%) for the dose ranging studies and 64% (extremes of 53%-76%) for the main study.

They were fed Spratt's Rabbit Diet supplied by Spillers and food and water were available ad libitum throughout the study.

c) Method

The application site, which is the entire trunk of the rabbit between the fore and hind limbs, was shaved and the skin abraded (where applicable) in such a way as to penetrate the stratum corneum but not the dermis by making abrasions every 2-3 cm longitudinally over the entire area of exposure, by means of a 'sterilin' blood lancet.

The test material was applied to the prepared skin on a piece of gauze of a length to cover approximately 10% of the body surface (approximately 22 x 12 cm for a 3 kg

rabbit). The gauze was covered with an impervious covering of Sleek occlusive tape. The test substance was kept in contact with the skin for 24 h. At the end of the exposure period, the wrapping was removed and the skin wiped to remove any remaining test material.

Animals were weighed on the day of administration and weekly thereafter, until death or sacrifice. They were observed frequently on the day of administration and in the morning and afternoon for 14 days following administration of the test substance, when surviving animals were sacrificed.

Phase 1 (29 July-13 August 1980)

In Phase 1, HMX in physiological saline at a constant concentration of 600 mg/ml was administered to one male and one female rabbit, on abraded skin, for each of the 3 dose levels by varying dose volumes. The dose levels investigated were 600, 1,800 and 3,000 mg dry HMX/kg/day body weight.

At death/sacrifice each animal was subjected to a gross post mortem examination where tissues were taken from selected animals. The selected tissues were brain, liver, kidney, spleen, heart and lungs and were fixed in 10% neutral buffered formalin. These were not examined further.

Phase 2 (10 August-25 August 1980)

Due to the unexpectedly high toxicity of HMX experienced in Phase 1 and some difficulties (homogeneity) encountered with physiological saline as the vehicle, Phase 2 was designed to confirm the high toxicity experienced in Phase 1 and investigate the suitability of 1% CMC as an alternative vehicle. Aliquots of the suspensions were taken at suitable intervals during dosing for analysis.

The dosing regime was as follows:-

i. HMX in physiological saline at a constant concentration of 600 mg/ml and varying dose volumes was administered to one male and one female rabbit on abraded skin for each of the 3 dose levels. The dose levels investigated were 600, 1,800 and 3,000 mg/kg.

ii. HMX in 1% CMC at a constant concentration of 600 mg/ml and varying dose volumes were administered to one male and one female rabbit, on abraded skin, for each of 4 dose levels. The dose levels investigated were 300, 600, 1,800 and 3,000 mg/kg.

At death, or sacrifice, selected tissues were taken for histopathological examination. The tissues taken were the liver, kidneys, spleen, heart, lungs, brain, spinal cord and 2 pieces of skin from the dorsal area and were fixed in 10% neutral buffered formalin.

Phase 3

In Phase 3 a dose ranging study was followed by the main study.

Dose Ranging (2 September-17 September 1980)

HMX in CMC at a constant concentration of 600 mg/ml and varying dose volumes was administered to one male and one female on non-abraded skin for each of 3 dose levels. The dose levels investigated were 600, 1,200 and 2,400 mg/kg.

Main Study (13 October-29 October 1980)

In the main study, 76 rabbits were randomly allocated to 8 test groups, each of 4 male and 4 female rabbits and 2 control groups, each of 3 male and 3 female rabbits. Four test groups and one control group (vehicle only) were abraded at the treatment site, the remaining 4 groups were non-abraded.

From the results of the Phase 1, Phase 2 and Phase 3 dose ranging studies, 4 dose levels were selected for both abraded and non-abraded groups. HMX in 1% CMC at a

constant concentration of 600 mg/ml and at varying dose volumes was administered dermally under occlusion. The dose levels selected were 168, 372, 816 and 1,788 mg/kg. Aliquots (3 per dose level) of the suspensions were taken at suitable intervals for analysis.

The control groups were dosed at a constant dose volume of 2.98 ml/kg 1% CMC.

At death or sacrifice, selected tissues were taken for histopathological examination. The tissues taken were the liver, kidneys, spleen, heart, lungs, brain, spinal cord and 2 pieces of skin from the dorsal area and these were fixed in 10% neutral buffered formalin.

d) Results

Phase 1 (Table 13)

HMX in physiological saline was investigated at dose levels of 600, 1,800 and 3,000 mg/kg in 3 groups, each of one male and one female rabbit. Mortality was 1/2, 2/2 and 2/2, respectively. Deaths occurred between 3 and 6 days after dosing.

Clinical signs included hyperkinesia, blood around nose, clonic convulsions, laboured breathing, vocalisation and loss of co-ordination.

At post mortem examination, the following observations were recorded; blood in nasal cavity, subdural haemorrhage between cerebellum and cerebrum, slight pitting of surface of kidneys and pale kidneys. Individual gross pathological findings are given in Appendix 1.

Histopathological examination of the tissues taken was not performed.

Phase 2 (Table 14)

This phase of dose ranging with HMX in either physiological saline or carboxymethylcellulose gave the following results:-

i. Treatment with HMX in physiological saline at dose levels of 600, 1,800 and 3,000 mg/kg resulted in 0/2, 2/2 and 2/2 deaths, respectively. Deaths occurred between 1 and 6 days after dosing.

Major clinical signs observed were clonic convulsions, hyperkinesia, miosis, laboured respiration, mydriasis, dyspnoea, cyanosis and hypokinesia.

Observations noted at the post mortem examination included red staining around nose, blood around mouth creamy particulate matter in thoracic cavity and pericardial sac fluid filled. Individual gross pathological findings are given in Appendix 2.

No histopathological examination of tissues was performed in this section.

ii. Treatment with HMX in high viscosity 1% carboxymethylcellulose at dose levels of 300, 600, 1,800 and 3,000 mg/kg resulted in mortality of 1/2, 1/2, 2/2 and 2/2, respectively.

Clinical signs noted were hyperkinesia, miosis, clonic convulsions, blood around nose, vocalisation, loss of mobility and laboured breathing.

Observations noted at post mortem examination included red staining around mouth and nose, subdural haemorrhage and small blood clot on the brain.

Individual gross pathological and histopathological findings are detailed in Appendix 3.

The analysis of HMX from the sampled aliquots is presented in Table 22.

Phase 3 (Table 15)

Dose Ranging

HMX suspended in 1% CMC was investigated in 3 groups, each of one male and one female rabbit in three dose levels 600, 1,200 and 2,400 mg/kg. Mortality was 1/2, 2/2 and 2/2, respectively. Deaths occurred 2 to 4 days after dosing.

Clinical signs noted were hyperkinesia, clonic convulsions, vocalisation and animal unable to move hind legs.

Observations noted at post mortem examination included extensive bruising of hind legs, broken femur, clotted blood in cervical region of spinal cord, clotted blood between cerebellum and cerebrum and dark red lungs.

Individual gross pathological and histopathological findings are given in Appendix 4.

Main Study

Two dose groups each of 4 male and 4 female rabbits were dosed at each of 4 dose levels viz: 168, 372, 816 and 1,788 mg HMX/kg body weight. One of each of the dose groups dosed at each dose level was abraded whilst the other remained non-abraded. In addition, 2 control groups, one abraded and one non-abraded were dosed at 2.98 ml/kg with the vehicle only.

Mortality in the non-abraded groups was 0/8, 0/8, 4/8 and 8/8 for each dose group, respectively. Mortality in the abraded groups was 0/8, 0/8, 3/8 and 7/8 for each dose group, respectively. One female non-abraded control animal died 10 days after dosing with vehicle only.

The clinical signs noted during the study and observations noted at the post mortem examination are given in Tables 16 and 17. An analysis of time of death/ sacrifice etc. is presented in Table 18. The incidence of paralysis and/or broken limbs is presented in Tables 19 and 20.

The group incidence of histopathological findings are detailed in Table 21.

Individual gross pathological and histopathological findings are detailed in Appendix 5.

The analysis of HMX in sampled aliquots is presented in Table 23. The method of analysis is detailed in Appendix 8.

Statistical Analysis

The Percutaneous Median Lethal Doses (LD50s) and 95% confidence limits in rabbits were calculated to be as follows:-

♂ non-abraded	-	634.12 (532.49-735.75)	mg/kg
abraded	-	673.81 (562.34-785.28)	mg/kg
♀ non-abraded	-	718.56 (595.59-841.53)	mg/kg
♀ abraded	-	1336.70 (414.56-1758.84)	mg/kg
♂+♀ non-abraded/abraded	-	982.03 (861.46-1102.60)	mg/kg

6. INTRAVENOUS TOXICITY (LD50) IN RATS

a) Materials

HMX dried from pots Nos. 25, 26, 31, 32, 35 and 36.

Vehicle - dimethylsulphoxide (Batch No. 6055410; BDH Chemicals Ltd) as a solvent.

b) Animals

Male and female rats of the Fischer 344 strain were used. The mean body weight on being dosed was 169 g (range 156-189 g) in the dose ranging study for males and 145 g (range 130-152 g) in the dose ranging study for females. The average body weight in the main study was 197 g (range 175-214 g) for males and 150 g (range 127-167 g) for females.

They were housed in groups of 5, by sex, in polypropylene cages with stainless steel grid tops and sterilised wood shavings. Mean environmental temperature and 22°C (extremes of 16°C-25°C) and mean humidity was 35% (extremes of 20%-48%).

The animals were fed on BP Nutrition expanded Rat and Mouse Maintenance Diet No. 1 and water was available ad libitum throughout the study.

c) Method

The rats were dosed once by intravenous injection (lateral tail vein) with freshly prepared dosing solutions of dried HMX in dimethylsulphoxide at a constant concentration of 250 mg/ml and at varying dose volumes to give the required dose levels. Dimethyl-sulphoxide was chosen as vehicle to avoid problems of flocculation. The dosing solutions were filtered through a millipore filter (pore size 0.22 μm) into a Vacutainer before dosing.

Dose Ranging (18 December-2 January 1981)

In the dose ranging study, HMX in dimethylsulphoxide was administered to one male and one female rat for each of 5 dose levels. The dose levels investigated were 10, 20, 30, 40 and 80 mg dry HMX/kg for males and 10, 20, 40, 80 and 160 mg dry HMX/kg for females. Dosing was carried out on successive days to give maximum information.

The rats were observed for clinical signs in the morning and afternoon for 14 days following administration of the test substance.

At death at the end of the observation period or at termination, each animal was subjected to a gross post mortem examination.

Main Study (23 December 1980-7 January 1981)

From the results of the dose ranging study, 5 dose levels were selected for the main study in which 5 groups of 5 male and 5 female rats were injected by the venous route. The dose levels investigated were 15.00, 19.50, 25.00, 32.50 and 42.50 mg dry HMX/kg in the males and 30.00, 45.00, 67.25, 101.50 and 152.50 mg dry HMX/kg in the females.

An additional group of 5 male and 5 female rats was injected with vehicle only (dimethylsulphoxide) at 0.17 ml/kg for the males and 0.61 ml/kg for the females.

The rats were observed for clinical signs in the morning and afternoon for 14 days following administration of the test substance. At death, or at the end of the observation period and sacrifice, each animal was subjected to a gross post mortem examination.

The LD50 was calculated using a method based on the following:-

Finney (1971), "Probit Analysis", Cambridge University Press.

d) Results

Dose Ranging (Table 24)

In the dose ranging study mortality was 0/1, 0/1, 1/1, 1/1 and 1/1 for the males in the 10, 20, 30, 40 and 80 mg/kg dose groups, respectively. For the females mortality was 0/1, 0/1, 0/1, 0/1 and 1/1 in the 10, 20, 40, 80 and 160 mg/kg dose groups, respectively.

Clinical signs included hypokinesia, clonic convulsions, sedation, coma, slight paralysis of hind limbs and unco-ordinated movements from 20 sec after injection.

No abnormalities were detected at post mortem examination.

Main Study (Table 25)

In the main study, mortality was 1/5, 0/5, 3/5, 3/5 and 4/5 for the males, in the 15.00, 19.50, 25.00, 32.50 and 42.50 mg/kg dose groups, respectively. For the females mortality was 2/5, 3/5, 4/5, 5/5 and 5/5 in the 30.00, 45.00, 67.25, 101.50 and 152.50 mg/kg dose groups, respectively.

Clinical signs included hyperkinesia, increased regular breathing, vocalisation, clonic convulsions, paralysis of hind limbs and coma from 30 sec after injection.

One male rat in the 42.50 mg/kg dose group received the dose subcutaneously not intravenously. The LD50 calculation was carried out excluding this animal.

Post mortem observations included red foci and dark red patches on all lung lobes.

There were no deaths in the control group and no clinical signs were recorded and no abnormalities were detected at post mortem.

The Intravenous Median Lethal Doses (with 95% confidence limits) of dry HMX in rats were calculated to be:-

♂ 25.13 (23.04-27.41) mg/kg
♀ 38.08 (32.25-43.91) mg/kg

7. INTRAVENOUS TOXICITY STUDY IN RABBITS (DOSE RANGING)

a) Materials

HMX (dried) from pots Nos. 19 and 23.

Vehicle - dimethylsulphoxide (Batch No. 6055410 BDH Chemicals Ltd) as a solvent.

b) Animals

Male and female rabbits of the New Zealand White strain were used. The mean body weight on being dosed was 3.0 kg (range 2.63-3.32 kg) for the males and 3.11 kg (range 2.83-3.28 kg) for the females.

They were housed individually in cages with a grid floor, beneath which was a peat moss filled tray. Mean environmental temperature was 19°C (extremes of 15°C-22°C) and mean humidity was 59% (extremes of 50%-79%).

The animals were fed on Spratt's Rabbit Diet supplied by Spillers and food and water were available ad libitum throughout the study.

c) Method

The test commenced on 26 November 1980 and was completed on 18 December 1980.

The rabbits were dosed once by intravenous injection with freshly prepared dosing solutions of dried HMX in dimethylsulphoxide at various concentrations and appropriate dose volumes to give the required dose levels. Dimethylsulphoxide was chosen as vehicle to avoid potential problems of flocculation. The dosing solutions were filtered through a millipore filter (pore size 0.22 µm) into a Vacutainer.

HMX was administered to one male and one female rabbit for each of 7 dose levels. The dose levels investigated were 10 and 1 mg dry HMX/kg at a concentration of 50 mg/ml and 5, 10, 20, 15 and 12.50 mg dry HMX/kg at a concentration of 250 mg/ml. Dosing was on successive days to achieve maximum information.

The concentration of HMX was increased from 50 to 250 mg/ml to reduce the volume of DMSO injected and therefore reduce the possible effects of injecting large volumes of DMSO.

An additional one male and one female rabbit were injected with vehicle only (dimethylsulphoxide) at 0.20 ml/kg.

The rabbits were observed in the morning and afternoon for 14 days following administration of the test substance. At the end of the observation period, a 5 ml whole blood sample was taken from surviving rabbits and centrifuged at 2,000 r.p.m. for 15 min. The plasma was then decanted and stored at -4°C for possible use in any future biochemical or metabolism study. At death or at the end of the observation period and sacrifice, each animal was subjected to a gross post mortem examination.

d) Results

Mortality was 2/2 and 0/2 in the 10 and 1 mg/kg dose groups, respectively and 0/2, 0/2, 1/2, 2/2 and 2/2 in the 5, 10, 12.50, 15 and 20 mg/kg dose groups, respectively.

Clinical signs included hyperkinesia, aggression, vocalisation, difficulty breathing, prostration, immobility, slight epistaxis, dyspnoea and clonic convulsions from 10 sec-5 days after administration. Details of these are given in Table 26.

There were no abnormalities detected at post mortem examination.

There were no deaths in the control group and no clinical signs were recorded. The only observation noted at post mortem was a 7 mm x 3 mm white patch on right hand lobe at posterior edge of the liver in the female of the control group.

8. PRIMARY SKIN IRRITATION IN RABBITS

a) Materials

HMX (dried) from pots Nos. 29 and 76 - 60% w/w in physiological saline

Sodium lauryl sulphate - 10% w/w in distilled water (positive control)

b) Animals

Three male and three female New Zealand White Rabbits, initially weighing between 2.50 and 3.0 kg were used.

They were housed individually in cages with a grid floor, beneath which was a peat moss filled tray. Mean environmental temperature was 20°C (extremes of 16°C-23°C) and mean humidity was 64% (extremes of 60%-74%).

The animals were fed on Spratt's Rabbit Diet supplied by Spillers and food and water were allowed ad libitum.

c) Method

The test was commenced on 22 July 1980 and completed on 26 July 1980.

The rabbits were prepared by clipping the skin of the back and flanks of all animals free from hair and 2 of the 4 test areas on each rabbit were abraded using a 'sterilin' blood lancet (see Table 27 for patch arrangement).

The test and control materials were tested on each rabbit and applied in the following manner: 0.5 ml of each of the test and positive control materials were applied under separate 2.5 cm^2 patches of filter paper to both intact and abraded skin of each animal. The patches were covered by an overlapping patch of impermeable plastic adhesive tape (Blenderm). The whole area was then bound by Sleek occlusive tape wrapped round the entire trunk of the animal. The patches were left in position for 24 h. At the end of the this period, they were removed, the site was gently wiped (not washed) to remove any remaining test substance.

The test sites were scored immediately and again after 48 h to give the required 24 h and 72 h readings. Readings were evaluated according to FDA scoring system (see Appendix 6 for scoring system).

d) Results

Detailed results are to be found in Tables 27 and 28.

HMX Treated Sites

The test product HMX in physiological saline (60% w/w), elicited very slight (score 1) to well defined erythema (score 2) at both abraded and non-abraded sites on 3/6 rabbits. Remaining rabbits showed no response to HMX at 24 h. At 72 h erythema persisted in one rabbit at both the abraded and non-abraded sites.

Very slight to slight (score 1 to 2) oedema was noted on both abraded and non-abraded sites on 2 rabbits, the remaining animals showing no response on either site at 24 h. At 72 h very slight oedema persisted in one rabbit at the non-abraded site.

Control Treated Site

The positive control product (sodium lauryl sulphate 10% w/w in distilled water) elicited very slight erythema (score 1) in one rabbit, well defined erythema (score 2) in 3 rabbits and moderate to severe erythema (score 3) in the remaining 2 rabbits at 24 h. Dryness was noted on 3 rabbits at 24 h on abraded and non-abraded sites. At 72 h one rabbit had well defined erythema, 3 rabbits moderate to severe erythema and 2 rabbits severe erythema on both abraded and non-abraded sites. Dryness had persisted on both abraded and non-abraded sites.

One rabbit showed very slight oedema (score 1), one rabbit slight oedema (score 2), one rabbit moderate oedema (score 3) and the remaining 3 rabbits severe oedema (score 4) on both abraded and non-abraded sites at 24 h. At 72 h, 2 rabbits had slight oedema, 3 rabbits moderate oedema and the remaining rabbit showed severe oedema on both abraded and non-abraded sites.

Calculations of Primary Skin Irritation Scores are detailed in Table 27 and were:-

HMX (dried) in physiological saline (60% w/w)	0.67
Sodium lauryl sulphate in distilled water (10% w/w).	5.59

These scores may be rated against the following system of grading as devised by Draize:-

0-2	Mild irritant
2-5	Moderate irritant
6+	Severe irritant.

9. EYE IRRITATION IN RABBITS (HMX IN DISTILLED WATER)

a) Materials

HMX (dried) from pots Nos. 76 (17.5% water) and 29 (18.7% water).

HMX was prepared in distilled water at 60% w/w.

b) Animals

Three male and three female New Zealand White rabbits, initially weighing between 2.5 and 3.0 kg were used.

They were housed individually in cages with a grid floor beneath which was a peat moss filled tray. Mean environmental temperature was 21°C (extremes of 16°C-23°C) and mean humidity was 65% (extremes of 60%-78%).

The animals were fed on Spratt's Rabbit Diet supplied by Spillers and food and water were allowed ad libitum.

c) Method

The test was commenced on 23 July and completed on 30 July 1980.

The quantity of test material instilled into each treated eye was 0.1 ml and instillation of the test material was by the following technique:-

The rabbit was held firmly but gently and the test material placed into the right eye by gently pulling the lower eyelid away from the eyeball to form a sac into which the test material was instilled. The other eye remained untreated to serve as a control.

The eyes were examined for irritation using standard illumination. The ocular reaction was recorded at 1 h, 24 h and 2, 3 and 7 days after treatment.

Ocular reactions were assessed numerically using the scoring system as detailed in Appendix 7.

d) Results

The test results are detailed in Table 29.

The test material elicited no corneal or iridial response at any stage of the test.

Slight redness (score 1) of the conjunctivae was noted in 2/6 treated eyes at 1 h. One treated eye showed slight redness (score 1) at 7 days. 5/6 treated eyes had returned to normal at 24 h.

Test material was evident as aggregations in the inner corner of the eye at 1 h in all treated eyes and in 1/6 treated eyes at 24 h.

10. EYE IRRITATION IN RABBITS (DRIED HMX)

a) Materials

HMX (dried) from pots Nos. 76 and 29.

b) Animals

Three male and three female New Zealand White rabbits, initially weighing between 2.5 and 3.0 kg were used.

They were housed individually in cages with a grid floor beneath which was a peat moss filled tray. Mean environmental temperature was 20°C (extremes of 16°C-23°C) and mean humidity was 66% (extremes of 58%-78%). The animals were fed on Spratt's Rabbit Diet supplied by Spillers and food and water were allowed ad libitum.

c) Method

The test was commenced on 13 April and completed on 20 August 1980.

The quantity of test material applied to each treated eye was 500 mg pre-weighed into capsules. Instillation of the test material was by the following technique:-

The rabbit was held firmly but gently and the test material placed into the right eye by gently pulling the lower eyelid away from the eyeball to form a sac into which the test material was dropped from a prepared capsule. The other eye remained untreated to serve as a control.

The eyes were examined for irritation using standard illumination. The ocular reaction was recorded at 1 and 24 h and at 2, 3 and 7 days after treatment.

Ocular reactions were assessed numerically using the scoring system as detailed in Appendix 7.

d) Results

The test results are detailed in Table 30.

The test material elicited no corneal or iridial response at any stage of the test.

Slight redness (score 1) of the conjunctivae was noted in 6/6 treated eyes at 1 h and in 2/6 treated eyes at 24 h. Very slight redness (score ½) was noted in 2/6 treated eyes at 24 h.

Very mild conjunctival chemosis was noted in 2/6 treated eyes at 1 h. All eyes had returned to normal at 2 days.

Although the nominal dose was 500 mg/treated eye, excess test material fell out of the eye immediately after instillation. In all animals the maximum possible volume of test material was instilled.

11. ALLERGENIC POTENTIAL IN GUINEA PIGS (MAGNUSSON-KLIGMAN MAXIMISATION TEST)

a) Materials

HMX from pots Nos. 2, 29, 54 and 76.

p-Phenylenediamine, Batch No. 2289790 (BDH Chemicals Ltd)

Sodium lauryl sulphate, Batch No. 15 (Fisons Laboratory Reagent)

Freunds Complete Adjuvant, Batch Nos. 645585 and 629620.

b) Animals

Female albino guinea pigs of the Dunkin-Hartley strain, within the weight range 350-400 g, were used.

For each of the 3 tests, 25 test group animals were allocated equally into 5 cages, 10 control group animals were allocated equally in 2 cages and 2 dose finding animals were housed in a single cage.

The cages had a grid floor beneath which was a peat moss filled tray.

The animals were fed on BP Nutrition FD1 Diet, supplemented with hay. They were allowed food and water ad libitum.

The mean environmental temperature was 21°C (extremes of 18°C-24°C) and mean humidity was 63% (extremes of 52%-74%).

c) Method

The test programme was commenced on 17 July and completed on 10 August 1980.

Guinea pigs were allocated to the following groups:-

Group I	25 guinea pigs	HMX (test group)
Group II	25 guinea pigs	p-Phenylenediamine (positive control group)

Group III	25 guinea pigs	Sodium lauryl sulphate (negative control group)
Group IV	10 guinea pigs	Irritancy controls for Group I
Group V	10 guinea pigs	Irritancy controls for Group II
Group VI	10 guinea pigs	Irritancy controls for Group III
Dose finding	6 guinea pigs	(one pair for each of groups I-III).

The maximisation test comprises 2 procedures. The induction procedure consists of an intradermal injection of the test material followed after 7 days by a topical application.

The challenge procedure, which consists of a topical application is carried out 21 days after commencement of the induction procedure.

Induction Procedure

i. Injection Phase

The hair was shaved from an area 4 x 6 cm across the scapular region with electric clippers. Two courses of intradermal injections were given, one on either side of the mid line in Groups I, II and III as follows:-

0.1 ml Freunds Complete Adjuvant
0.1 ml test material alone
0.05 ml test material emulsified with 0.05 ml Freunds Complete Adjuvant

The test materials and concentrations injected were as follows:-

Group I HMX - 6.67% w/v in distilled water

Group II p-Phenylenediamine 2% w/v in distilled water

Group III Sodium lauryl sulphate - 1% w/w in distilled water

In addition, 36 guinea pigs were injected with Freund's Complete Adjuvant only (2 injections of 0.1 ml). Six of these were used for determining the maximum non-irritant concentration of the test materials in their solvents in Groups, I, II and III in a dose ranging experiment. Thirty were used as controls for irritancy of the test materials at the challenge phase.

ii. Topical Induction

Six days after the injection phase, the injection site of animals in Groups I, II and III was shaved again. Twenty four hours later a 2 x 4 cm patch of Whatman No. 3 mm filter paper saturated with the test material was applied to the pre-treated area and the patch was covered by an overlapping patch of impermeable plastic adhesive tape (Blenderm) firmly secured in position by an elastic adhesive bandage wound round the torso of the animal. This dressing was left in place for 48 h. The control guinea pigs (Groups IV-VI) remained untreated at this stage. The concentrations of test material applied were as follows:-

Group I	HMX - 60% w/v in distilled water.
Group II	p-Phenylenediamine - 2% w/v in distilled water.
Group III	Sodium lauryl sulphate - 1% w/w in distilled water.

Dose Ranging Experiment

A maximum non-irritant concentration of HMX, p-Phenylenediamine and sodium lauryl sulphate was determined each in a pair of guinea pigs, hitherto treated with 2 injections of Freunds Complete Adjuvant only.

The test materials were applied at a series of concentrations to the shaved flanks of the guinea pigs under the same occlusive patch system used for the topical

induction. The test materials were tested at the following concentrations:-

Group I HMX - 60% w/v and 30% w/v in paraffin oil.

Group II p-Phenylenediamine - 2% w/v and 1% w/v in distilled water.

Group III Sodium lauryl sulphate - 1% w/w and 0.5% w/w in distilled water.

No irritant responses were observed in any group at any concentration and, therefore, the non-irritant concentrations for each group selected for application in the challenge phase were as follows:-

Group I HMX - 60% w/v in paraffin oil.

Group II p-Phenylenediamine - 2% w/v in distilled water.

Group III Sodium lauryl sulphate - 0.5% w/w in distilled water.

Challenge Procedure

All animals in Groups I-VI were challenged 3 weeks after the injection phase. Hair was removed from a 5 x 5 cm area on the left flank using an electric clipper. The test material, at the pre-selected concentration, was then applied on a 2 x 2 cm patch of filter paper as for the topical induction, occluded as before with 'Blenderm' and held in place with an elastic adhesive bandage for 24 h. The control guinea pigs (Groups IV-VI) which were pre-treated with Adjuvant only at the injection phase, were included to check on the irritancy of the test materials at the challenge concentration.

The degree of response was determined by trained assessors 24 h after removal of the challenge patch, when any allergic reaction would have been at a peak. Any erythema at the challenge site was considered to be a positive response.

The following system was used to score reactions:-

No visible change	0
Slight or discrete erythema	1
Moderate and confluent erythema	2
Intense erythema and smelling	3

d) Results

Challenge scores for Groups I-VI are detailed in Tables 31-32.

Groups I and IV

Preliminary tests indicated that HMX should be non-irritant at a concentration of 60% w/v in paraffin oil on adjuvant pre-treated guinea pigs when applied to the shaved flank under occlusion.

HMX did not elicit positive responses in the test group (Group I) after challenge of the 25 guinea pigs by topical application.

HMX was tested at the challenge phase on 10 guinea pigs which were Adjuvant pre-treated only.

None of these irritancy control group animals (Group IV) reacted positively to this topical application, so supporting the dose ranging results that at a concentration of 60% w/v in paraffin oil this test material would be non-irritant.

Groups II and V

After being challenged with p-Phenylenediamine at a concentration of 2% w/v in distilled water, 24/25 test group (Group II) animals showed moderate and confluent erythema (score 2) while 1/25 test group animals showed slight erythema (score 1).

None of the control group (Group V) animals showed erythema when challenged with p-Phenylenediamine at a concentration of 2% w/v in distilled water, so supporting the dose ranging results that at that concentration the test material would be non-irritant.

All challenge sites were noted as being stained dark brown on assessment.

Groups III and VI

After challenge with sodium lauryl sulphate at a concentration of 0.5% w/w in distilled water, no test group animals (Group III) showed erythema at challenge sites.

None of the control group (Group VI) animals showed erythema when challenged with sodium lauryl sulphate at a concentration of 0.5% w/w in distilled water, so supporting the dose ranging results that at that concentration the test material would be non-irritant.

DISCUSSION AND CONCLUSIONS

A programme of acute toxicity studies was carried out at Inveresk Research International to determine the acute oral, intravenous and percutaneous toxicity potential of HMX, as well as the potential to irritate skin or eye or sensitise.

1. Acute Oral Toxicity

In acute oral toxicity tests on rats and mice HMX was of low toxicity. HMX was slightly more toxic to mice than rats and there were also signs of sex difference in toxicity between male and female mice which was not as obvious in rats.

An oral dosing study in rabbits indicated that HMX was slightly toxic with a possible Oral Median Lethal Dose (LD50) of between 100 and 200 mg/kg in males and a possible value below 50 mg/kg for females.

In rabbits the most severe clinical signs indicated that treatment with HMX caused CNS effects which were delayed and prolonged.

2. Acute Percutaneous Toxicity

An acute dermal study in rats with HMX in physiological saline resulted in no deaths at the one dose level investigated which was 5 g HMX/kg body weight. HMX may be considered to be non-toxic to rats by the dermal route.

The rabbit, again, was much more susceptible to HMX than the rat. HMX was applied to abraded and non-abraded skin and was found to be toxic in both. In males and females with non-abraded skin there were no obvious sex difference in response with LD50 values in the region of 675 mg/kg. On abraded skin there was a sex difference with males being more susceptible than females (LD50 values of 673 mg/kg - males and 1,336 mg/kg - females).

Clinical signs were delayed and included convulsions and behavioural changes indicating a CNS effect.

Gross necropsy and histopathology did not reveal any specific cause for the observed clinical signs and mortality. There was some evidence of dose related incidence of heptocyte eosinophilic staining. A similar sporadic response was found for splenic white pulp cell depletion.

3. Acute Intravenous Toxicity

The acute intravenous toxicity of HMX was established in rats and rabbits. Dimethylsulphoxide (DMSO) was used as the solvent, and in order to minimise the solvent effects dosing was carried out with HMX at the highest concentration possible (i.e. 250 mg/ml). Dose levels were varied by varying the dose volume. Deaths occurred almost immediately after dosing and HMX by the intravenous route was toxic to both male and female rats (31 mg/kg - males, and 38 mg/kg for females).

A similar result was shown in the rabbit intravenous study with the intravenous LD50 of HMX being in the region of 10-15 mg/kg.

Further studies are required to determine the reasons for the differing effects of HMX, as seen (i) between species (e.g. dermally in rat and rabbits) and (ii) between routes (e.g. dermally/orally and intravenously in rats).

4. Dermal Toxicity (Irritation/Sensitisation)

HMX in physiological saline elicited only mild irritation under 24 h occlusive patch conditions.

The Magnusson-Kligman guinea pig maximisation test for delayed contact hypersensitivity gave no indication that HMX might be a sensitiser.

These tests indicate that there should be no such problems associated with the handling of HMX.

5. Ocular Irritation

There was no indication from rabbit eye irritation tests that HMX (which was tested in distilled water as a suspension and as the dried powder) is an eye irritant.

TABLE 1

HMX: Acute Oral Toxicity (LD50) Study in Rats

Dose Ranging Study

Animal No./Sex	Dose Group	Dose Level (mg/kg)		Clinical Signs (time affected after dosing)	Deaths (time after dosing)	PM Observations
		Wet	Dry			
1♂ 6♀	1	300	255	No abnormality detected.	0/1 (S) 0/1 (S)	Patchy lungs.
2♂ 7♀	2	700	596	No abnormality detected.	0/1 (S) 0/1 (S)	Patchy lungs.
3♂ 8♀	3	1500	1277	No abnormality detected.	0/1 (S) 0/1 (S)	Lungs red.
4♂ 9♀	4	5000	4255	No abnormality detected.	0/1 (S) 0/1 (S)	Lungs patchy and red.
5♂ 10♀	5	15000	12765	Hypokinesia, ataxia (1 h-2 h).	0/1 (S) 1/1 (3 h)	White fluid in stomach and gastro-intestinal tract. Lungs red.

(S) = Sacrificed at end of 14 day observation period

TABLE 2

HMX: Acute Oral Toxicity (LD50) Study in Rats

Main Study

Animal No./Sex	Dose Group	Dose Level (mg/kg)		Clinical Signs (days affected)	Deatns (time after dosing)	PM Observations
		Wet	Dry			
11-15♂ 16-20♀	Control 1	20 ml/kg CMC		No abnormality detected.	0/5 (S) 0/5 (S)	No abnormality detected.
21-25♂ 26-30♀	2	3000	2421	Piloerection, hyperkinesia (2-4 days).	0/5 (S) 0/5 (S)	No abnormality detected.
31-35♂ 36-40♀	3	4500	3632	Piloerection, hyperkinesia (1 h-4 days).	0/5 (S) 0/5 (S)	No abnormality detected.
41-45♂ 46-50♀	4	6750	5447	Piloerection, hyperkinesia, ataxia, hypokinesia (1 h-5 days).	2/5 (2 days) 2/5 (2 days, 3 days)	White fluid in stomach and gastro-intestinal tract. Lungs pink. Kidneys pale.
51-55♂ 56-60♀	5	10125	8171	Piloerection, hyperkinesia. Animal bleeding from bite on nose (½ h-2 days).	5/5 (2 days) 3/5 (2 days)	White fluid in stomach and gastro-intestinal tract.
61-65♂ 66-70♀	6	15188	12256	Piloerection, hypokinesia, ataxia (2 h).	5/5 (3 h, 2 days) 5/5 (3 h, 2 days)	Stomach and gastro-intestinal tract filled with white fluid. Lungs pink.

(S) = Sacrificed at end of 14 day observation period

TABLE 3

HMX: Acute Oral Toxicity (LD50) Study in Rats
Dose Ranging Study
Analysis of HMX

Sample No.	Dose Level (mg Wet HMX/kg)	Wet/(Dry) Nominal Concentration (mg/ml)	HMX Dried Concentration Found (mg/ml)	HMX Dried Average Concentration (mg/ml)	Deviation From Nominal (mg/ml)
101	300	15.0	11.79		
102		(12.0)	13.03	12.16	0.16
103			11.65		(1%)
104	700	35.0	28.43		
105		(28.0)	29.40	29.45	1.45
106			30.51		(5%)
107	1500	75.0	54.77		
108		(60.0)	62.40	61.47	1.47
109			67.25		(2%)
110	5000	250.0	205.23		
111		(200.0)	188.59	201.53	1.53
112			210.78		(1%)
113	15000	750.0	533.87		
114		(600.0)	568.54	561.61	38.39
115			582.41		(6%)

TABLE 4

HMX: Acute Oral Toxicity (LD50) Study in Rats
Main Study
Analysis of HMX

Sample No.	Dose Level (mg Wet HMX/kg)	Wet/(Dry) Nominal Concentration (mg/ml)	HMX Dried Concentration Found (mg/ml)	HMX Dried Average Concentration (mg/ml)	Deviation From Nominal (mg/ml)
1	3000	150.00	127.11		
2		(121.05)	132.37		
3			122.28		
4			130.58		
5			137.51	127.83	6.78
6			135.20		(5.6%)
7			128.27		
8			128.96		
9			108.16		
10	4500	225.00	186.21		
11		(181.58)	196.12		
12			168.39		
13			176.80		
14			169.87	184.20	2.62
15			201.07		(1.4%)
16			169.87		
17			205.23		
18			184.23		

TABLE 4 (continued)

Sample No.	Dose Level (mg Wet HMX/kg)	Wet/(Dry) Nominal Concentration (mg/ml)	HMX Dried Concentration Found (mg/ml)	HMX Dried Average Concentration (mg/ml)	Deviation From Nominal (mg/ml)
19	6750	337.50	252.38		
20		(272.36)	244.40		
21			291.21		
22			261.49		
23			293.98	272.48	0.12
24			288.43		(0%)
25			261.16		
26			295.83		
27			263.47		
28	10125	506.25	390.59		
29		(408.54)	395.21		
30			369.21		
31			403.53		
32			403.53	396.75	11.79
33			400.76		(3%)
34			441.43		
35			351.88		
36			414.62		
37	15187.5	759.38	665.62		
38		(612.82)	637.88		
39			679.48		
40			603.21		
41			672.55	639.42	26.6 (4.3%)
42			NO SAMPLE		
43			621.24		
44			654.52		
45			582.41		

TABLE 5

HMX: Acute Oral Toxicity (LD50) Study in Mice
Dose Ranging Study

Animal No./Sex	Dose Group	Dose Level (mg/kg)		Clinical Signs (time affected after dosing)	Deaths (time after dosing)	PM Observations
		Wet	Dry			
1♂ 6♀	1	300	247	No abnormality detected.	0/1 (S) 0/1 (S)	No abnormality detected.
2♂ 7♀	2	700	577	No abnormality detected.	0/1 (S) 0/1 (S)	No abnormality detected.
3♂ 8♀	3	1500	1236	Hypokinesia, piloerection, ataxia (3 days).	1/1 (5 days) 0/1 (S)	Autolysed.
4♂ 9♀	4	5000	4120	Hypokinesia, piloerection (2 days).	1/1 (4 days) 0/1 (S)	Autolysed.
5♂ 10♀	5	15000	12360	Hypokinesia (½ h-4 h).	0/1 (S) 1/1 (4 h)	White fluid in stomach and upper gastro-intestinal tract. Kidneys pale and mottled.

(S) = Sacrificed at end of 14 day observation period

TABLE 6

HMX: Acute Oral Toxicity (LD50) Study in Mice
Main Study

Animal No./Sex	Dose Group	Dose Level (mg/kg)		Clinical Signs (time affected after dosing)	Deaths (time after dosing)	PM Observations
		Wet	Dry			
1-15♂ 16-20♀	1	20 ml/kg CMC		No abnormality detected.	0/5 (S) 0/5 (S)	No abnormality detected.
21-25♂ 26-30♀	2	1200.00	956	Hyperkinesia (½ h-3 days).	0/5 (S) 0/5 (S)	No abnormality detected.
31-35♂ 36-40♀	3	2040.00	1626	Hyperkinesia, piloerection, ataxia. Penis protruded, constant stream of urine. Did not blink when eyes touched. (½ h-6 days).	4/5 (5 days, 7 days) 0/5 (S)	Lungs red, gut contents fluid.
41-45♂ 46-50♀	4	3468.00	2764	Hyperkinesia, hypokinesia, ataxia, piloerection, vocalisation, sedation. Soiled coat. Eyes half shut and sunken into head, would not run or jump as normal when touched. Penis swollen and red stained with blood/urine. (1-6 days).	5/5 (2 days, 5 days, 6 days) 0/5 (S)	Stomach and gastro intestinal tract filled with white liquid. Lungs red. Gut contents fluid. Blood filled gut. Stomach wall white. Penis extended and dark red.
51-55♂ 56-60♀	5	5895.60	4699	Hyperkinesia, hypokinesia, piloerection, ataxia. (½ h-2 h).	5/5 (2 days) 5/5 (2 days)	Stomach and gastro-intestinal tract filled with white fluid. Lungs red.
61-65♂ 66-70♀	6	10022.50	7988	Hyperkinesia, hypokinesia, sedation, piloerection, (½ h-2 h).	5/5 (2 days) 5/5 (2 h, 2 days)	Stomach and upper gastro-intestinal tract filled with white fluid. Lungs red.

(S) = Sacrificed at end of 14 day observation period

TABLE 7

HMX: Acute Oral Toxicity (LD50) Study in Mice
Dose Ranging Study
Analysis of HMX

Sample No.	Dose Level (mg Wet HMX/kg)	Wet/(Dry) Nominal Concentration (mg/ml)	HMX Dried Concentration Found (mg/ml)	HMX Dried Average Concentration (mg/ml)	Deviation From Nominal (mg/ml)
116	300	15.00	12.33		
117		(12.00)	13.56	13.25	1.25
118			13.87		(10%)
119	700	35.00	30.23		
120		(28.00)	29.89	29.98	1.98
121			29.81		(7%)
122	1500	75.00	65.17		
123		(60.00)	56.16	61.24	1.24
124			62.40		(2%)
125	5000	250.00	208.00		
126		(200.00)	220.49	205.23	5.23
127			187.20		(3%)
128	15000	750.00	540.81		
129		(600.00)	644.81	584.72	15.28
130			568.54		(3%)

TABLE 8

HMX: Acute Oral Toxicity (LD50) Study in Mice
Main Study
Analysis of HMX

Sample No.	Dose Level (mg Wet HMX/kg)	Wet/(Dry) Nominal Concentration (mg/ml)	HMX Dried Concentration Found (mg/ml)	HMX Dried Average Concentration (mg/ml)	Deviation From Nominal (mg/ml)
46	1200	60.00	51.59		
47		(48.00)	45.76		
48			41.60		
49			49.37		
50			44.49	45.90	2.10
51			40.68		(4%)
52			52.00		
53			45.30		
54			42.29		
55	2040	102.00	75.81		
56		(81.60)	72.11		
57			72.11		
58			77.66		
59			89.67	79.15	2.45
60			74.88		(3%)
61			86.67		
62			84.36		
63			79.04		

TABLE 8 (continued)

Sample No.	Dose Level (mg Wet HMX/kg)	Wet/(Dry) Nominal Concentration (mg/ml)	HMX Dried Concentration Found (mg/ml)	HMX Dried Average Concentration (mg/ml)	Deviation From Nominal (mg/ml)
64	3468	173.40	124.80		
65		(138.72)	115.10		
66			133.47		
67			130.14		
68			144.22	126.88	11.84
69			128.96		(8.5%)
70			126.34		
71			119.26		
72			119.60		
73	5896	294.78	240.36		
74		(235.82)	244.56		
75			249.61		
76			242.67		
77			249.61	240.16	4.34
78			227.42		(2%)
79			244.98		
80			235.74		
81			226.49		
82	10023	501.13	419.97		
83		(400.90)	404.12		
84			459.59		
85			454.14		
86			388.28	413.34	12.44
87			366.09		(3%)
88			NO SAMPLE		
89			435.82		
90			382.73		

TABLE 9

HMX: Acute Oral Toxicity Study in Rabbits

Animal No./Sex	Dose Group	Dose Level (mg Dry HMX/kg)	Clinical Signs (days affected after dosing)	Deaths (time after dosing)	PM Observations
278♂	2	250.0	Slight hyperkinesia, miosis, laboured respiration, slight clonic convulsion, slight immobility in hind legs. (2-4).	4 days	External - nose reddened, pink staining round mouth. Kidneys - pale and surface blood vessels prominent. Lungs - irregular reddening.
279♀	2	250.0	Slight hyperkinesia, mydriasis. (2-3).	3 days	Lungs dark red in colour and black patch 4 x 5 mm on left bottom lung lobe. Spleen - light brown streak running along side.
280♂	3	428.5	Hypokinesia, hyperkinesia, slight immobility in hind legs. (2-4).	*4 days	Liver - one 5 mm diameter yellow nodule.
281♀	3	428.5	Hypokinesia, timid, mydriasis. (2-4).	4 days	Lungs - irregular reddening of all lobes.
276♂	1	2000.0	No abnormality detected.	2 days	Lungs - all lobes dark red with irregular black patches.
277♀	1	2000.0	Slight hypokinesia, facial muscles moving as if chewing or having difficulty breathing. Heart beat loud and slow, shallow quick respiration. (2-3).	3 days	Thoracic Cavity contains brown fluid and white particulate material. Lungs - left lobes brown and firm plus many white granules. Severe pleurisy as well. Kidneys mottled. Stomach ruptured - probably PM effect as no inflammatory reaction present in stomach wall.

* Sacrificed to prevent further distress

TABLE 9 (continued)

Animal No./Sex	Dose Group	Dose Level (mg/kg)	Clinical Signs (days affected after dosing)	Deaths (time after dosing)	PM Observations
282♂	4	1000.00	Hypokinesia, loose faeces, hyperkinesia. (2-4.	*4 days	No abnormality detected.
283♀	4	1000.00	Slight hypokinesia, immobile, blood staining around face, hind quarters paralysed. (2-3).	*3 days	Lungs red. Trachea blood filled.
284♂	5	50.00	Hyperkinesia, mild clonic convulsion, mydriasis, blood on nose, hypokinesia, left hind leg immobile (2-14).	14 (S)	Lungs - irregular reddening of all lobes and also occasional firm white nodules up to 10 mm diameter.
285♀	5	50.00	Hyperkinesia, mydriasis, mild clonic convulsion. Suspected broken back. (2-3).	*3 days	Kidneys - surface blood vessels prominent.
286♂	6	100.00	Hyperkinesia, mydriasis, mild clonic convulsions, timid (2-14).	14 (S)	No abnormalities detected.
287♀	6	100.00	Hyperkinesia, mydriasis, (2-3).	3 days	Kidneys - occasional 1 mm bright red depressed foci particularly at the poles. Lungs - dark red and frothy. ?PM change.

* Sacrificed to prevent further distress

(S) = Sacrified at end of 14 day observation period

TABLE 10

HMX: Acute Dermal Toxicity (LD50) Study in Rats
Body Weights - Dose Ranging Study

Animal No./Sex	Dose Level (g/kg)	Body Weight			
		On Dosing (g)	At 7 Days' Post Dosing (g)	At Sacrifice (g)	Gain/ (loss) (g)
71♂	0.5	242	251	276	34
75♀		156	178	194	38
72♂	1.0	234	256	280	46
76♀		140	156	180	40
73♂	2.0	235	254	274	39
77♀		154	168	181	27
74♂	5.0	224	248	260	36
78♀		160	178	191	31

TABLE 11

HMX: Acute Dermal Toxicity (LD50) Study in Rats (Abraded Skin)
Body Weights: Main Study

Animal No./Sex	Dose Level (g/kg)	Body Weight			
		On Dosing (g)	At 7 Days' Post Dosing (g)	At Sacrifice (g)	Gain/ (loss) (g)
89♂	5.0	243	254	262	19
90		239	245	253	14
91		246	250	254	8
92		244	248	252	8
93		236	251	260	24
94		237	239	248	11
95		221	229	239	18
96		250	241	235	(15)
97♀		155	155	155	0
98		149	146	143	(6)
99		138	144	153	15
100		157	158	161	4
101		144	142	140	(4)
102		149	144	145	(4)
103		157	157	156	(1)
104		167	166	164	(3)

TABLE 12

Acute Dermal Toxicity (LD50) Study in Rats
Body Weights - Control Group

Animal No./Sex	Body Weight			
	On Dosing (g)	At 7 Days' Post Dosing (g)	At Sacrifice (g)	Gain/ (loss) (g)
79♂	204	258	284	80
80	256	266	278	22
81	255	263	270	15
82	231	248	252	21
83	223	225	227	4
84♀	160	164	167	7
85	162	160	151	(9)
86	149	156	162	13
87	143	142	142	(1)
88	147	147	147	0

TABLE 13

HMX: Acute Dermal Toxicity (LD50) Study in Rabbits

Phase 1 (Abraded Skin)

Animal No./Sex	Dose Level	Clinical Signs (days affected after dosing)	Deaths (time after dosing)	PM Observations
13♂	1 ml (0.6 g)/kg	Blood around nose, very hyperkinetic (4-5).	6 days	No abnormality detected.
14♀	1 ml (0.6 g)/kg	Hyperkinesia (5-8).	14 (S)	No abnormality detected.
15♂	3 ml (1.8 g)/kg	Hyperkinesia, clonic convulsions (3).	*3 days	A little blood in nasal cavity otherwise NAD.
16♀	3 ml (1.8 g)/kg	Hyperkinesia, clonic convulsions, laboured breathing, vocalisation, loss of co-ordination (3).	*3 days	Brain - area of subdural haemorrhage between cerebellum and cerebrum. Kidneys - slight pitting on surface.
17♂	5 ml (3.0 g)/kg	Clonic convulsions (3).	4 days	Subdural haemorrhage between cerebellum and cerebrum.
18♀	5 ml (3.0 g)/kg	Clonic convulsions (3-4).	*4 days	Kidneys pale.

* Sacrificed in extremis

(S) = Sacrificed at end of 14 day observation period

Brain, liver, kidney, spleen, heart and lung taken from some of the animals but not all: later deatns only.

Vehicle - Physiological saline

TABLE 14

HMX: Acute Dermal Toxicity (LD50) Study in Rabbits
Phase 2 (Abraded Skin)

Animal No./ Sex	Dose Level (mg/kg)	Clinical Signs (days affected after dosing)	Deaths (time after dosing)	PM Observations
19♂	600 mg/kg (phys. saline)	Clonic convulsions, hyperkinesia, laboured breathing, subdued. (3-7).	14 (S)	No abnormality detected
20♀		Clonic convulsion, hyperkinesia, miosis, left hind leg stiff, animal reluctant to move about. (3-14).	14 (S)	No abnormality detected
21♂	1800 mg/kg (phys. saline)	Hyperkinesia, clonic convulsions, unable to move hind limbs. (2-4).	*4 days	Red staining around nose (21♂).
22♀		Hyperkinesia, mydriasis, clonic convulsions, dyspnoea, not eating, cyanosis. (2-9).	*9 days	Thoracic cavity contains creamy particulate fluid, lungs entire right side coated with creamy white material, heart coated with same material, pericardial sac fluid filled
23♂	3000 mg/kg (phys. saline)	Hyperkinesia, severe clonic convulsions (2).	*2 days	Blood around mouth
24♀		Hyperkinesia, clonic convulsions, hypokinesia. (2-4).	4 days	Red staining around mouth and nose

(S) = Sacrified at end of 14 day observation period

* = Sacrificed in extremis

TABLE 14 (continued)

Animal No./ Sex	Dose Level (mg/kg)	Clinical Signs (days affected after dosing)	Deaths time after dosing)	PM Observations
25♂	300 mg/kg (1% CMC)	Hyperkinesia, miosis, clonic convulsions. (2-5).	5 days	No abnormality detected
26♀		Hyperkinesia. (2-8).	14 (S)	No abnormality detected
27♂	600 mg/kg (1% CMC)	Clonic convulsions, hyperkinesia. (3-8).	14 (S)	No abnormality detected
28♀		Clonic convulsions, hyperkinesia. (3-4).	4 days	No abnormality detected
29♂	1900 mg/kg (1% CMC)	Hyperkinesia, clonic convulsions, blood around nose, vocalisation. (2-3).	*3 days	Red staining around nose, subdural haemorrhage, small blood clot on surface of brain
30♀		Hyperkinesia, clonic convulsions. (2-5).	*5 days	No abnormality detected
31♂	3000 mg/kg (1% CMC)	Clonic convulsions, complete loss of mobility, blood around nose, laboured breathing. (3-4).	*4 days	Red staining around nose and mouth
32♀		Clonic convulsions. (3).	4 days	Red staining around nose and mouth

(S) = Sacrificed at end of 14 day observation period

* = Sacrificed in extremis

TABLE 15

HMX: Acute Dermal Toxicity (LD50) Study in Rabbits
Phase 3 (Non-Abraded Skin)

Animal No./ Sex	Dose Level (mg/kg)	Clinical Signs (days affected after dosing)	Deaths (time after dosing)	PM Observations
39♂	600	Clonic convulsions, hyperkinesia, unable to move hind limbs, vocalisation. (3-5).	*5 days	Extensive bruising of both hind legs
40♀		Hyperkinesia. (4-8).	14 (S)	No abnormality detected
41♂	1200	Clonic convulsions. (2).	3 days	Left femur broken
42♀		Clonic convulsions. (3).	3 days	Bladder full of semi-solid substance
43♂	2400	Hyperkinesia, blood in cage. (3-4).	5 days	Blood staining around nose and mouth, spinal cord - clotted blood in cervical region, brain - clotted blood between cerebellum and cerebrum
44♀		Hyperkinesia. (3).	4 days	Bladder dark red, lungs dark red, sponge like to touch, red staining around nostrils

(S) = Sacrified at end of 14 day observation period

* = Sacrificed in extremis

Vehicle = 1% CMC

TABLE 16

HMX: Acute Dermal Toxicity (LD50) Study in Rabbits
Main Study - Non-Abraded

Animal No./ Sex	Dose Group	Dose Level	Clinical Signs (Days affected after dosing)	Deaths	P.M Observations (Day of Death After Dosing)
200-203♂ 204-207♀	14	0.28 ml/kg 168 mg/kg	Hyperkinesia, hypokinesia, miosis, clonic convulsions, aggressive and timid. (3-10).	0/4 (S) 0/4 (S)	Spleen enlarged, tracheal mucosa dark red in colour.
216-219♂ 220-223♀	16	0.62 ml/kg 372 mg/kg	Hyperkinesia, mydriasis, hypokinesia, clonic convulsions, aggressive, timid, left hind limb stiff and unable to move it, miosis. (4-14).	0/4 (S) 0/4 (S)	Tracheal mucosa dark red in colour. Lungs redder than normal. Mottled liver - fissures prominent. Spleen pale in colour. Kidneys mottled in appearance.
232-235♂ 236-239♀	18	1.36 ml/kg 816 mg/kg	Severe clonic convulsions, miosis, hyperkinesia, hypokinesia, timid, mydriasis, unable to move hind limbs, aggressive. (3-12).	3/4 1/4	232♂(5) no abnormality detected. 233♂(3) right hind leg broken. Kidneys - both show prominent surface blood vessels. 235♂(3) kidneys - numerous 2-4 mm areas of dark red depression. 239♀(5) liver - fissuring prominent. Trachea - mucosa dark red. Lungs dark red in colour. --- *Lungs irregular dark areas on all lobes. Tracheal mucosa dark red in colour. Spleen large.
248-251♂ 252-255♀	20	2.98 ml/kg 1788 mg/kg	Hyperkinesia, blood around nose, timid, clonic convusion, aggressive, vocalisation, prostration. (2-9).	4/4 4/4	248♂(3) abdominal muscle ruptured on left side. Two small 0.5 cm diameter perforations in ileum which lay in subcutaneous tissue. Left femur broken - associated muscle bruising. Lungs deep red. 249♂(3) kidneys - several 1-2 mm dark red foci. Lungs - several 1-2 mm dark red foci on all lobes. Watery discharge from mouth. 250♂(5) lungs - numerous dark areas in all lobes. Trachea - mucosa dark red in colour. 251♂(3) pink staining around nose and mouth. Liver mottled. Kidneys irregular reddening. Lungs - occasional 1-2 mm red foci on all lobes.

(S) = Sacrificed at end of 14 day observation period

* Group observation of survivors

TABLE 16 (continued)

Animal No./ Sex	Dose Group	Dose Level	Clinical Signs (days affected after dosing)	Deaths	PM Observations (Day of Death After Dosing)
248-251♂ 252-255♀ (cont).					252♀(3) pink staining around mouth. Kidneys - irregular reddening. Lungs - all lobes show many pinpoint bright red foci. Heart - left ventricle dilated. 253♀(9) blood around nose and mouth. Front tooth broken. Kidneys mottled. Lungs - edge of left lobe 10 mm dark red area. 254♀(4) blood around nose. Liver mottled. Kidneys pale and mottled. Spleen small. Lungs dark red. Subcutaneous bruising on top of skull. 255♀(3) pink staining around nose and mouth. Lungs dark red in colour. Kidneys - appear to be areas of bruising. Skull - extensive bruising on top of skull.
264-266♂ 267-269♀	22	2.98 ml/kg Control vehicle	Diarrhoea, piloerection. (4-9).	0/3 (S) 1/3 (10)*	269♀(10) no abnormality Tracheal mucosa dark red in colour. Spleen large. Liver fissuring prominent.

S - Sacrificed at end of 14 day observation period
*Group observation of survivors

TABLE 17

HMX: Acute Dermal Toxicity (LD50) Study in Rabbits
Main Study - Abraded

Animal No./ Sex	Dose Group	Dose Level	Clinical Signs (days affected after dosing)	Deaths	PM Observations (Day of Death After Dosing)
208-211♂ 212-215♀	15	0.28 ml/kg 168 mg/kg	Hyperkinesia, hypokinesia, miosis, clonic convulsions, aggressive, timid, mydriasis, vocalisation. (3-10).	0/4 (S) 0/4 (S)	Lungs - irregular dark areas on all lobes. Tracheal mucosa slightly red in colour; red froth exuding from trachea. Kidneys pale.
224-227♂ 228-231♀	17	0.62 ml/mg 372 mg/kg	Hyperkinesia, mydriasis, miosis, hypokinesia, clonic convulsions, aggressive, timid. (3-11).	0/4 (S) 0/4 (S)	Liver lobulation prominent. Kidneys mottled. Tracheal mucosa dark red in colour. Auxiliary lobe to spleen 14 mm x 8 mm.
240-243♂ 244-247♀	19	1.36 ml/kg 816 mg/kg	Severe clonic convulsions, miosis, hyperkinesia, hypokinesia, timid, mydriasis, unable to move hind limbs, aggressive. (3-14).	2/4 1/4	240♂(4) kidneys pitted surface. Lungs irregular dark areas all lobes. Skull - areas of bruising on top of head, subdural haemorrhage between cerebellum and cerebral lobes. 243♂(3) as for 247♀ 247♀(3) irregular dark red patches on all lobes of lungs.
256-259♂ 260-263♀	21	2.98 ml/kg 1788 mg/kg	Hyperkinesia, miosis, mydriasis, timid, clonic convulsion, vocalising, blood around nose and mouth, unable to move hind limbs, difficulty in breathing, cyanosis. (2-14).	4/4 3/4	256♂(4) spleen small, tracheal mucosa dark red. Bruising on top of skull. 257♂(3) no abnormality detected. 258♂(5) no abnormality detected. 259♂(3) liver fissuring obvious. Bladder very large. 261♀(3) spleen small. Lungs irregular reddening. 262♀(3) bladder grossly distended. 263♀(4) tracheal mucosa red. Lungs dark red in colour. Bruising on top of skull. --- *Tracheal mucosa dark red in colour.
270-272♂ 273-275♀	23	2.98 ml/kg Control vehicle	No abnormality detected.	0/3 (S) 0/3 (S)	Tracheal mucosa red in colour. Lungs extensive irregular reddening of all lobes. Spleen 7 x 5 mm auxiliary lobe to spleen. Spleen large with irregular edges.

S - Sacrificed at end of 14 day observation period
*Group observation of survivors

TABLE 18

HMX: Acute Dermal Toxicity (LD50) Study in Rabbits
Main Study - Death or Sacrifice Summary

Non-Abraded					Abraded				
Animal No./Sex	Dose Group	Dose Level	Deaths	How Died (Time/Days after Dosing)	Animal No./Sex	Dose	Dose Level	Deaths	How Died (Time/Days after Dosing)
200-203♂ 204-207♀	14	0.28 ml/kg 168 mg/kg	0/4 0/4	Sacrificed	208-211♂ 212-215♀	15	0.28 ml/kg 168 mg/kg	0/4 0/4	Sacrificed
216-219♂ 220-223♀	16	0.62 ml/kg 372 mg/kg	0/4 0/4	Sacrificed	224-227♂ 228-231♀	17	0.62 ml/kg 372 mg/kg	0/4 0/4	Sacrificed
232-235♂ 236-239♀	18	1.36 ml/kg 816 mg/kg	3/4 1/4	232♂(5)KIE 233♂(3)KIE 235♂(3)FDC 239♀(5)KIE Others sacrificed	240-243♂ 244-247♀	19	1.36 ml/kg 816 mg/kg	2/4 1/4	240♂(4)FDC 243♂(3)KIE 247♀(3)KIE Others sacrificed
248-251♂ 252-255♀	20	2.98 ml/kg 1788 mg/kg	4/4 4/4	248♂(3)KIE 249♂(3)FDC 250♂(5)KIE 251♂(3)KIE 252♀(3)FDC 253♀(9)KIE 254♀(4)KIE 255♀(3)FDC	256-259♂ 260-263♀	21	2.98 ml/kg 1788 mg/kg	4/4 3/4	256♂(4)KIE 257♂(3)KIE 258♂(5)KIE 259♂(3)FDC 261♀(3)KIE 262♀(3)KIE 263♀(4)KIE Others sacrificed
264-266♂ 267-269♀	22	2.98 ml/kg Control vehicle	0/3 1/3	269♀(10)FDC Others sacrified	270-272♂ 273-275♀	23	2.98 ml/kg Control vehicle	0/3 0/3	Sacrificed

Sacrificed - killed at end of 14 day observation period
FDC - found dead in cage
KIE - killed for humane reasons i.e. broken bones or damage to head etc.

TABLE 19

HMX: Acute Dermal Toxicity (LD50) Study in Rabbits

Main Study - Non-Abraded Group
Incidence of Paralysis/Broken Limbs†

Dose Group	Dose Level	Animal No./Sex	Clinical Sign†	PM Observation
14	168 mg/kg	-	Nil.	Nil.
16	372 mg/kg	223♀	Left hind limb stiff and unable to move it.	Nil.
18	816 mg/kg	232♂	Unable to move hind limbs.	No evidence of broken back.
		233♂	Broken right hind limb (*)	Broken right hind limb.
		239♀	Unable to move hind limbs. Suspected broken back (*).	No evidence of broken back.
20	1788 mg/kg	248♂	Unable to move left hind leg (*).	Left hind femur broken.
		250♂	Unable to move hind limbs. Suspected broken back (*).	No evidence of broken back.
22	Vehicle Control	-	Nil.	Nil.

(*) - Sacrificed in extremis

TABLE 20

HMX: Acute Dermal Toxicity (LD50) Study in Rabbits

Main Study - Abraded Group

Incidence of Paralysis/Broken Limbs +

Dose Group	Dose Level	Animal No./Sex	Clinical Sign+	PM Observation
15	168 mg/kg	-	Nil.	Nil.
17	372 mg/kg	-	Nil.	Nil.
19	816 mg/kg	243♂	Unable to move hind limbs, suspected broken back (*).	No evidence of broken back.
		247♀	Unable to move hind limbs (*).	Nil.
21	1788 mg/kg	257♂	Unable to move hind limbs (*).	Nil.
		261♀	Unable to move hind limbs, suspected broken back (*).	No evidence of broken back.
		263♀	Unable to move hind limbs, suspected broken back (*).	No evidence of broken back.
23	Vehicle control	-	Nil	Nil

(*) - Sacrificed in extremis

TABLE 21

HMX: Acute Dermal Toxicity (LD50) Study in Rabbits
Group Incidence of Histopathological Findings

Organ	Lesion / Dose Group/ (Dose Level mg HMX/kg)	Group Incidence									
		14 (168)		16 (372)		18 (816)		15 (168)		17 (372)	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Liver	Congestion	0/4	1/4	0/4	1/4	2/4	2/4	0/4	1/4	0/4	3/4
	Centrilobular necrosis /degeneration	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
	Increased cellularity	0/4	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
	Eosinophilic cytoplasm	0/4	0/4	1/4	0/4	3/4	1/4	0/4	0/4	0/4	0/4
Kidney	Congestion	0/4	0/4	0/4	0/4	3/4	0/4	0/4	1/4	1/4	0/4
	Chronic nephropathy	0/4	2/4	3/4	4/4	2/4	1/4	2/4	3/4	2/4	3/4
Lung	Congestion	2/4	1/4	4/4	2/4	4/4	3/4	4/4	1/4	4/4	2/4
	Alveolar haemorrhage	0/4	0/4	0/4	0/4	3/4	0/4	1/4	0/4	0/4	1/4
	Alveolar exudation	0/4	0/4	1/4	2/4	3/4	1/4	1/4	2/4	2/4	1/4
	Alveolar degeneration	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Spleen	White pulp depletion	0/4	0/4	0/4	0/4	3/4	1/4	0/4	0/4	0/4	0/4
	Red pulp depletion	0/4	0/4	1/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4

Groups 14, 16 and 18 - non-abraded
Groups 15 and 17 - abraded

TABLE 21 (continued)

Organ	Lesion \ Dose Group/ (Dose Level mg HMX/kg)	Group Incidence										Totals
		19 (816)		21 (1788)		23 (Control)		20 (1788)		22 (Control)		
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
Liver	Congestion	2/4	2/4	4/4	3/4	0/3	0/3	3/4	1/4	0/3	0/3	25/76
	Centrilobular necrosis/degeneration	0/4	0/4	0/4	0/4	0/3	0/3	1/4	1/4	0/3	0/3	3/76
	Increased cellularity	0/4	0/4	0/4	0/4	0/3	0/3	1/4	1/4	0/3	0/3	3/76
	Eosinophilic cytoplasm	2/4	2/4	3/4	4/4	0/3	0/3	4/4	2/4	0/3	0/3	22/76
Kidney	Congestion	2/4	1/4	4/4	3/4	0/3	0/3	4/4	3/4	0/3	1/3	23/76
	Chronic nephropathy	2/4	2/4	1/4	1/4	1/3	0/3	1/4	1/4	2/3	0/3	33/76
Lung	Congestion	4/4	3/4	4/4	3/4	2/3	1/3	4/4	4/4	3/3	2/3	57/76
	Alveolar haemorrhage	2/4	1/4	2/4	3/4	0/3	1/3	3/4	1/4	0/3	0/3	18/76
	Alveolar exudation	2/4	0/4	1/4	3/4	1/3	1/3	2/4	2/4	0/3	0/3	25/76
	Alveolar degeneration	0/4	1/4	1/4	3/4	0/3	0/3	3/4	1/4	0/3	0/3	10/76
Spleen	White pulp depletion	2/4	0/4	1/4	2/4	0/3	0/3	0/4	1/4	0/3	0/3	10/76
	Red pulp depletion	0/4	0/4	0/4	1/4	0/3	0/3	0/4	0/4	0/3	0/3	3/76

Groups 20 and 22 - non-abraded
Groups 19, 21 and 23 - abraded

TABLE 22

HMX: Acute Dermal Toxicitv (LD50) Study in Rabbits

Phase 2

Analysis of HMX

Sample No.	Vehicle	Nominal Concentration	Concentration Found (mg/ml)	Average Found (mg/ml)	Group Average	Deviation from Nominal
1	Physiological Saline	600 mg/ml	525.0 549.1	537.1	537 mg/ml	63 mg/ml (10.5%)
2			524.1 535.9	530.0		
3			529.3 548.6	539.0		
4			541.0 543.7	542.4		
5	High Viscosity 1% CMC	600 mg/ml	575.6 559.2	567.4	539 mg/ml	61 mg/ml (10%)
6			527.5 539.1	533.3		
7			530.4 511.7	521.1		
8			543.2 527.4	535.3		

TABLE 23

HMX: Acute Dermal Toxicity (LD50) Study in Rabbits
Main Study
Analysis of HMX

Date Prepared	Nominal Value	Sample No.	Concentration Found (%)	Mean Value (%)	Standard Deviation
(Non-abraded group) 14 October 1980	60% HMX in 1% CMC	1	66.1	63.0	± 3.7
		2	68.8		
		3	60.7		
		4	63.1		
		5	62.3		
		6	61.3		
		7	58.2		
		8	58.9		
		9	67.5		
(Abraded group) 15 October 1980	60% HMX in 1% CMC	10	65.6	64.5	± 2.0
		11	66.1		
		12	65.3		
		13	63.5		
		14	63.5		
		15	65.8		
		16	65.2		
		17	60.0		
		18	64.7		

TABLE 24

HMX: Intravenous Toxicity (LD50) Study in Rats
Dose Ranging Study

Animal No./Sex	Dose Group	Dose Level		Clinical Signs (Duration after dosing)	Deaths (time after dosing)	PM Observations
		mg/kg	ml/kg			
328♂	1	10	0.04	No abnormality detected	(S)	No abnormality detected
329♀	1	10	0.04	No abnormality detected	(S)	No abnormality detected
330♂	2	20	0.08	Hypokinesia (1 min-2 h)	(S)	No abnormality detected
331♀	2	20	0.08	Hypokinesia (1 min-2 h)	(S)	No abnormality detected
332♂	3	40	0.16	Clonic convulsions (20 sec after dosing)	1½ min	No abnormality detected
333♀	3	40	0.16	Hypokinesia (1 min)	(S)	No abnormality detected
334♂	4	80	0.32	+	2 min	No abnormality detected
335♀	4	80	0.32	Hypokinesia (1 min)	(S)	No abnormality detected
336♂	5	30	0.12	+	2 min	No abnormality detected
337♀	5	160	0.64	+	1 min	No abnormality detected

(S) = Sacrificed at end of 14 day observation period

\+ = Animals back limbs showed slight paralysis, some movement in hind quarters after 30 sec. Movements uncoordinated, slight clonic convulsion followed rapidly by sedation and coma.

TABLE 25

HMX: Intravenous Toxicity (LD50) Study in Rats

Main Study

Animal No./Sex	Dose Group	Dose Level		Clinical Signs (Duration after dosing)	Deaths (time after dosing)	PM Observations
		mg/kg	ml/kg			
338-342♂	1	Control DMSO	0.17	No abnormality detected.	0/5 (S)	No abnormality detected.
343-347♀	1	Control	0.61	No abnormality detected	0/5 (S)	No abnormality detected.
348-352♂	2	15.00	0.06	Clonic convulsion, increased irregular breathing (2 min-1 h).	1/5 (2 min)	Lungs - red foci on all lobes.
353-357♀	2	30.00	0.12	Clonic convulsion, vocalisation, irregular breathing, hyperkinesia, coma (1 min-1 h).	2/5 (2 min)	Lungs - dark red patches on all lobes.
358-362♂	3	19.50	0.078	Increased irregular breathing, hyperkinesia. (2 min-½ h).	0/5 (S)	No abnormality detected.
363-367♀	3	45.00	0.18	Increased irregular breathing, hyperkinesia, mild clonic convulsion, coma (2 min-1 h), end of tail bruised (4-14 days).	3/5 (2 min)	Lower 1/4" of tail black and hard.
368-372♂	4	25.00	0.10	Increased irregular breathing, vocalisation, hyperkinesia, clonic convulsion, paralysis of hind limbs, coma (30 sec-1½ h).	3/5 (1½-2 min)	No abnormality detected.
373-377♀	4	67.25	0.27	As for 368-372♂ (30 sec-½ h).	4/5 (1½-2 min)	No abnormality detected.
378-382♂	5	32.50	0.13	As for 25.00 mg/kg (20 sec-1½ h).	3/5 (1½-2 min)	No abnormality detected.
383-387♀	5	101.50	0.41	As for 25.00 mg/kg (1½-2 min).	5/5 (1½-2 min)	No abnormality detected.
388-392♂	6	42.50	0.17	Hypokinesia, paralysis of hind limbs, increased irregular breathing, clonic convulsions (20 sec-2min)	4/5 (1½-2 min)	No abnormality detected.
393-397♀	6	152.50	0.61	Increased irregular breathing, vocalisation, paralysis of hind limbs, hyperkinesia, coma (10 sec-1 day).	5/5 (1½ min-1 day)	No abnormality detected.

(S) = Sacrificed at end of 14 day observation period

TABLE 26

HMX: Intravenous Toxicity Study in Rabbits
Dose Ranging Findings

Animal No./Sex	Dose Group	Dose Level mg/kg	Concentration mg/ml	Clinical Signs (Duration after dosing)	Deaths (time after dosing)	PM Observations
288♂ 289♀	1	10.00	50	Clonic convulsions, vocalisation, prostrate (10-30 sec)	1/1 (30 sec) 1/1 (30 sec)	No abnormality detected.
290♂ 291♀	2	1.00	50	Hyperkinesia. (1 h-1 day)	0/1 (S) 0/1 (S)	No abnormality detected.
292♂ 293♀	3	5.00	250	No abnormality detected.	0/1 (S) 0/1 (S)	No abnormality detected.
294♂ 295♀	4	10.00	250	Hyperkinesia, aggressive. (1-4 days)	0/1 (S) 0/1 (S)	No abnormality detected.
298♂ 299♀	6	20.00	250	Clonic convulsions, hyperkinetic, vocalisation, immobility (30 sec-2 min).	1/1 (2 min) 1/1 (2 min)	Post mortem not carried out.
300♂ 301♀	7	15.00	250	Clonic convulsions, vocalisation, dyspnoea (3-5 min).	1/1 (5 min) 1/1 (5 min)	Post mortem not carried out.
302♂ 303♀	8	12.50	250	Clonic convulsions, hyperkinetic, slight epistaxis (2-5 days).	1/1 (3 min) 0/1 (S)	No abnormality detected.
296♂ 297♀	5	DMSO Control 0.20 ml/kg		No abnormality detected.	0/1 (S) 0/1 (S)	Liver - 7 mm x 3 mm white patch on right hand lobe at posterior edge.

(S) = Sacrificed

TABLE 27

HMX: Primary Skin Irritation Test in Rabbits

Patch Arrangement and Reaction Scores

	Rabbit No./ Sex	Patch Arrangement*	Skin Score	
			Erythema & Eschar	Oedema
24 HOURS	1♂	A A / B B	0 0 / 1 1	0 0 / 1 1
	2♀	B B / A A	2 2 ↕ / 0 2	2 2 ↕ / 0 2
	3♂	A B / A B	1 2d / 1 2d	0 4 / 0 4
	4♀	B A / B A	3d 0 / 3d 0	4 0 / 4 0
	5♂	A A / B B	0 0 / 2 2	0 0 / 3 3
	6♀	B B / A A	↕ 3d 3d ↕ / 2 2	↕ 4 4 ↕ / 1 1
72 HOURS	1♂	A A / B B	0 0 / 2 2	0 0 / 2 2
	2♀	B B / A A	3 3 / 1 2d	2 2 / 0 1
	3♂	A B / A B	0 3d / 0 3d	0 4 / 0 4
	4♀	B A / B A	4d 0 / 4d 0	3 0 / 3 0
	5♂	A A / B B	0 0 / 3d 3d	0 0 / 3 3
	6♀	B B / A A	4d 4d / 0 0	3 3 / 0 0

* Top right and bottom left abraded

TEST MATERIALS

A = 60% w/w HMX in physiological saline

B = 10% aqueous sodium lauryl sulphate

d = dry

↕ = test areas merged

TABLE 28

HMX: Primary Skin Irritation in Rabbits

Mean Scores and Primary Irritation Scores

	Exposure Time (Hours)	Reaction Score	
		A	B
Erythema & Eschar Formation			
Intact skin	24	0.83	2.17
Intact skin	72	0.33	3.17
Abraded skin	24	0.50	2.17
Abraded skin	72	0.17	3.17
Sub total		1.83	10.68
Oedema Formation			
Intact skin	24	0.50	3.00
Intact skin	72	0.17	2.83
Abraded skin	24	0.17	3.00
Abraded skin	72	0.00	2.83
Sub total		0.84	11.66
Total		2.67	22.34
Primary Irritation Score		0.67	5.59

A 60% w/w HMX in physiological saline

B 10% aqueous sodium lauryl sulphate

The reaction score is the average value of results from the 6 test animals. The primary irritation score is obtained in the following way: values for erythema and eschar formation at 24 h and 72 h (4 values) are added to the values for oedema at 24 h and 72 h (4 values) and the resulting figure is divided by 4.

A material which has a score of 5 or more is a primary irritant under the definitions in the Code of Federal Regulations of the U.S.A.

TABLE 29

HMX: Eye Irritation in Rabbits
60% HMX w/w in Distilled Water

Rabbit No. and Sex	Scores							
	7♂	8♀	9♂	10♀	11♂	12♀	Total	Mean
Zero Time Total	0	0	0	0	0	0	0	0
1 Hour	*	*	*	*	*	*		
Cornea	0	0	0	0	0	0		
Iris	0	0	0	0	0	0		
Conjunctivae								
redness	1	0	0	0	0	1		
chemosis	0	0	0	0	0	0		
discharge	0	0	0	0	0	0		
Total	1	0	0	0	0	1	2	0.33
24 Hours						*		
Cornea	0	0	0	0	0	0		
Iris	0	0	0	0	0	0		
Conjunctivae								
redness	0	0	0	0	0	0		
chemosis	0	0	0	0	0	0		
discharge	0	0	0	0	0	0		
Total	0	0	0	0	0	0	0	0
48 Hours								
Cornea	0	0	0	0	0	0		
Iris	0	0	0	0	0	0		
Conjunctivae								
redness	0	0	0	0	0	0		
chemosis	0	0	0	0	0	0		
discharge	0	0	0	0	0	0		
Total	0	0	0	0	0	0	0	0
72 Hours								
Cornea	0	0	0	0	0	0		
Iris	0	0	0	0	0	0		
Conjunctivae								
redness	0	0	0	0	0	0		
chemosis	0	0	0	0	0	0		
discharge	0	0	0	0	0	0		
Total	0	0	0	0	0	0	0	0
7 Day								
Cornea	0	0	0	0	0	0		
Iris	0	0	0	0	0	0		
Conjunctivae								
redness	0	1	0	0	0	0		
chemosis	0	0	0	0	0	0		
discharge	0	0	0	0	0	0		
Total	0	1	0	0	0	0	1	0.17

* Test material in corner of eye

TABLE 30

HMX: Eye Irritation in Rabbits
Dried HMX

Rabbit No. and Sex	Scores							
	33♂	34♀	35♂	36♀	37♂	38♀	Total	Mean
Zero Time Total	0	0	0	0	0	0	0	0
1 Hour	*	*	*	*	*	*		
Cornea	0	0	0	0	0	0		
Iris	0	0	0	0	0	0		
Conjunctivae								
redness	1	1	1	1	1	1		
chemosis	½	0	0	0	½	0		
discharge	0	0	0	0	0	0		
Total	1½	1	1	1	1½	1	7.00	1.17
24 Hours								
Cornea	0	0	0	0	0	0		
Iris	0	0	0	0	0	0		
Conjunctivae								
redness	1	½	0	0	½	1		
chemosis	0	0	0	0	0	0		
discharge	0	0	0	0	0	0		
Total	1	½	0	0	½	1	3.00	0.50
48 Hours								
Cornea	0	0	0	0	0	0		
Iris	0	0	0	0	0	0		
Conjunctivae								
redness	0	0	0	0	0	0		
chemosis	0	0	0	0	0	0		
discharge	0	0	0	0	0	0		
Total	0	0	0	0	0	0	0	0
72 Hours								
Cornea	0	0	0	0	0	0		
Iris	0	0	0	0	0	0		
Conjunctivae								
redness	0	0	0	0	0	0		
chemosis	0	0	0	0	0	0		
discharge	0	0	0	0	0	0		
Total	0	0	0	0	0	0	0	0
7 Day								
Cornea	0	0	0	0	0	0		
Iris	0	0	0	0	0	0		
Conjunctivae								
redness	0	0	0	0	0	0		
chemosis	0	0	0	0	0	0		
discharge	0	0	0	0	0	0		
Total	0	0	0	0	0	0	0	0

* Test material in corner of eye

TABLE 31

HMX: Sensitisation Potential in Guinea Pigs
Challenge Results - Groups I and IV

HMX Concentration w/v in paraffin oil)	Group/Cage/Animal No.																																
	Group I																									Group IV								
	1					2					3					4					5					16					17			
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
60%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 32

p-phenylenediamine: Sensitisation Potential in Guinea Pigs
Challenge Results - Groups II and V

PPDA Concentration (w/v in distilled water	Group/Cage/Animal No.																															
	Group II																									Group V							
	6					7					8					9					10					18					19				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
2%	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	0	0	0	0	0	0	0	0	0	0

TABLE 33

Sodium Lauryl Sulphate: Sensitisation Potential in Guinea Pigs
Challenge Results - Groups III and VI

SLS Concentration (w/w in distilled water)	Group/Cage/Animal No.																																	
	Group III																										Group VI									
	11					12					13					14					15					20					21				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
0.5%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

APPENDIX 1

HMX: Acute Dermal Toxicity (LD50) Study in Rabbits
Dose Ranging - Phase 1
Gross Pathology Findings in Individual Animals

Abbreviations used:

TK = Terminal kill
FD = Found dead
KIE = Killed in extremis

APPENDIX 1 (continued)

Project No: 415669 AC **Group:** 1
Animal No: 13 **Sex:** ♂

Time on Study	Death
6 days	FD

Clinical History	Sample	Histopathology	Number of Sections Examined	
				HE
Slightly hyperkinetic on Day 4. Blood around nose, very hyperkinetic on Day 5. Found dead in cage on Day 6		Not performed.		
Necropsy Findings				
NAD				

Project No: 415669 AC **Group:** 1
Animal No: 14 **Sex:** ♀

Time on Study	Death
2 weeks	TK

Clinical History	Sample	Histopathology	Number of Sections Examined	HE
Very hyperkinetic on Days 5 and 6. Hyperkinetic on Day 7. Slightly hyperkinetic on Day 8.		Not performed.		

Necropsy Findings
NAD

APPENDIX 1 (continued)

Project No: 415669 AC **Group:** 2
Animal No: 15 **Sex:** ♂

Time on Study	Death
3 days	KIE

Clinical History	Sample	Histopathology	Number of Sections Examined: HE
Very hyperkinetic, clonic convulsions, sacrificed on Day 3.		Not performed.	

Necropsy Findings
Face, neck and front paws covered in blood. A little blood in the nasal cavity.

Project No: 415669 AC **Group:** 2
Animal No: 16 **Sex:** ♀

Time on Study	Death
3 days	KIE

Clinical History	Sample	Histopathology	Number of Sections Examined: HE
Hyperkinetic, blood around nose, laboured breathing, clonic convulsions, loss of co-ordination, vocalisation, sacrificed on Day 3.		Not performed.	

Necropsy Findings

Kidneys Small depressions in surface.

Brain Area of subdural haemorrhage between cerebellum and cerebrum.

Blood on face and forepaws.

APPENDIX 1 (continued)

Project No: 415669 AC **Group:** 3
Animal No: 17 **Sex:** ♂

Time on Study	Death
4 days	FD

Clinical History	Sample	Histopathology	Number of Sections Examined: HE
Clonic convulsions on Day 3. Found Dead in cage on Day 4.		Not performed.	
Necropsy Findings			
Brain Subdural haemorrhage between cerebellum and cerebrum.			

AD-A171 598 HMX: ACUTE TOXICITY TESTS IN LABORATORY ANIMALS(U) 2/3
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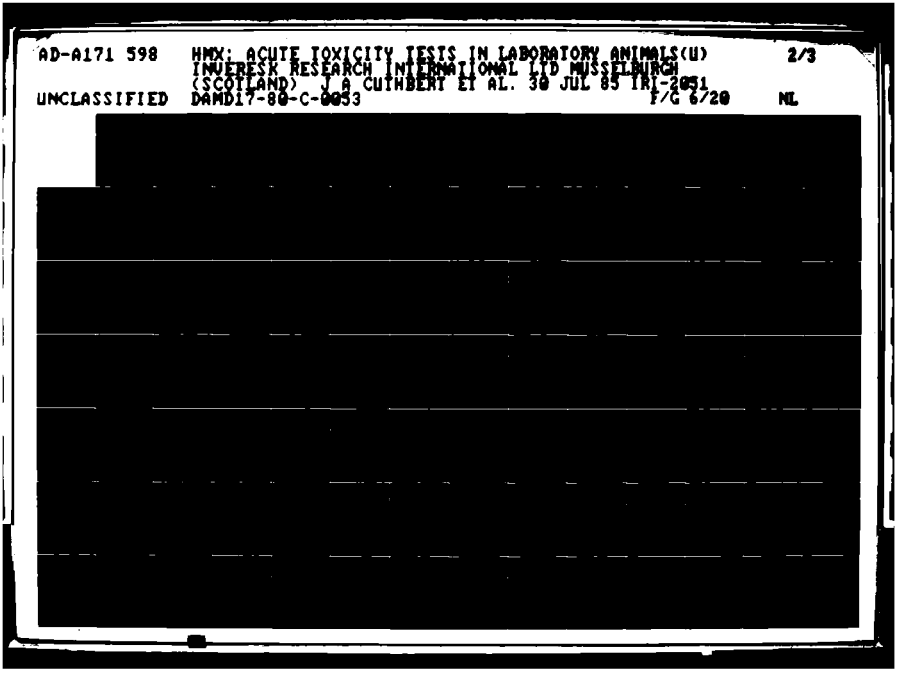

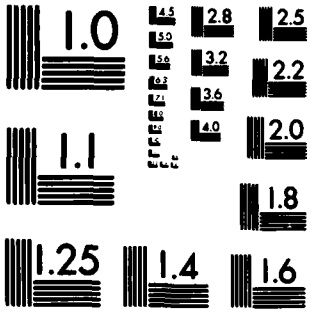

APPENDIX I (continued)

Project No: 415669 AC **Group:** 3
Animal No: 18 **Sex:** ♀

Time on Study	Death
4 days	KIE

Clinical History	Sample	Histopathology	Number of Sections Examined	HE
Clonic convulsions on Day 3. Severe clonic convulsions, blood around nose, sacrificed on Day 4.		Not performed.		

Necropsy Findings

Kidneys Very pale.

Blood around nose.

APPENDIX 2

HMX: Acute Dermal Toxicity (LD_{50}) Study in Rabbits
Dose Ranging - Phase 2 (Section i)
Gross Pathology Findings in Individual Animals

Abbreviations Used

TK = Terminal Kill
FD = Found dead
KIE = Killed in extremis

APPENDIX 2 (continued)

Project No: 415669 AC **Group:** 4
Animal No: 19 **Sex:** ♀

Time on Study	Death
2 weeks	TK

Clinical History	Sample	Histopathology	Number of Sections Examined: HE
Clonic convulsions on Day 3. Hyperkinetic, laboured breathing on Day 4. Hyperkinetic on Day 5. Very subdued on Days 6 and 7.		Not performed.	

Necropsy Findings
NAD

APPENDIX 2 (continued)

Project No: 415669 AC **Group:** 4
Animal No: 20 **Sex:** ♀

Time on Study	Death
2 weeks	TK

Clinical History	Sample	Histopathology	Number of Sections Examined: HE
Clonic convulsions, hyperkinetic, miosis on Day 3. Miosis, hyperkinetic on Day 4. Slight hyperkinesia on Day 5. Left hind leg stiff, animal reluctant to move on Day 8.		Not performed.	

Necropsy Findings
NAD

APPENDIX 2 (continued)

Project No: 415669 AC **Group:**
Animal No: 21 **Sex:** ♀

Time on Study	Death
4 days	KIE

Clinical History

Hyperkinetic on Day 2. Clonic convulsions on Day 3. Severe clonic convulsions, unable to move hind limbs - sacrificed on Day 4.

Necropsy Findings

Blood around nose.

Sample	Histopathology	Number of Sections Examined: HE
	Not performed.	

APPENDIX 2 (continued)

Project No: 415669 AC **Group:** 5
Animal No: 22 **Sex:** ♀

Time on Study	Death
9 days	KIE

Clinical History

Hyperkinetic on Day 2. Very hyperkinetic mydriasis on Day 3. Clonic convulsions, hyperkinetic on Day 4. Hyperkinetic, dyspnoea on Days 5-7. Hyperkinetic, dyspnoea, not eating on Day 8. Hyperkinetic, dyspnoea, not eating and cyanosed - sacrificed on Day 9.

Necropsy Findings

Lungs Entire right side coated with creamy white material. Similar material exuded from cut surfaces.

Heart Pericardium coated with creamy material. Pericardial sac filled with fluid.

Creamy particulate fluid present in thoracic cavity.

Sample	Histopathology	Number of Sections Examined: HE
	Not performed.	

APPENDIX 2 (continued)

Project No: 415669 AC **Group:**
Animal No: 23 **Sex:** ♂

Time on Study	Death
2 days	KIE

Clinical History	Sample	Histopathology	Number of Sections Examined	HE
Hyperkinetic, severe clonic convulsions - sacrificed on Day 2		Not performed.		

Necropsy Findings
Blood around mouth.

APPENDIX 2 (continued)

Project No: 415669 AC **Group:** 6
Animal No: 24 **Sex:** ♀

Time on Study	Death
4 days	FD

Clinical History	Sample	Histopathology	Number of Sections Examined: HE
Hyperkinetic, clonic convulsions on Days 2 and 3. Found dead in cage on Day 4.		Not performed.	
Necropsy Findings			
Blood on face.			

APPENDIX 3

HMX: Acute Dermal Toxicity (LD50) Study in Rabbits
Dose Ranging - Phase 2 (Section ii)
Gross Pathology and Histopathological Findings
in Individual Animals

Abbreviations used:

FD = Found dead
KIE = Killed in extremis
TK = Terminal Kill
BALT = Bronchus Associated Lymphoid Tissue
VALT = Vascular Associated Lymphoid Tissue

APPENDIX 3 (continued)

Project No: 415669 AC **Group:** 7
Animal No: 25 **Sex:** ♂

Time on Study	Death
4 days	FD

Clinical History

Hyperkinetic on Day 2. Hyperkinetic miosis on Day 3. Clonic convulsions on Day 4. Found dead in cage on Day 5.

Necropsy Findings

NAD

Sample	Histopathology
Liver	Periportal foci of lymphocytes and macrophages.
Kidney	Small interstitial foci of lymphocytes.
Heart	One small focus of lymphocytes in septum.
Lung	BALT and VALT present.
Brain	Small granuloma in cerebrum, hippocampus and mid-brain. Small perivascular lymphoid cuffing in all parts of brain.

Number of Sections Examined	HE
Liver	1
Kidney	2
Heart	2
Spleen	1
Lung	1
Brain	3
Spinal Cord	1
Skin	2

APPENDIX 3 (continued)

Project No: 415669 AC **Group:** 7
Animal No: 26 **Sex:** ♀

Time on Study	Death
2 weeks	TK

Clinical History

Hyperkinetic on Days 2-8.

Necropsy Findings

NAD

Sample	Histopathology
Liver	Periportal foci of lymphocytes.
Kidney	Small interstitial foci of lymphocytes.
Lungs	BALT and VALT present.
Brain	Two granulomata in cerebrum. Very mild perivascular lymphoid cuffing. Granus and Giemsa stains failed to identify any micro organisms in these lesions.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	3
Spinal Cord	1
Skin	2

APPENDIX 3 (continued)

Project No: 415669 AC Group: 8
Animal No: 27 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History
Clonic convulsions, hyperkinetic on Day 3. Hyperkinetic on Days 4-8.

Necropsy Findings
NAD

Sample	Histopathology
Liver	Hepatocyte cytoplasm very "lacy" in appearance. A few periportal lymphocytes.
Kidneys	One very small focus of lymphocytes in interstitium.
Lung	BALT present.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	3
Spinal Cord	1
Skin	2

APPENDIX 3 (continued)

Project No: 415669 AC **Group:** 8
Animal No: 28 **Sex:** ♀

Time on Study	Death
4 days	FD

Clinical History

Hyperkinetic, clonic convulsions on Day 3. Found dead in cage on Day 4.

Necropsy Findings

NAD

Sample	Histopathology
Liver	Moderately excessive periportal infiltration by lymphocytes and macrophages. Periportal hepatocytes pale.
Kidneys	Small foci of lymphocytes in interstitium.
Brain	Granulomata in cerebrum, hippocampus and medulla. Perivascular lymphoid cuffing also present in these areas plus in the meringes. Gram positive protozoa identified in one hippocampal granuloma. These organisms stained navy blue with Giemsa stain.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	1
Spleen	1
Lung	1
Brain	3
Spinal Cord	1
Skin	2

APPENDIX 3 (continued)

Project No: 415669 AC **Group:** 9
Animal No: 29 **Sex:** ♂

Time on Study	Death
3 days	KIE

Clinical History

Very hyperkinetic on Day 2. Clonic convulsions, vocalisation - sacrificed on Day 3.

Necropsy Findings

Brain

Sub-dural haemorrhage with small blood clot on surface of brain.

Blood around nose and mouth.

Sample	Histopathology
Liver	A few periportal lymphoid cells.
Lung	BALT present. Alveoli very congested.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	1
Spleen	1
Lung	1
Brain	3
Spinal Cord	1
Skin	2

APPENDIX 3 (continued)

Project No: 415669 AC **Group:** 9
Animal No: 30 **Sex:** ♀

Time on Study	Death
4 days	KIE

Clinical History

Hyperkinetic on Day 2. Clonic convulsions on Days 3 and 4. Severe clonic convulsions - sacrificed on Day 5.

Necropsy Findings

NAD

Sample	Histopathology
Liver	Mild periportal infiltration of lymphocytes and macrophages.
Lung	BALT present.
Brain	Small area of meningeal haemorrhage in cerebrum.
Skin	One small pustule.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	3
Spinal Cord	1
Skin	2

APPENDIX 3 (continued)

Project No: 415669 AC **Group:** 10
Animal No: 31 **Sex:** ♂

Time on Study	Death
4 days	KIE

Clinical History

Clonic convulsions on Day 3. Clonic convulsions, complete loss of mobility and laboured breathing - sacrificed on Day 4.

Necropsy Findings

Red staining around nose.

Sample	Histopathology
Liver	Mild vacuolation of all hepatocytes.
Lungs	Marked alveolar congestion. BALT present.
Brain	Meningeal haemorrhage in cerebrum and cerebellum.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	3
Spinal Cord	1
Skin	2

APPENDIX 3 (continued)

Project No: 415669 AC **Group:** 10
Animal No: 32 **Sex:** ♀

Time on Study	Death
4 days	FD

Clinical History

Severe clonic convulsions on Day 3. Found dead in cage on Day 4.

Necropsy Findings

Blood around nose and mouth.

Sample	Histopathology
Liver	A few small periportal foci of lymphocytes and macrophages. Centrilobular hepatocytes had a "lacy" appearance.
Lung	BALT present.
Brain	Small perivascular lymphoid cuff and small area of gliosis in mid brain.

Number of Sections Examined	ME
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	3
Spinal Cord	1
Skin	2

APPENDIX 4

HMX: Acute Dermal Toxicity (LD50) Study in Rabbits
Dose Ranging (Phase 3)
Gross Pathology and Histopathological Findings
in Individual Animals

Abbreviations Used

FD = Found Dead
KIE = Killed in extremis
TK = Terminal Kill
BALT = Bronchus Associated Lymphoid Tissue

Project No: 415669 AC **Group:** 11
Animal No: 39 **Sex:** ♂

Time on Study	Death
4 days	KIE

Clinical History

Clonic convulsions on Day 3. Hyperkinetic on Day 4. Very hyperkinetic, vocalisation, unable to move hind limbs - sacrificed on Day 5.

Necropsy Findings

Extensive bruising of both hind legs.

Sample	Histopathology
Liver	Centrilobular hepatocytes had a "lacy" appearance.
Lung	BALT present plus mild acute bronchitis.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	3
Spinal Cord	1
Skin	2

APPENDIX 4 (continued)

Project No: 415669 AC **Group:** 11
Animal No: 40 **Sex:** ♀

Time on Study	Death
2 weeks	TK

Clinical History

Hyperkinetic on Days 4-8.

Necropsy Findings

NAD

Sample	Histopathology
Liver	Periportal infiltration by macrophages and a few fibroblasts.
Lung	BALT present.

Number of Sections Examined	ME
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	3
Spinal Cord	1
Skin	2

APPENDIX 4 (continued)

Project No: 415669 AC **Group:** 12
Animal No: 41 **Sex:** ♂

Time on Study	Death
3 days	FD

Clinical History

Clonic convulsions on Day 2. Found dead in cage on Day 3.

Necropsy Findings

Left femur

Fractured.

Sample	Histopathology
Liver	A few periportal macrophages and fibroblasts.
Lung	BALT present plus mild acute bronchitis.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	3
Spinal Cord	1
Skin	2

APPENDIX 4 (continued)

Project No: 415669 AC **Group:** 12
Animal No: 42 **Sex:** ♀

Time on Study	Death
3 days	FD

Clinical History

Clonic convulsions - died on Day 3.

Necropsy Findings

Bladder

Full of semi-solid white matter.

Sample	Histopathology
Lung	BALT present.

Number of Sections Examined	HE
Liver	1
Kidney	2
Heart	2
Spleen	1
Lung	1
Brain	3
Spinal Cord	1
Skin	2
Bladder	2

Project No: 415669 AC **Group:** 13
Animal No: 43 **Sex:** ♂

Time on Study	Death
5 days	FD

Clinical History

Hyperkinetic on Day 3. Blood in cage, very hyperkinetic on Day 4. Found dead in cage on Day 5.

Necropsy Findings

Brain

Clotted blood between cerebellum and cerebrum.

Spinal Cord

Clotted blood in cervical region.

Blood around nose and mouth.

Sample	Histopathology
Lung	A very little BALT present.
Brain	NAD.
Spinal Cord	Not examined.

Number of Sections Examined	ME
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	3
Spinal Cord	0
Skin	2

APPENDIX 4 (continued)

Project No: 415669 AC **Group:** 13
Animal No: 44 **Sex:** ♀

Time on Study	Death
4 days	FD

Clinical History

Hyperkinetic on Day 3. Found dead in cage on Day 4.

Necropsy Findings

Bladder

Dark red and contained white milky substance.

Blood around nostrils.

Sample	Histopathology
Lung	BALT present.

Number of Sections Examined	HE
Liver	1
Kidney	2
Heart	2
Spleen	1
Lung	1
Brain	1
Spinal Cord	1
Skin	2

APPENDIX 5

HMX: Acute Dermal Toxicity (LD50) Study in Rabbits
Main Study
Gross Pathology and Histopathological Findings in
Individual Animals

ABBREVIATIONS USED

KIE = Killed in extremis
FD = Found Dead
TK = Terminal Kill
CN = Chronic Nephropathy

APPENDIX 5 (continued)

Project No: 415669 AC Group: 14
Animal No: 200 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History

Hypokinetic on Day 4. Hyperkinetic on Days 5 and 6.

Necropsy Findings

NAD

Sample	Histopathology	Number of Sections Examined	HE
	NAD	Liver	1
		Kidney	1
		Heart	2
		Spleen	1
		Lung	1
		Brain	2
		Spinal Cord	1
		Cerebellum	1
		Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 14
Animal No: 201 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History

Hypokinetic on Day 4. Mild clonic convulsion on Day 5. Very hyperkinetic, aggressive on Days 6 and 7. Hyperkinetic on Days 8-10.

Necropsy Findings

NAD

Sample	Histopathology
Lungs	Congestion. Focus perivascular lymphocytes and polymorphs.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 14
Animal No: 202 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History

Mild clonic convulsion on Day 4. Hypokinetic, mild clonic convulsion, hyperkinetic on Day 5. Hyperkinetic on Day 6. Hypokinetic on Day 8. Hyperkinetic on Days 9 and 10.

Necropsy Findings

NAD

Sample	Histopathology
	NAD

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 14
Animal No: 203 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History	Sample	Histopathology	Number of Sections Examined	HE
Hypokinetic on Days 4 and 5. Hypokinetic, miosis on Day 6. Hypokinetic on Day 7.	Lung	Congested.	Liver	1
			Kidney	1
			Heart	2
			Spleen	1
			Lung	5
			Brain	1
			Spinal Cord	1
			Cerebellum	1
			Skin	2

Necropsy Findings
NAD

APPENDIX 5 (continued)

Project No: 415669 AC Group: 14
Animal No: 204 Sex: ♀

Time on Study	Death
2 weeks	TK

Clinical History	Sample	Histopathology	Number of Sections Examined	
				HE
Hypokinetic on Days 4 and 5. Hyper-kinetic on Day 6.		NAD	Liver	1
			Kidney	1
			Heart	2
			Spleen	1
			Lung	1
			Brain	2
			Spinal Cord	1
			Cerebellum	1
			Skin	2
Necropsy Findings				
NAD				

APPENDIX 5 (continued)

Project No: 415669 AC Group: 14
Animal No: 205 Sex: ♀

Time on Study	Death
2 weeks	TK

Clinical History
Hyperkinetic on Day 3. Hypokinetic on Day 4. Hyperkinetic on Day 5.

Necropsy Findings
NAD

Sample	Histopathology
Kidneys	Area tubular dilation, some tubular and glomerular atrophy and one focus lymphocyte infiltration.

Number of Sections Examined	
	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 14
Animal No: 206 Sex: ♀

Time on Study	Death
2 weeks	TK

Clinical History
Hypokinetic on Day 4. Clonic convulsion, hyperkinetic on Day 5. Hyperkinetic on Days 6, 7 and 8. Hypokinetic, timid on Days 9 and 10.

Necropsy Findings
Spleen enlarged.

Sample	Histopathology
Spleen	Sinuses dilated. Some extra medullary haemopoiesis.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 14
Animal No: 207 Sex: ♀

Time on Study	Death
2 weeks	TK

Clinical History

Hypokinetic on Days 4, 5 and 6. Hypokinetic on Day 9.

Necropsy Findings

Spleen enlarged.

Tracheal mucosa dark red.

Sample	Histopathology
Kidneys	Area tubular dilation, tubular and glomerular atrophy.
Liver	Foci periportal congestion. Some bile duct hyperplasia. Extra medullary haemopoiesis. Lymphocyte infiltration.
Lungs	Foci congestion, increased interstitial cellularity and lymphocyte infiltration.
Spleen	NAD
Trachea	Congested.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 15
Animal No: 208 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History	Sample	Histopathology
Hyperkinetic on Days 3, 4 and 5.	Lungs	Foci congested.
	Trachea	Congested.

Necropsy Findings
Tracheal mucosa dark red.
Lungs mottled.

Number of Sections Examined	
	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

Project No: 415669 AC Group: 15
Animal No: 209 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History

Hypokinetic on Day 3. Hyperkinetic on Days 4 and 5. Hyperkinetic, clonic convulsion on Day 6. Slightly hyperkinetic, timid on Day 7.

Necropsy Findings

Tracheal mucosa dark red.

Sample	Histopathology
Kidneys	Small foci tubular dilation.
Lungs	Foci chronic interstitial reaction, foci congestion.
Trachea	Congested.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 15
Animal No: 210 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History

Hyperkinetic on Days 3, 4 and 5.

Necropsy Findings

Lungs - irregular dark areas on all lobes.

Trachea - mucosa dark red.

Sample	Histopathology
Lungs	Area of congestion/haemorrhage.
Trachea	Congested.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 15
Animal No: 211 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History

NAD.

Necropsy Findings

Lungs - irregular dark areas on all lobes.

Trachea - mucosa red and red froth in trachea.

Kidneys - pale.

Sample	Histopathology
Kidneys	Areas tubular dilation, tubular and glomerular atrophy and fibrosis, particularly in sub capsular area.
Lungs	Areas of congestion, alveolar exudation.
Trachea	Congested. Lymphocytes, plasma cells in lamina propria; polymorphs in lamina propria and migrating through epithelium.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 15
Animal No: 212 Sex: ♀

Time on Study	Death
2 weeks	TK

Clinical History
Miosis, hyperkinetic on Day 3. Hyperkinetic, timid on Days 4 and 5. Hyperkinetic on Days 6, 7 and 8.

Necropsy Findings
NAD

Sample	Histopathology
Kidneys	Foci tubular atrophy.
Lungs	Alveolar exudation.

Number of Sections Examined	
	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 15
Animal No: 213 Sex: ♀

Time on Study	Death
2 weeks	TK

Clinical History	Sample	Histopathology	Number of Sections Examined	HE
Hypokinetic, hyperkinetic on Day 3. Hyperkinetic, mydriasis on Day 4. Hyperkinetic on Day 5.	Kidneys	Small foci tubular atrophy and tubular dilation.	Liver	1
			Kidney	1
			Heart	1
			Spleen	1
			Lung	1
			Brain	2
			Spinal Cord	1
			Cerebellum	1
			Skin	2

Necropsy Findings
NAD

Project No: 415669 AC Group: 15
Animal No: 214 Sex: ♀

Time on Study	Death
2 weeks	TK

Clinical History	Sample	Histopathology	Number of Sections Examined	
				HE
Hyperkinetic on Day 3.	Kidneys	Area tubular dilation, atrophy and fibrosis. Increased cellularity in glomeruli.	Liver	1
			Kidney	1
			Heart	1
			Spleen	1
			Lung	1
			Brain	1
			Spinal Cord	1
			Cerebellum	1
			Skin	1
			Trachea	1
Necropsy Findings				
Tracheal mucosa slightly red.				

APPENDIX 5 (continued)

Project No: 415669 AC Group: 15
Animal No: 215 Sex: ♀

Time on Study	Death
2 weeks	TK

Clinical History

Hyperkinetic on Day 3. Hyperkinetic, miosis on Day 4. Hyperkinetic on Days 5 and 6. Hyperkinetic, timid on Day 7. Hyperkinetic, very aggressive, vocalisation on Day 8. Hyperkinetic on Days 9 and 10.

Necropsy Findings

Lungs - irregular dark areas on all lobes.

Sample	Histopathology
Liver	Congested. Extra medullary haemopoiesis.
Kidneys	Congested.
Lungs	Congested. Foci polymorphs round blood vessels. Alveolar exudation.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 16
Animal No: 216 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History

Hypokinetic on Days 4 and 5. Hyperkinetic, clonic convulsions on Day 6. Hyperkinetic on Days 7, 8, 9 and 10.

Necropsy Findings

Kidneys - mottled in appearance with several dark areas over surface.

Lungs - mottled.

Tracheal mucosa dark red.

Sample	Histopathology
Kidneys	Areas tubular and glomerular atrophy and lymphocyte infiltration.
Lungs	Congested. Alveolar exudation and degeneration.
Brain	Foci perivascular cuffing. Nosematosis.
Trachea	Congested.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 16
Animal No: 217 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History

Mydriasis, hyperkinetic on Day 3. Hypokinetic on Day 4. Hypokinetic, hyperkinetic on Day 5. Hypokinetic on Day 6. Hyperkinetic on Day 7. Hyperkinetic, slight mydriasis on Day 8. Hyperkinetic on Days 9, 10 and 11.

Necropsy Findings

Lungs slightly motted.

Sample	Histopathology
Liver	Cytoplasm fairly eosinophilic.
Kidneys	Foci tubular dilation and tubular atrophy.
Lungs	Congested. Interstitial polymorphs.

Number of Sections Examined	
	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 16
Animal No: 218 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History

Miosis on Day 3. Hypokinetic on Day 4. Hyperkinetic on Day 5. Hypokinetic on Days 6, 7, 8, 9, 10, 11.

Necropsy Findings

Spleen pale.

Tracheal mucosa dark red.

Sample	Histopathology
Kidneys	Small foci tubular dilation and some tubular atrophy. Some lymphocytic infiltration.
Spleen	Red pulp depletion.
Trachea	Congested.
Lungs	Slight congestion.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 16
Animal No: 219 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History

Miosis, hyperkinetic, clonic convulsions on Day 3. Hypokinetic on Day 4. Hypokinetic, timid on Days 5, 6, 7, 8, 9 and 10. Hypokinetic on Day 11.

Necropsy Findings

Tracheal mucosa dark red.

Sample	Histopathology
Lungs	Slight congestion.
Brain	Foci perivascular cuffing.
Trachea	Congested.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 16
Animal No: 220 Sex: ♀

Time on Study	Death
2 weeks	TK

Clinical History

Hyperkinetic on Day 3. Hypokinetic on Days 4 and 5. Hypokinetic, timid on Day 6. Hypokinetic, slight clonic convulsion on Day 7. Slight clonic convulsion on Days 8 and 9. Hyperkinetic on Day 10.

Necropsy Findings

Liver - fissures prominent.

Tracheal mucosa red.

Sample	Histopathology
Kidneys	Foci tubular dilation, tubular atrophy.
Lungs	Congested. Alveolar exudation.
Liver	NAD.
Skin	Small foci inflammation in dermis.
Trachea	Congested.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	1
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 16
Animal No: 221 Sex: ♀

Time on Study	Death
2 weeks	TK

Clinical History

Slightly hyperkinetic on Day 3. Hypokinetic, miosis on Day 4. Hypokinetic on Day 5. Hyperkinetic on Day 6. Hyperkinetic, hypokinetic on Day 7. Hypokinetic on Day 8.

Necropsy Findings

Lungs redder than usual.

Tracheal mucosa red.

Sample	Histopathology
Kidnevs	Foci tubular atrophy.
Lungs	Congested, alveolar exudation.
Trachea	Slight congestion.

Number of Sections Examined	
	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 16
Animal No: 222 Sex: ♀

Time on Study	Death
2 weeks	TK

Clinical History

Hyperkinetic, aggressive on Days 4, 5, 6, 7 and 8. Aggressive on Day 9. Hyperkinetic, aggressive on Day 10. Hyperkinetic on Day 11.

Necropsy Findings

Tracheal mucosa dark red.

Sample	Histopathology
Kidneys	Foci tubular dilation and atrophy.
Trachea	Congested.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 16
Animal No: 223 Sex: ♀

Time on Study	Death
2 weeks	TK

Clinical History

Clonic convulsion on Day 3. Hypokinetic on Day 4. Hyperkinetic on Day 5. Hyperkinetic, aggressive on Day 6. Hyperkinetic, slight clonic convulsion on Day 7. Very hyperkinetic on Day 8. Very hyperkinetic, unable to move left hind limb on Day 9. Unable to move left hind limbs on Days 10-14.

Necropsy Findings

Tracheal mucosa dark red.

Sample	Histopathology
Kidneys	Area of tubular atrophy. Fibrosis and lymphocytic infiltration.
Liver	Congestion in centrilobular areas.
Trachea	Congested.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 17
Animal No: 224 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History
Hyperkinetic on Day 4. Slight clonic convulsion on Day 5. Hyperkinetic, miosis on Day 6. Hyperkinetic, clonic convulsion on Day 7.

Necropsy Findings
Tracheal mucosa dark red.

Sample	Histopathology
Lungs	Area congestion.
Trachea	Slight congestion.

Number of Sections Examined	
	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5(continued)

Project No: 415669 AC Group: 17
Animal No: 225 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History

Hyperkinetic, slight clonic convulsion on Day 4. Hyperkinetic, timid on Day 5. Slightly hyperkinetic on Day 6.

Necropsy Findings

Tracheal mucosa dark red.

Sample	Histopathology
Kidneys	Foci tubular dilation and atrophy. Congested.
Lungs	Slight congestion.
Trachea	Congested.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 17
Animal No: 226 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History

Slightly hyperkinetic on Day 3. Clonic convulsions, hyperkinetic, timid on Day 4. Hyperkinetic, timid, hypokinetic on Day 5. Hypokinetic, clonic convulsions on Day 6. Hypokinetic on Days 7 and 8.

Necropsy Findings

Spleen - extra lobe to spleen 14 mm x 8 mm.

Tracheal mucosa dark red.

Sample	Histopathology
Kidneys	Congested. Areas tubular dilation, tubular and glomerular atrophy, fibrosis, lymphocyte infiltration.
Liver	Foci lymphocyte infiltration especially in portal areas.
Lungs	Congestion. Areas alveolar exudation.
Spleen	NAD.
Brain	Perivascular cuffing. Nosematosis.
Trachea	Congested. Lymphocyte infiltration lamina propria. Epithelium active.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 17
Animal No: 227 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History

Hyperkinetic on Days 3 and 4. Clonic convulsions on Day 5.

Necropsy Findings

Tracheal mucosa dark red.

Sample	Histopathology
Lungs	Slight congestion and alveolar exudation.
Trachea	Congested.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 17
Animal No: 228 Sex: ♀

Time on Study	Death
2 weeks	TK

Clinical History

Hypokinetic, miosis, slightly hyperkinetic on Day 3. Hyperkinetic on Day 4. Hyperkinetic, aggressive on Day 5.

Necropsy Findings

Tracheal mucosa dark red.

Sample	Histopathology
Kidneys	Areas tubular dilation, tubular and glomerular atrophy, fibrosis.
Lungs	Congested. Areas of haemorrhage.
Trachea	Congested.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 17
Animal No: 229 Sex: ♀

Time on Study	Death
2 weeks	TK

Clinical History	Sample	Histopathology
Slightly hyperkinetic on Days 3 and 4. Hypokinetic, timid on Days 6, 7, 8 and 9. Hypokinetic on Days 10 and 11.	Liver	Congested. Extra medullary haemopoiesis.

Necropsy Findings
NAD

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 17
Animal No: 230 Sex: ♀

Time on Study	Death
2 weeks	TK

Clinical History

Hypokinetic, mydriasis on Day 3. Clonic convulsion, hypokinetic on Day 4. Hypokinetic, timid on Days 5 and 6.

Necropsy Findings

Liver - lobulation prominent.

Sample	Histopathology
Liver	Mild bile duct hyperplasia. Centrilobular congestion and slight cellular degeneration.
Kidneys	Foci tubular atrophy.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 17
Animal No: 231 Sex: ♀

Time on Study	Death
2 weeks	TK

Clinical History

Slightly hyperkinetic on Day 3. Hyperkinetic, miosis on Day 4. Slightly hyperkinetic on Day 5.

Necropsy Findings

Kidneys - both mottled.

Sample	Histopathology
Kidneys	Areas tubular dilation, tubular and glomerular atrophy, fibrosis and lymphocyte infiltration.
Liver	Centrilobular congestion.
Lungs	Congestion. Alveolar exudation.
Brain	Perivascular cuffing. Nosematosis.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 18
Animal No: 232 Sex: ♂

Time on Study	Death
5 days	KIE

Clinical History

Hyperkinetic on Day 3.

Clonic convulsion, unable to move hind limbs. Sacrificed.

Necropsy Findings

NAD

Sample	Histopathology
Liver	Congested. Cytoplasm eosinophilic.
Spleen	White pulp depletion.
Lungs	Area congestion.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 18
Animal No: 233 Sex: ♂

Time on Study	Death
3 days	KIE

Clinical History
Broken leg on Day 3.

Necropsy Findings
Right hind leg broken. Kidneys - both show prominent surface blood vessels.

Sample	Histopathology
Kidneys	Congested. Foci tubular atrophy.
Liver	Cytoplasm fairly eosinophilic.
Lungs	Congested. Areas haemorrhage and alveolar exudation.
Spleen	Degree of white pulp depletion.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 18
Animal No: 234 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History

Blood in cage, severe clonic convulsions on Day 3. Clonic convulsion, hyperkinetic on Day 4. Hyperkinetic, miosis, clonic convulsion, aggressive on Days 5 and 6. Clonic convulsion, hyperkinetic on Days 7 and 8. Clonic convulsions on Day 9. Hyperkinetic on Day 10. Hypokinetic on Day 11. Hyperkinetic on Day 12.

Necropsy Findings

Lungs - irregular dark areas on all lobes.

Sample	Histopathology
Kidneys	Congested. Some tubules contain pink globules.
Liver	Some congestion, especially in centrilobular areas.
Lungs	Congested. Areas haemorrhage and alveolar exudation.

Number of Sections Examined	
	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 18
Animal No: 235 Sex: ♂

Time on Study	Death
3 days	FD

Clinical History

Found dead in cage on Day 3.

Necropsy Findings

Pink staining round nose and mouth.

Kidneys - numerous.
2-4 mm areas of dark red depression.

Sample	Histopathology
Kidneys	Congested. Area tubular and glomerular atrophy, fibrosis, lymphocyte infiltration.
Liver	Cytoplasm eosinophilic.
Lungs	Congested. Areas haemorrhage and alveolar exudation.
Spleen	White and red pulp depletion.
Brain	Perivascular cuffing. Gliosis. Nosematosis.
Spinal Cord	Perivascular cuffing.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 18
Animal No: 236 Sex: ♀

Time on Study	Death
2 weeks	TK

Clinical History

Clonic convulsion, hyperkinetic on Day 3. Clonic convulsion on Day 4. Hyperkinetic, timid, clonic convulsion on Day 5. Clonic convulsion on Day 6. Hyperkinetic, clonic convulsion on Days 7 and 8. Hyperkinetic on Day 9.

Necropsy Findings

Slight reddening of tracheal mucosa.

Sample	Histopathology
Liver	Congested. Extra medullary haemopoiesis.
Lungs	Congested. Alveolar exudation.
Trachea	NAD.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

Project No: 415669 AC Group: 18
Animal No: 237 Sex: ♀

Time on Study	Death
2 weeks	TK

Clinical History

Hyperkinetic, miosis on Day 3. Hyperkinetic, clonic convulsions on Day 4. Clonic convulsions, miosis on Day 5. Hyperkinetic, timid on Day 6. Hypokinetic, timid on Days 7 and 8. Hypokinetic on Day 9. Hyperkinetic on Days 10 and 11.

Necropsy Findings

Spleen large.

Tracheal mucosa dark red.

Sample	Histopathology
Kidneys	Foci tubular dilation and tubular atrophy.
Spleen	Sinuses dilated.
Lungs	Foci polymorphs and lymphocytes.
Trachea	Congested.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

Project No: 415669 AC Group: 18
Animal No: 238 Sex: ♀

Time on Study	Death
2 weeks	TK

Clinical History

Hyperkinetic on Day 3. Hyperkinetic, hypokinetic, mydriasis on Day 4. Hyperkinetic, slight clonic convulsions on Day 5. Hyperkinetic, miosis on Day 6. Slight clonic convulsions on Days 7 and 8. Hyperkinetic, clonic convulsions on Days 9 and 10. Hyperkinetic on Day 11.

Necropsy Findings

Tracheal mucosa dark red.

Sample	Histopathology
Lungs	Congested.
Trachea	Congested.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

Project No: 415669 AC Group: 18
Animal No: 239 Sex: ♀

Time on Study	Death
5 days	KIE

Clinical History

Hyperkinetic Day 3 and 4.
Unable to move hind limbs, suspect broken back therefore sacrificed.

Necropsy Findings

Liver - fissuring prominent.

Lungs - dark red.

Trachea - mucosa dark red.

Sample	Histopathology
Liver	Congested. Cytoplasm eosinophilic, particularly in portal areas. Increased cellularity in portal areas.
Lungs	Congested.
Spleen	White pulp depletion.
Trachea	Congested.
Kidneys	Increased Bowmans space containing pink material.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 19
Animal No: 240 Sex: ♀

Time on Study	Death
4 days	FD

Clinical History

Clonic convulsions on Day 3.

Necropsy Findings

Blood staining round nose, mouth and left side of face.

Kidneys - pitted surfaces.

Lungs - irregular dark areas all lobes.

Skull - areas of bruising on top of head.

Brain - subdural haemorrhage between cerebellum and cerebral lobe.

Sample	Histopathology
Kidneys	Areas tubular atrophy, fibosis, lymphoid infiltration. Congestion.
Liver	Congestion. Eosinophlic cytoplasm.
Lungs	Congested but autolytic.
Spleen	White pulp depletion.
Brain	Sub arachnoid haemorrhage, meningitis. Haemorrhage in ventricles. Perivascular cuffing. Nosematosis.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 19
Animal No: 241 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History

Hyperkinetic, clonic convulsion on Day 3. Clonic convulsion on Days 4 and 5. Hyperkinetic, miosis on Day 6. Hyperkinetic on Day 7.

Necropsy Findings

Lungs - irregular reddening on all lobes.

Trachea mucosa dark red.

Sample	Histopathology
Liver	Small foci polymorphs.
Lungs	Congested. Interstitial polymorphs. Alveolar haemorrhage and exudation.
Trachea	Congested.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 19
Animal No: 242 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History

Hyperkinetic, miosis on Day 3. Hyperkinetic, clonic convulsion, aggressive on Day 4. Slight clonic convulsion, hyperkinetic on Day 5. Hypokinetic, timid on Days 6, 7, 8, 9 and 10. Hypokinetic on Days 11 and 12.

Necropsy Findings

NAD

Sample	Histopathology
Lungs	Congested. Epithelium of bronchus active. Polymorphs in epithelium and lamina propria. Alveolar haemorrhage and exudation.
Brain	Small foci perivascular cuffing and gliosis.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

Project No: 415669 AC Group: 19
Animal No: 243 Sex: ♂

Time on Study	Death
3 Days	KIE

Clinical History

Unable to move limbs on Day 3.

Necropsy Findings

Lungs - irregular dark areas on all lobes.

Sample	Histopathology
Liver	Congested. Cytoplasm eosinopilic.
Kidneys	Foci tubular atrophy, lymphoid infiltration. Congestion.
Lungs	Congestion.
Spleen	Some white pulp depletion.
Brain	Perivascular cuffing. Nosematosis.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 19
Animal No: 244 Sex: ♀

Time on Study	Death
2 Weeks	TK

Clinical History

Hyperkinetic, clonic convulsion, mydriasis on Day 3. Hyperkinetic on Day 4. Hyperkinetic, clonic convulsion on day 5, Hyperkinetic on Day 6.

Necropsy Findings

Kidneys - both mottled.

Tracheal mucosa - red.

Sample	Histopathology
Liver	Cytoplasm eosinophilic in portal areas.
Kidneys	Areas tubular dilation, tubular and glomerular atrophy and fibrosis, particularly in sub capsular area.
Lungs	Area congestion.
Skin	Slight inflammatory reaction in dermis.
Trachea	Congested. Lymphoid infiltration of lamina propria and epithelium.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC **Group:** 19
Animal No: 245 **Sex:** ♀

Time on Study	Death
2 weeks	TK

Clinical History

Clonic convulsions on Day 3.
Clonic convulsion, hyperkinetic on Day 4.
Hyperkinetic on Day 5.

Necropsy Findings

Liver - large, fissuring prominent, lobulation prominent.

Tracheal mucosa red.

Sample	Histopathology
Liver	Congested especially in portal areas.
Kidneys	*Foci tubular dilation and tubular atrophy.*
Lungs	Foci congestion and lymphoid areas.
Trachea	Congested.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 19
Animal No: 246 Sex: ♀

Time on Study	Death
2 weeks	TK

Clinical History

Hyperkinetic on Day 3. Clonic convulsion, miosis on Day 4. Clonic convulsion on Day 5. Hyperkinetic on Day 6 and 7. Hyperkinetic, clonic convulsion on Day 8, 9 and 10. Hyperkinetic on Day 11.

Necropsy Findings

NAD

Sample	Histopathology
Lungs	Congested.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 19
Animal No: 247 Sex: ♀

Time on Study	Death
3 days	KIE

Clinical History
Unable to move hind limbs on Day 3.

Necropsy Findings
Lungs - irregular dark red patches on all lobes.

Sample	Histopathology
Liver	Congested. Cytoplasm eosinophilic particularly in portal areas.
Kidneys	Congested.
Lungs	Autolytic. Possible haemorrhage and alveolar degeneration.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 20
Animal No: 248 Sex: ♂

Time on Study	Death
3 days	KIE

Clinical History

Severe clonic convulsion on Day 3. Left hind leg broken - sacrificed.

Necropsy Findings

Abdominal muscle ruptured on left side.

Left femur broken.

Lungs deep red.

Two small 0.5 cm diameter perforations in ileum which lay in subcutaneous tissue.

Sample	Histopathology
Liver	Congested. Cytoplasm eosinophilic. Increased cellularity portal areas.
Kidneys	Congested.
Lungs	Congested. Alveolar haemorrhage and exudation. Degeneration of alveoli. Autolysis.
Ileum	Congestion and haemorrhage in lamina propria.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Ileum	2

APPENDIX 5 (continued)

Project No: 415669 AC **Group:** 20
Animal No: 249 **Sex:** ♂

Time on Study	Death
3 days	FD

Clinical History

Clonic convulsion on Day 3.

Necropsy Findings

Watery discharge round mouth.

Kidneys - several 1-2 mm dark red depressed foci.

Lungs - several 1-2 mm dark red foci on all lobes.

Sample	Histopathology
Liver	Cytoplasm eosinophilic - Foci lymphocytes.
Kidneys	Congested. Areas tubular and glomerular atrophy and fibrosis. Lymphocyte infiltration.
Lungs	Congested.
Brain	Perivascular cuffing. Nosematosis.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 20
Animal No: 250 Sex: ♂

Time on Study	Death
5 days	KIE

Clinical History

Hyperkinetic on Day 2. Clonic convulsions on Day 3 and 4. Unable to move hind limbs, suspected broken back - sacrificed.

Necropsy Findings

Lungs - numerous dark areas on all lobes.

Trachea - mucosa dark red.

No evidence of broken back at autopsy.

Sample	Histopathology
Kidneys	Congested. Increased cellularity in glomeruli.
Liver	Congested. Cytoplasm eosinophilic.
Lungs	Congested. Autolytic but possible alveolar haemorrhage and degeneration.
Brain	Some perivascular cuffing.
Trachea	Congested.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

Project No: 415669 AC Group: 20
Animal No: 251 Sex: ♂

Time on Study	Death
3 days	KIE

Clinical History

Hyperkinetic on Day 2. Clonic convulsions, blood round nose on Day 3.

Necropsy Findings

Pink staining round nose and mouth.

Liver - mottled.

Kidneys - irregular reddening.

Lungs - occasional 1-2 mm red foci on all lobes.

Sample	Histopathology
Kidneys	Congested.
Liver	Congested. Cytoplasm eosinophilic. Centrilobular necrosis.
Lungs	Congested. Areas alveolar haemorrhage, exudation and degeneration.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 20
Animal No: 252 Sex: ♀

Time on Study	Death
3 days	FD

Clinical History

Hyperkinetic on Day 2.
Found dead on Day 3.

Necropsy Findings

Pink staining round mouth.

Kidneys - irregular reddening.

Lungs - all lobes show many periportal red foci.

Heart - left ventricle dilated.

Sample	Histopathology
Kidneys	Congested. Areas tubular and glomeruli atrophy. Areas fibrosis. Lymphocyte infiltration.
Lungs	Congested. Areas increased interstitial cellularity.
Heart	NAD.
Brain	Perivascular cuffing. Meningitis. Nosematosis.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 20
Animal No: 253 Sex: ♀

Time on Study	Death
9 days	KIE

Clinical History

Hyperkinetic on Day 2. Hyperkinetic, mydriasis on Day 3. Aggressive, clonic convulsions, hyperkinetic on Day 4, 5 and 6. Hyperkinetic, hypokinetic on Day 7. Hyperkinetic timid on Day 8. Severe clonic convulsion, hyperkinetic, timid - sacrificed on Day 9.

Necropsy Findings

Blood round nose and mouth.
Front tooth broken.

Kidneys mottled.

Lungs - edge of left lobe shows 10 mm dark red area.

Sample	Histopathology
Liver	Cytoplasm fairly eosinophilic. Increased cellularity in portal areas. Mild centrilobular necrosis.
Kidneys	Congested.
Lungs	Congested.
Heart	Few foci fibrosis in ventricle and septum.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 20
Animal No: 254 Sex: ♀

Time on Study	Death
4 days	KIE

Clinical History

Hyperkinetic on Day 2. Clonic convulsion, very aggressive Day 3. Severe clonic convulsions on Day 4. Blood round nose and prostrated - sacrificed.

Necropsy Findings

Slight blood staining round nose.

Liver - mottled.

Kidneys - pale and mottled.

Spleen - small.

Lungs - dark red.

Subcutaneous bruising on top of skull.

Sample	Histopathology
Kidneys	Congested.
Liver	Congested. Cytoplasm eosinophilic.
Spleen	NAD.
Lungs	Congested. Autolytic but probable alveolar haemorrhage, exudation and degeneration.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

Project No: 415669 AC Group: 20
Animal No: 255 Sex: ♀

Time on Study	Death
3 days	FD

Clinical History	Sample	Histopathology	Number of Sections Examined: HE
Hyperkinetic on Day 2. Found dead on Day 3.	Lungs	Congestion; alveolar exudation.	Liver 1
	Kidneys	NAD on section.	Kidney 1
	Spleen	Lymphocytic depletion.	Heart 2
			Spleen 1
			Lung 1
			Brain 2
			Spinal Cord 1
			Cerebellum 1
			Skin 2

Necropsy Findings
Pink staining round nose and mouth. Lungs dark red. Kidneys - appear to be areas of bruising. Skull - extensive bruising on top of skull.

APPENDIX 5 (continued)

Project No: 415669 AC Group: 21
Animal No: 256 Sex: ♂.

Time on Study	Death
4 days	KIE

Clinical History

Hyperkinetic on day 2. Clonic convulsions on day 3. Severe clonic convulsions, blood around mouth, difficulty in breathing. Damaging itself when convulsing - sacrificed.

Necropsy Findings

Red staining of fur round nose and mouth.

Trachea - mucosa dark red.

Spleen - small.

Skull - bruising on top of head.

Sample	Histopathology
Liver	Blood vessels and sinusoids congested cytoplasm and hepatocytes very eosinophilic.
Kidneys	Blood vessels congested.
Lungs	Congested and autolytic but evidence of alveolar haemorrhages and degeneration.
Spleen	Some loss of lymphocytes in germinal centres.
Trachea	Congested.

Number of Sections Examined	
	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 21
Animal No: 257 Sex: ♂

Time on Study	Death
3 days	KIE

Clinical History

Hyperkinetic on Day 2. Clonic convulsions, blood round nose and mouth, unable to move hind limbs - sacrificed.

Necropsy Findings

Red staining round nose and mouth.

Sample	Histopathology
Liver	Congested. Cytoplasm of hepatocytes very eosinophilic.
Kidneys	Congested. Small areas of tubular atrophy, interstitial fibrosis and lymphocyte infiltration.
Lungs	Congested and autolytic. Possible alveolar exudation/haemorrhage.
Brain	Congestion and perivascular cuffing. Meningitis and foci nosematosis.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 21
Animal No: 258 Sex: ♂

Time on Study	Death
5 days	KIE

Clinical History
Hyperkinetic on Day 3. Clonic convulsions on Day 4. Severe clonic convulsion, difficulty in breathing, cyanosis - sacrificed.

Necropsy Findings
NAD

Sample	Histopathology
Liver	Congested. Cytoplasm of hepatocytes very eosinophilic.
Kidneys	Congested.
Lungs	Congested.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 21
Animal No: 259 Sex: ♂

Time on Study	Death
3 days	FD

Clinical History

Found dead in cage on Day 3.

Necropsy Findings

Red streaks, varying in size from 10-30 mm in length, distributed over shaved area of skin.

Liver - fissuring obvious.

Bladder very large.

Sample	Histopathology
Kidneys	Congested.
Liver	Congested.
Lungs	Congested, increased interstitial cellularity and polymorphs.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 21
Animal No: 260 Sex: ♀

Time on Study	Death
2 weeks	TK

Clinical History
Clonic convulsions, hyperkinetic miosis, timid, hypokinetic.

Necropsy Findings
Tracheal mucosa dark red.

Sample	Histopathology
Liver	Areas of hepatocytes with very eosinophilic cytoplasm. Some cells with vacuolated cytoplasm.
Lungs	Areas of congestion.
Heart	Foci lymphocytic and fibrosis.
Trachea	Congested.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 21
Animal No: 261 Sex: ♀

Time on Study	Death
3 days	KIE

Clinical History
Blood on nose and mouth. Hyperkinetic on Day 2. Clonic convulsions, unable to move hind limbs - sacrificed.

Necropsy Findings
Red staining round nose and mouth. Spleen small. Lungs - irregular reddening.

Sample	Histopathology
Kidneys	Congested.
Liver	Congested. Very eosinophilic cytoplasm.
Lungs	Autolytic, possible alveolar haemorrhage, exudation, degeneration.
Spleen	Depletion of white and red pulp.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 21
Animal No: 262 Sex: ♀

Time on Study	Death
3 days	KIE

Clinical History
Severe clonic convulsions.

Necropsy Findings
Bladder, grossly distended.

Sample	Histopathology
Kidneys	Small foci tubular and glomerular atrophy. Congestion.
Liver	Congested. Cytoplasm eosinophilic, some vacuolation of cytoplasm.
Lungs	Congested. Areas of alveolar haemorrhage, exudation, degeneration.

Number of Sections Examined	
	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 21
Animal No: 263 Sex: ♀

Time on Study	Death
4 days	KIE

Clinical History

Clonic convulsions on Day 4, unable to move hind limbs, suspect broken back - sacrificed.

Necropsy Findings

Trachea - mucosa red.

Lungs - dark red.

Head - bruising on top of skull.

Sample	Histopathology
Kidneys	Congested.
Liver	Congested. Cytoplasm very eosinophilic.
Lungs	Congested but autolytic, possible alveolar haemorrhage, exudation, degeneration.
Trachea	Congested.
Spleen	White pulp depletion.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (Continued)

Project No: 415669 AC Group: 22
Animal No: 264 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History	Sample	Histopathology
NAD.	Lungs	Areas slight congestion. Polymorphs migrating through bronchial epithelium.
	Trachea	NAD.

Necropsy Findings

Tracheal mucosa dark red in colour.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 22
Animal No: 265 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History
NAD.

Necropsy Findings
Tracheal mucosa dark red.

Sample	Histopathology
Liver	Foci lymphocytes and occasional polymorphs.
Kidneys	Small foci tubular atrophy and some lymphocyte infiltration.
Lungs	Foci lymphocytes - areas of congestion.
Trachea	Epithelial hyperplasia, many polymorphs in epithelium and lamina propria.

Number of Sections Examined	
	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 22
Animal No: 266 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History
NAD.

Necropsy Findings
Spleen large. Trachea - mucosa dark red.

Sample	Histopathology
Liver	Foci lymphocytes, particularly in portal areas.
Kidneys	Large areas tubular atrophy and fibrosis. Lymphocyte infiltration. Many plasma cells in pelvis.
Lungs	Foci lymphocytes and some eosinophils. Small degree epithelial hyperplasia of bronchioles. Occasional giant cell. Areas congestion.
Trachea	Congestion otherwise NAD.
Spleen	NAD.
Brain	Foci nosematosis in cerebrum and cerebellum. Perivascular cuffing.

Number of Sections Examined	
	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 22
Animal No: 267 Sex: ♀

Time on Study	Death
2 weeks	TK

Clinical History

NAD.

Necropsy Findings

Liver fissuring prominent.

Sample	Histopathology
Liver	NAD.
Kidneys	Slight congestion.
Lungs	Slight congestion. Increased interstitial cellularity.

Number of Sections Examined	
	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC Group: 22
Animal No: 268 Sex: ♀

Time on Study	Death
2 weeks	TK

Clinical History

NAD.

Necropsy Findings

Tracheal mucosa dark red.

Spleen large.

Sample	Histopathology
Spleen	NAD.
Trachea	NAD.
Lungs	Slight congestion.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 22
Animal No: 269 Sex: ♀

Time on Study	Death
10 days	FD

Clinical History

Diarrhoea for 5 Days.

Necropsy Findings

Brown staining of fur round nose and mouth.

Faecal encrustation round anus and tail.

Sample	Histopathology	Number of Sections Examined	HE
	Sections autolytic.	Liver	1
		Kidney	1
		Heart	2
		Spleen	1
		Lung	1
		Brain	2
		Spinal Cord	1
		Cerebellum	1
		Skin	2

Project No: 415669 AC Group: 23
Animal No: 270 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History

NAD.

Necropsy Findings

Spleen large with irregular edges.

Tracheal mucosa dark red.

Sample	Histopathology
Liver	Foci lymphocytes mainly periportal.
Kidneys	Areas tubular atrophy, fibrosis, lymphocyte infiltration.
Spleen	White pulp hyperplasia.
Lungs	Foci lymphocytes.
Trachea	Congestion.
Brain	Perivascular cuffing and nosematosis in cerebrum and cerebellum.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

AD-A171 598 HMX: ACUTE TOXICITY TESTS IN LABORATORY ANIMALS(U) 3/3
INVERESK RESEARCH INTERNATIONAL LTD MUSSELBURGH
(SCOTLAND) J A CUTHBERT ET AL. 30 JUL 85 IRI-2051
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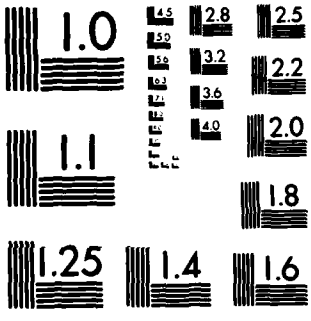

APPENDIX 5 (continued)

Project No: 415669 AC Group: 23
Animal No: 271 Sex: ♂

Time on Study	Death
2 weeks	TK

Clinical History

NAD.

Necropsy Findings

NAD.

Sample	Histopathology
Lungs	Congested.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2

APPENDIX 5 (continued)

Project No: 415669 AC **Group:** 23
Animal No: 272 **Sex:** ♂

Time on Study	Death
2 weeks	TK

Clinical History

NAD.

Necropsy Findings

Spleen - 7 x 5 mm extra portion to spleen.

Tracheal mucosa slightly red.

Lungs mottled.

Sample	Histopathology
Lungs	Slight congestion and alveolar exudation.
Spleen	NAD.
Trachea	NAD.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC **Group:** 23
Animal No: 273 **Sex:** ♀

Time on Study	Death
2 weeks	TK

Clinical History

NAD.

Necropsy Findings

Lungs - extensive irregular reddening of all lobes.

Tracheal mucosa dark red.

Sample	Histopathology
Liver	Focus of lymphocytes and polymorphs.
Lungs	Extensive haemorrhage - possibly back bleeding.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC Group: 23
Animal No: 274 Sex: ♀

Time on Study	Death
2 weeks	TK

Clinical History
NAD.

Necropsy Findings
Tracheal mucosa red.

Sample	Histopathology
Liver	Mild bile duct hyperplasia.
Trachea	Blood vessels congested.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 5 (continued)

Project No: 415669 AC **Group:** 23
Animal No: 275 **Sex:** ♀

Time on Study	Death
2 weeks	TK

Clinical History

NAD.

Necropsy Findings

Tracheal mucosa slightly reddened.

Sample	Histopathology
Lungs	Congestion, alveolar exudation.
Trachea	NAD.

Number of Sections Examined	HE
Liver	1
Kidney	1
Heart	2
Spleen	1
Lung	1
Brain	2
Spinal Cord	1
Cerebellum	1
Skin	2
Trachea	1

APPENDIX 6

Primary Skin Irritation in Rabbits
FDA recommended scoring system

	Grade
Erythema and Eschar Formation	
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Total possible erythema score	4
Oedema Formation	
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (area raised approximately 1 mm)	3
Severe oedema (raised by more than 1 mm and extending beyond area of exposure)	4
Total possible oedema score	4
Total possible score for primary irritation	8

The averages of the 24 and 72 h scores for the intact and for the abraded skin for each material were combined to give a primary irritation score. Materials with a primary irritation score of 2 or less are classified as only mildly irritant, those of 2-5 as being moderately irritant and those of 6 or more as being severely irritant.

The term "primary irritant" applies to a substance that is not corrosive, and that all available data of human experience indicate to be a primary irritant or to a substance with a primary irritation score of 5 or more.

APPENDIX 7

Ocular Irritancy in Rabbits
FDA recommended scoring system

	Grade
Cornea	
No ulceration or opacity	0
Scattered or diffuse areas of opacity, details of iris clearly visible	(1)*
Easily discernible translucent areas of opacity, details of iris slightly obscured	2
Nacreous areas of opacity, no details of iris visible, size of pupil barely discernible	3
Complete corneal opacity, iris not discernible	4
Ulceration, absence of a gross patch of corneal epithelium	4
Iris	
Normal	0
Markedly, deepened folds, congestion, swelling, moderate circumcorneal injection (any of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)	(1)*
No reaction to light, haemorrhage, gross destruction (any or all of these)	2
Conjunctivae	
Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris):	
Vessels normal	0
Some vessels definitely injected	1
Diffuse crimson red, individual vessels not easily discernible	(2)*
Diffuse beefy red	3

APPENDIX 7 (continued)

	Grade
Chemosis	
No swelling	0
Any swelling above normal (including nictitating membrane)	1
Obvious swelling with partial eversion of lids	(2)*
Swelling with lids about half closed	3
Swelling with lids more than half closed	4
Ulceration or necrosis of palpebral and bulbar conjunctivae or nictitating membrane	4
Discharge	
Mild	1
Moderate	2
Severe	3

* Bracketed figures indicate the lowest grades considered positive under section 19-12 of the Federal Hazardous Substances Labelling Act Regulations of the USA.

Note: A score ½ denotes a response considered to be less than 1.

Federal Hazardous Substances Act Regulations Quote:-

"An animal shall be considered as exhibiting a positive reaction if the test substance produces at any of the readings ulceration of the cornea (other than fine stippling), or opacity of the cornea (other than slight dulling of the normal lustre), or inflammation of the iris (other than slight deepening of the folds, or slight circumcorneal injection of the blood vessels) or if such substances produce in the conjunctivae (excluding the cornea and iris) an obvious swelling with partial eversion of the lids, or a diffuse crimson red with individual vessels not discernible."

APPENDIX 8

Analysis of HMX in Gavage Formulations

Method

(i) The supplied gavage formulation (suspended in 0.5% low viscosity CMC) was shaken thoroughly on a "Whirlimixer". A 1 ml aliquot was immediately removed and transferred to a 100 ml volumetric flask and made to the mark with acetonitrile (Rathburn HPLC grade). The flask was shaken thoroughly for at least 1 min for complete solution of the HMX (if necessary an ultrasonic bath is used). A suitable aliquot (V_1 ml) was removed and transferred to a second volumetric flask (V_2, 100 ml or 250 ml) and made to the mark with acetonitrile (Rathburn S grade). The ultraviolet spectrum was recorded and the absorption at 228 nm calculated using for reference material a 1 ml aliquot of 0.5% low viscosity CMC treated as above.

The extinction coefficient of HMX was calculated from the absorption of HMX solutions of known concentration (the value 21346 was used in the calculations).

Calculation:

$$\text{Conc (mg/ml)} = \frac{\Delta A}{V_1} \times 138.67 \text{ (where } V_2 = 100 \text{ ml)}$$

$$\text{or} \quad \frac{\Delta A}{V_1} \times 346.67 \text{ (where } V_2 = 250 \text{ ml)}$$

(ii) Where the dosing vehicle was 1% high viscosity or physiological saline the method was modified to allow for the difficulty in transferring accurately measured amounts of the suspensions. The density of suspensions of HMX in the above media was calculated by transferring aliquots to pre-weighed 1 ml volumetric flasks. After making to the mark the flasks were reweighed and the density calculated.

Results: In 1% CMC d = 1.244
In Saline d = 1.126

Analysis

Aliquots of the formulation were transferred to pre-weighed flasks (100 ml) and the flasks reweighed. The weight of suspension was calculated (w.g.), the suspension was then dissolved in acetonitrile (Rathburn HPLC grade) and suitable aliquots

APPENDIX 8 (continued)

(V ml) removed and transferred to 100 ml volumetric flasks and made to the mark with acetonitrile (Rathburn S grade). The ultraviolet spectrum was recorded.

Calculation:

High viscosity CMC

$$\text{Conc (mg/ml)} = \frac{\Delta A \times 172.5}{w \times v}$$

Physiological Saline

$$\text{Conc (mg/ml)} = \frac{\Delta A \times 156.1}{w \times v}$$

PERSONNEL INVOLVED IN PROJECT 415669AC

Principal Investigator:	A.B. Wilson, B.V.Sc., M.R.C.V.S.
Project Leader:	J.A. Cuthbert, B.Sc.
Technicians:	S. Carr P. Hose, B.Sc. K. D'Arcy-Burt, H.N.D.
Animal Services Manager:	A. Dick, F.I.A.T.
Veterinary Officer:	A.J. Spencer, Ph.D., B.V.M.S., M.R.C.V.S.
Pathologists:	A.J. Spencer, Ph.D., B.V.M.S., M.R.C.V.S. R. Aitken, Ph.D., B.V.M.S., M.R.C.V.S. B. Rushton, Ph.D., B.V.M.S., M.R.C.V.S., R.C.V.S. A.K.A. Rushton, B.V.M.S., M.R.C.V.S. F. Macnaughtan, B.Vet.Med., M.R.C.V.S. M. Jones, B.V.M.S., M.R.C.V.S.
Quality Assurance:	A.W. Waddell, B.Sc., Ph.D. E.M. Baxendine, B.Sc. N.C. McLachlan, B.Sc.
Analytical Chemistry:	J.N. Done, B.Sc., Ph.D. D.W. Carmichael, B.Sc. J.D. Gilbert, B.Sc., Ph.D., C.Chem., F.R.C.S.
Animal Technicians:	D. Carrigan A. Young H. Ramage L. Punton
Test Substance Formulator:	A.T. Soden
Research Assistant:	A. Trench
Autopsy Room Supervisor:	E.P. Hall, F.I.M.L.S.

PERSONNEL INVOLVED (continued)

Pathology Assistants:	V. Archibald
	L. Brett, A.I.M.L.S.
	M. Brown, B.Sc.
	H. Duffy
	D. Fraser
	J. Houlihan
	R. Johnson, H.N.C.
	A. Kirkwood
	D. McBride
	F. MacLean
	C. Petrie, B.Sc.
	S. Rutherford
	G. Ash, B.Sc.
	L. Spratt

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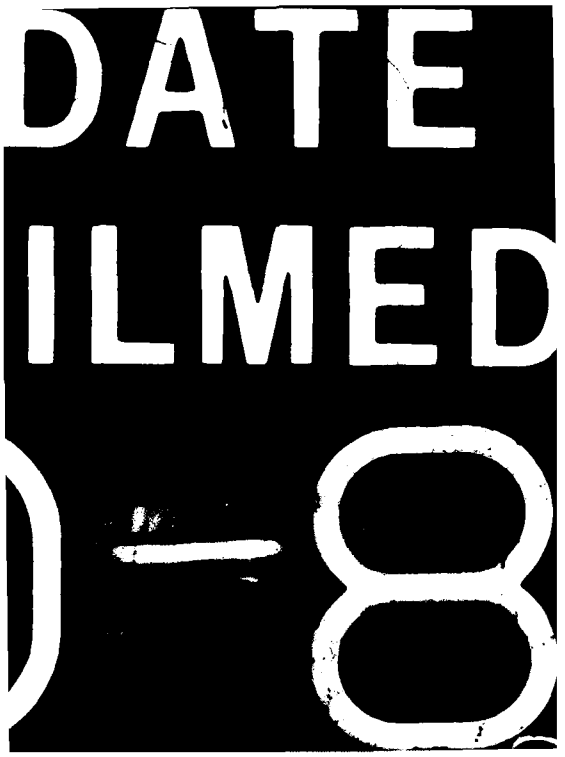
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