

STUDY TITLE

Dimethoate
Effects On Cholinesterase
In The CD Rat (Adult And Juvenile)By Oral
Gavage Administration

DATA REQUIREMENT

US EPA Subdivision F, OPPTS 870.6300

STUDY DIRECTOR/AUTHOR

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STUDY COMPLETION DATE

September 27, 2001

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CHV/070 (Report 012226)

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

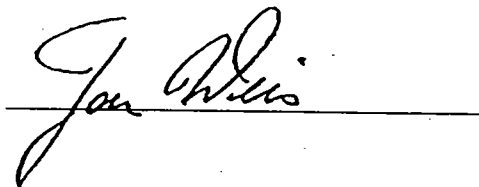
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Company: Cheminova A/S

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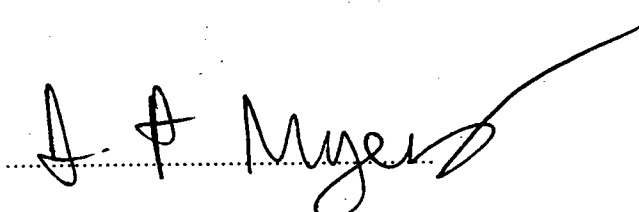
GOOD LABORATORY PRACTICE STATEMENT
DIMETHOATE
EFFECTS ON CHOLINESTERASE
IN THE CD RAT (ADULT AND JUVENILE) BY ORAL
GAVAGE ADMINISTRATION

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

The UK Good Laboratory Practice Regulations 1999 (Statutory Instrument No 3106).

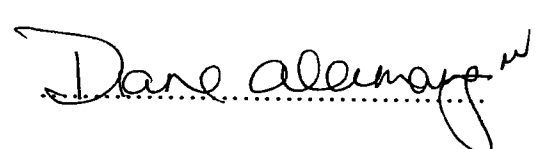
EC Commission Directive 1999/11/EC of 8 March 1999 (Official Journal No L 77/8).

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17.


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FLAGGING STATEMENT

Dimethoate
Effects On Cholinesterase
In The CD Rat (Adult And Juvenile) By Oral
Gavage Administration

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

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QUALITY ASSURANCE STATEMENT

DIMETHOATE

EFFECTS ON CHOLINESTERASE

IN THE CD RAT (ADULT AND JUVENILE) BY ORAL

GAVAGE ADMINISTRATION

The following inspections and audits have been carried out in relation to this study

Study Phase	Date of Inspection	Date of Reporting
Protocol Audit	14.07.00	14.07.00
Study Based Inspections		
Dose Preparation	14.07.00	14.07.00
Dosing & post dose obs	17.07.00	20.07.00
Female necropsy – day 20	01.08.00	01.08.00
Fetal blood sampling – day 20	01.08.00	01.08.00
Blood sampling – day 4 pups	07.08.00	08.08.00
Brain cholinesterase activity	07.08.00	08.08.00
Dosing of offspring	14.08.00	21.08.00
Report Audit	26.09.01	26.09.01

Protocol Audit: An audit of the protocol for this study was conducted and reported to the Study Director and Company Management as indicated above.

Study based inspections: Inspections of phases of this study were conducted and reported to the Study Director and Company Management as indicated above.

Process based inspections: At or about the time this study was in progress inspections of other routine and repetitive procedures employed on this type of study were carried out. These were promptly reported to appropriate Company Management.

Report Audit: This report has been audited by the Quality Assurance Department. This audit was conducted and reported to the Study Director and Company Management as indicated above.

The methods, procedures and observations were found to be accurately described and the reported results to reflect the raw data.



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27 September 2001.

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DIMETHOATE

EFFECTS ON CHOLINESTERASE

IN THE CD RAT (ADULT AND JUVENILE) BY ORAL

GAVAGE ADMINISTRATION

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SUMMARY

Procedures

The purpose of this study was to assess the effect in rats of acute and/or repeated dosing with dimethoate on plasma, erythrocyte and brain acetyl cholinesterase activity in gestation females and their pre-term fetuses at GD 20 (Gestation day 20), in pre-weaning offspring (PNDs 4, 11 and 21 (post natal days 4, 11, and 21) and in young rats (7-8 weeks of age). Recovery of cholinesterase activity was also assessed in adults at PND 60, 39 days after cessation of repeated-dose treatment.

Groups of animals were dosed by oral gavage at 0.1, 0.5 or 3 mg/kg/day as follows:

Groups of 19 mated females were dosed with ten females per group treated from GD 6 to PND 10 and the remaining nine females treated to GD 20. A similarly constituted control group received the vehicle, water for formulation, using the same treatment regime.

For the females treated up to GD 20, eight dams per group were killed 3 hours after dosing on that day; litter data was assessed and cholinesterase activity determined for maternal and fetal plasma, erythrocyte and brain.

For the females which were allowed to litter and treated up to PND 10, offspring in eight litters per group were dosed from PND 11 to PND 21 inclusive in order to assess effects on survival, weight gain and plasma, erythrocyte and brain cholinesterase activity. Selected offspring from these litters were killed on PNDs 4, 21, and 60 and cholinesterase activity determined for plasma, erythrocyte and brain.

A group of 8 pregnant females was undosed throughout the study. On PND 11, one male and one female offspring per litter were assigned to each experimental group for treatment on PND 11. Eight male and eight female offspring per group (one male and one female from each litter) were killed 2 hours after dosing and cholinesterase activity determined for plasma, erythrocyte and brain.

Groups of 16 male and 16 female naïve adult rats were dosed as follows: 8 males and 8 females per group were dosed for one day and killed 2 hours after dosing and cholinesterase activity determined for plasma, erythrocyte and brain. The remaining 8 males and 8 females per group were dosed for 11 consecutive days and were killed 2 hours after dosing and cholinesterase activity determined for plasma, erythrocyte and brain.

In order to gain experience in a specific method of perfusion fixation of tissue, and in histological sectioning of brains from young-adult rats, five offspring were killed on PND 61, perfused and the brain embedded, sectioned and subjected to light microscopy examination.

Results

3 mg/kg/day

There were no clinical signs considered to be related to treatment and no deaths. There was no adverse effect of treatment on weight gain of adult males and females, weight change of dams during gestation and lactation or, macroscopic necropsy findings.

Litter data on GD 20 and bodyweight, weight gain and survival of offspring up to PND 11 were unaffected by treatment.

There was no effect of direct treatment on the growth or survival of the offspring.

Plasma, RBC (red blood cell) and brain cholinesterase activity were decreased among dams (44%, 58% and 60% lower than in controls) and fetuses (43%, 31% and 33% lower than in controls) on GD 20.

Among male offspring on PND 4 there were slight but significant decreases in RBC and brain cholinesterase activity compared with controls (17% and 13% lower respectively). Among female offspring there were slight decreases in plasma and RBC and brain cholinesterase activity compared with controls (10%, 14% and 7% lower than in controls respectively) with only the difference for plasma cholinesterase activity attaining significance. These minor differences are considered to be of doubtful biological significance.

There were slight but significant decreases in plasma and brain cholinesterase activity among male offspring dosed once on PND 11 (19% and 17% lower than in controls respectively). Among female offspring dosed once there were slight decreases in plasma, RBC and brain cholinesterase activity compared with controls (18%, 26% and 18% lower than in controls respectively) with the difference for brain cholinesterase activity attaining significance.

There was a marked decrease in levels of plasma, RBC and brain cholinesterase activity among dosed male (39%, 59% and 45% lower than in controls) and female offspring (38%, 65% and 42% lower than in controls) killed on PND 21. In contrast, offspring killed on PND 60 showed complete recovery of cholinesterase activity 39 days after the end of dosing with no biologically significant decreases in activity recorded.

Treatment of naïve adult animals with one dose of 3 mg/kg/day dimethoate was associated with decreases in plasma, RBC and brain cholinesterase activity among males (19%, 17% and 12% lower respectively) and among females (13%, 27% and 14% lower respectively); differences attained significance for all parameters in males and RBC and brain cholinesterase activity among females.

Treatment of naïve adult males with eleven consecutive doses of dimethoate was associated with marked and statistically significant decreases in plasma, RBC and brain cholinesterase activity (37%, 58% and 47% lower than in controls respectively).

Treatment of naïve adult females with eleven doses of 3 mg/kg/day was associated with marked and significant decreases in erythrocyte and brain cholinesterase activity (63% and 58% lower than in controls respectively); plasma cholinesterase activity was also decreased compared with controls (21% lower) but the difference did not attain significance.

0.5 mg/kg/day

There were no clinical signs considered to be related to treatment and no deaths. There was no adverse effect of treatment on weight gain of adult males and females, weight change of dams during gestation and lactation or, macroscopic necropsy findings.

Litter data on GD 20 and bodyweight, weight gain and survival of offspring up to PND 11 were unaffected by treatment.

There was no effect of direct treatment on the growth or survival of the offspring.

A number of minor/slight decreases in cholinesterase activity were recorded among naïve adult males and females, dams and fetuses on GD 20 and offspring on PNDs 4, 11 and 21. The greatest degree of inhibition of cholinesterase activity recorded was the statistically significant 23% inhibition for RBC activity among female offspring on PND 21 and this was part of a dosage-related inhibition. All other decreases at this dose level in cholinesterase activity were less than 20% and thus considered not biologically significant. Offspring killed on PND 60 showed complete recovery of cholinesterase activity 39 days after the end of dosing with no biologically significant decreases in cholinesterase activity recorded.

0.1 mg/kg/day

There were no clinical signs considered to be related to treatment and no deaths. There was no adverse effect of treatment on weight gain of adult males and females, weight change of dams during gestation and lactation or, macroscopic necropsy findings.

Litter data on GD 20 and bodyweight, weight gain and survival of offspring up to PND 11 were unaffected by treatment.

There was no effect of direct treatment on the growth or survival of the offspring.

A number of minor decreases in cholinesterase activity were recorded but these were considered to be of no biological significance.

Conclusion

Within the context of this study it is concluded that the no-observed-adverse-effect-level (NOAEL) for decreased cholinesterase activity in gestating dams and their fetuses is 0.5 mg/kg/day. The magnitude and progression of decreases in cholinesterase activity in juveniles and young adults is comparable and the NOAEL for juvenile and young rats is concluded to be 0.1 mg/kg/day.

Complete recovery of cholinesterase activity was demonstrated at PND 60, 39 days after the end of repeat dosing.

TABULAR SUMMARY

Parameter (group mean values)	Dosage (mg/kg/day)			
	Control	0.1	0.5	3
MATERNAL DATA (Groups 1-4)				
Bodyweight on Day 6 of gestation (g)	273	275	275	276
Bodyweight on Day 20 of gestation (g)	401	411	400	409
Bodyweight gain Days 6-20 (g)	128	136	126	133
Plasma CHE on Day 20 (U/L)	1381	1216 (12)	1184 (14)	776** (44)
Erythrocyte CHE on Day 20 (U/L)	1669	1563 (6)	1459 (13)	709** (58)
Brain CHE on Day 20 (U/kg)	12838	13044 (-2)	11563* (10)	5094** (60)
Bodyweight gain Days 1-21 of lactation (g)	30	22	24	42
DAY 20 LITTER DATA/FETAL CHE (Groups 1-4)				
Implantations	14.8	16.0	15.3	16.2
Resorptions	0.6	2.6	0.8	0.9
Live young	14.2	13.4	14.6	15.3
Mean fetal weight (g)	3.89	3.94	3.93	4.06
Plasma CHE on Day 20 - fetuses (U/L)	258	257 (0)	239 (7)	147** (43)
Erythrocyte CHE on Day 20 - fetuses (U/L)	1213	1225 (-1)	1181 (3)	834** (31)
Brain CHE on Day 20 - fetuses (U/kg)	1781	1569* (12)	1600* (10)	1188** (33)

CHE Cholinesterase activity; * $p < 0.05$, ** $p < 0.01$ compared with control.

() Percentage inhibition of CHE compared with control.

TABULAR SUMMARY

Parameter (group mean values)	Dosage (mg/kg/day)			
	Control	0.1	0.5	3
LITTERING PHASE/CHE ON DAY 4 OF AGE (Groups 1-4)				
Total litter size Day 1	13.3	14.2	13.3	13.7
Mean pup weight on Day 1 (g) - males	6.6	6.4	6.6	6.7
Mean pup weight on Day 1 (g) - females	6.4	6.1	6.2	6.2
Offspring viability index (%)	100.0	98.5	98.5	99.3
Bodyweight gain Days 1-11 of age (g) - male offspring	18.9	19.2	19.1	18.6
Bodyweight gain Days 1-11 of age (g) - female offspring	18.1	18.6	18.4	18.1
Bodyweight gain Days 11-21 of age (g) - dosed male offspring	26.3	25.7	26.2	26.9
Bodyweight gain Days 11-21 of age (g) - dosed female offspring	25.0	25.6	25.4	25.7
Bodyweight gain Days 11-60 of age (g) - dosed male offspring	325.7	331.8	348.8	353.9
Bodyweight gain Days 11-60 of age (g) - dosed female offspring	189.9	204.0	205.7	196.6
Plasma CHE on Day 4 - male offspring (U/L)	612	607 (1)	588 (4)	566 (8)
Plasma CHE on Day 4 - female offspring (U/L)	640	605 (5)	591* (8)	576** (10)
Erythrocyte CHE on Day 4 - male offspring (U/L)	1291	1403 (-9)	1254 (3)	1071** (17)
Erythrocyte CHE on Day 4 - female offspring (U/L)	1260	1261 (0)	1352 (-7)	1088 (14)
Brain CHE on Day 4 - male offspring (U/kg)	3137	2817* (10)	2889* (8)	2744** (13)
Brain CHE on Day 4 - female offspring (U/kg)	2823	2941 (-4)	2650 (6)	2638 (7)

CHE Cholinesterase activity; * $p < 0.05$, ** $p < 0.01$ compared with control.

() Percentage inhibition of CHE compared with control.

TABULAR SUMMARY

Parameter (group mean values)	Dosage (mg/kg/day)			
	Control	0.1	0.5	3
LITTERING PHASE/CHE ON DAYS 21 and 60 OF AGE (Groups 1-4)				
Plasma CHE on Day 21 - male offspring (U/L)	506	535 (-6)	478 (6)	307** (39)
Plasma CHE on Day 21 - female offspring (U/L)	487	507 (-4)	463 (5)	304** (38)
Erythrocyte CHE on Day 21 - male offspring (U/L)	1638	1659 (-1)	1494 (9)	669** (59)
Erythrocyte CHE on Day 21 - female offspring (U/L)	1900	1619 (15)	1466* (23)	663** (65)
Brain CHE on Day 21 - male offspring (U/kg)	10375	9944* (4)	9044** (13)	5675** (45)
Brain CHE on Day 21 - female offspring (U/kg)	10275	9906 (4)	9019** (12)	5956** (42)
Plasma CHE on Day 60 - male offspring (U/L)	373	369 (1)	340 (9)	337 (10)
Plasma CHE on Day 60 - female offspring (U/L)	907	915 (-1)	945 (-4)	846 (7)
Erythrocyte CHE on Day 60 - male offspring (U/L)	1075	1100 (-2)	1100 (-2)	1038 (3)
Erythrocyte CHE on Day 60 - female offspring (U/L)	1109	1119 (-1)	991 (11)	1044 (6)
Brain CHE on Day 60 - male offspring (U/kg)	13000	13100 (-1)	12988 (0)	13044 (0)
Brain CHE on Day 60 - female offspring (U/kg)	13275	12950 (2)	12738* (4)	12744* (4)
CHE ON DAY 11 OF AGE (Group 5)				
Plasma CHE on Day 11 - male offspring (U/L)	756	748 (1)	688 (9)	614** (19)
Plasma CHE on Day 11 - female offspring (U/L)	742	700 (6)	720 (3)	609 (18)
Erythrocyte CHE on Day 11 - male offspring (U/L)	1663	1634 (2)	1597 (4)	1544 (7)
Erythrocyte CHE on Day 11 - female offspring (U/L)	1997	1647 (18)	1894 (5)	1475 (26)
Brain CHE on Day 11 - male offspring (U/kg)	6475	6363 (2)	6144* (5)	5375** (17)
Brain CHE on Day 11 - female offspring (U/kg)	6256	6350 (-2)	6125 (2)	5144** (18)

CHE Cholinesterase activity; * p < 0.05, ** p < 0.01 compared with control.

() Percentage inhibition of CHE compared with control.

TABULAR SUMMARY

Parameter (group mean values)	Dosage (mg/kg/day)			
	Control	0.1	0.5	3
ADULT MALES AND FEMALES (Groups 6-9)				
Bodyweight gain Days 1-11 of treatment (g) - males	66	54	67	52
Bodyweight gain Days 1-11 of treatment (g) - females	37	29	30	30
Plasma CHE after 1 dose (U/L) - males	375	387 (-3)	364 (3)	305* (19)
Plasma CHE after 1 dose (U/L) - females	688	657 (5)	729 (-6)	602 (12)
Erythrocyte CHE after 1 dose (U/L) - males	1122	1247 (-11)	1131 (-1)	928* (17)
Erythrocyte CHE after 1 dose (U/L) - females	1209	1128 (7)	1106 (9)	881** (27)
Brain CHE after 1 dose (U/kg) - males	13794	13544 (2)	13294* (4)	12131**(12)
Brain CHE after 1 dose (U/kg) - females	14150	13625 (4)	13850 (2)	12106**(14)
Plasma CHE after 11 consecutive doses (U/L) - males	343	327 (5)	302 (12)	215** (37)
Plasma CHE after 11 consecutive doses (U/L) - females	790	949 (-20)	770 (3)	624 (21)
Erythrocyte CHE after 11 consecutive doses (U/L) - males	1094	1169 (-7)	903 (17)	456** (58)
Erythrocyte CHE after 11 consecutive doses (U/L) - females	1019	991 (3)	950 (7)	375** (63)
Brain CHE after 11 consecutive doses (U/kg) - males	14100	13988 (1)	12700* (10)	7469** (47)
Brain CHE after 11 consecutive doses (U/kg) - females	14869	13913 (6)	12881**(13)	6188** (58)

CHE Cholinesterase activity; * $p < 0.05$, ** $p < 0.01$ compared with control.

() Percentage inhibition of CHE compared with control.

INTRODUCTION

Objective

The purpose of this study was to assess the effect in rats of acute and/or repeated dosing with dimethoate on plasma, erythrocyte and brain acetyl cholinesterase activity in gestation females and their pre-term fetuses (at GD 20), in pre-weaning offspring (PNDs 4, 11 and 21) and in young rats (7-8 weeks of age). Recovery of cholinesterase activity was also assessed in adults PND 60, 39 days after cessation of repeated-dose treatment.

Groups of animals were dosed by oral gavage at 0.1, 0.5 or 3 mg/kg/day as follows:

Groups of 19 mated females were dosed with ten females per group treated from GD 6 to PND 10 and the remaining nine females treated to GD 20. A similarly constituted control group received the vehicle, water for formulation, using the same treatment regime.

For the females treated up to GD 20, eight dams per group were killed 3 hours after dosing on that day; litter data were assessed and cholinesterase activity determined for maternal and fetal plasma, erythrocyte and brain.

For the females which were allowed to litter and treated up to PND 10, offspring in eight litters per group were dosed from PND 11 to PND 21 inclusive in order to assess effects on survival, weight gain and plasma, erythrocyte and brain cholinesterase activity. Selected offspring from these litters were killed on PNDs 4, 21 and 60 and cholinesterase activity determined for plasma, erythrocyte and brain.

A group of 8 pregnant females was undosed throughout the study. On PND 11, one male and one female offspring per litter were assigned to each experimental group for treatment on PND 11. Eight male and eight female offspring per group (one male and one female from each litter) were killed 2 hours after dosing and cholinesterase activity determined for plasma, erythrocyte and brain.

Groups of 16 naïve male and 16 naïve female adult rats were dosed as follows: 8 males and 8 females per group were dosed for one day and killed 2 hours after dosing and cholinesterase activity determined for plasma, erythrocyte and brain. The remaining 8 males and 8 females per group were dosed for 11 consecutive days and were killed 2 hours after dosing and cholinesterase activity determined for plasma, erythrocyte and brain.

In order to gain experience in a specific method of perfusion fixation of tissue, and in histological sectioning of brains from young-adult rats, five offspring were killed on PND 61, perfused and the brain embedded, sectioned and subjected to light microscopy examination.

Justification for the test system

The rat was chosen because it is the species required for the Developmental Neurotoxicity Study. The CD strain was chosen as this strain is suitable for studies of this type and is extensively used at these laboratories.

Justification for the treatment regimen

The oral route was selected to simulate the conditions of potential human exposure. Gavage was chosen to allow accurate administration of doses to dams and pups.

Dosages of 0.1, 0.5 and 3 mg/kg/day were selected by the Sponsor, based on the results from a dose finding study in CD rats preliminary to developmental neurotoxicity study (Huntingdon Life Sciences Report no. CHV068/000129). A dosage of 3 mg/kg/day was considered suitable as the high dosage for the present study, as there was a clear reduction in maternal weight gain during gestation and a decrease in cholinesterase activity among dams and fetuses at GD 20, but no effect on survival, growth or development of the fetuses or offspring through to PND 10. Direct dosing of offspring between PND 11 and PND 21 was without obvious toxicity, but decreased cholinesterase activity was demonstrated at PND 21.

Study organisation

Testing facilities:

The principal laboratory was:

Huntingdon Life Sciences Ltd
Eye
Suffolk IP23 7PX
England

Formulation analysis and measurement of cholinesterase activity was performed at Huntingdon Research Centre, Huntingdon, Cambridgeshire PE28 4HS, England.

Study timing:

Protocol approval	:	10 July 2000
Animals arrived	:	5 July 2000
Pairing commenced	:	10 July 2000
Treatment of mated dams commenced	:	17 July 2000
Treatment of adult males and females commenced:	:	17 July 2000
Necropsy completed	:	3 October 2000

Archives

Following completion of this study all raw data, specimens and samples, except those generated or used during any Sponsor's or supplier's analysis, were stored in the archives of Huntingdon Life Sciences. A copy of the final report was also retained. Quality Assurance records relating to this study were also retained indefinitely.

Huntingdon Life Sciences shall retain the above-mentioned data, specimens and samples in its archive for a period of 5 years from the date of issue of the final report. After such time, the Sponsor will be contacted and their advice sought on the return, disposal or further retention of the materials. Under no circumstances will any items be discarded without the Sponsors knowledge.

MATERIALS AND METHODS

DESIGN CONDITIONS

Animals

One hundred and four virgin female rats of the CrI: CD[®] (SD) IGS BR strain (ordered at 9-10 weeks of age and 200-220 g bodyweight) were obtained from Charles River UK Limited, Margate, Kent, England, and mated with stock males from the same strain and source. Females were allowed a minimum of 5 days acclimatisation, during which time they were inspected daily to check their physical condition. At commencement of the study (Day 0 of gestation) the eighty four animals allocated were in the weight range 216 to 260 g (approximately 10 to 11 weeks of age).

Seventy male and seventy female rats of the CrI: CD[®] (SD) IGS BR strain (ordered at 5-6 weeks of age and 120-175 g bodyweight for males and 100-150 g for females) were obtained from Charles River UK Limited, Margate, Kent, England. The animals were allowed a minimum of 12 days acclimatisation, during which time they were inspected daily to check their physical condition. At commencement of the study (Day before treatment commenced) the sixty four young adults of each sex allocated were in the weight range 221 to 286 g for males and 166 to 210 g for females (approximately 7 to 8 weeks of age).

Environmental control

The animals were housed inside a barriered, limited access, rodent facility.

Each animal room was kept at positive pressure with respect to the outside by its own supply of filtered fresh air which was passed to atmosphere and not re-circulated. The system was designed to change the air at least 15 times per hour and this is checked periodically. Target ranges within the study room were 19-25°C for temperature and 40-70% for relative humidity. There were no excursions from these ranges that were considered to have affected the outcome of the study. Lighting was controlled to provide a 12-hour light : 12-hour dark cycle, with the lights on at 06:00 GMT.

The facility was designed and operated to minimise the entry of external biological and chemical agents and to minimise the transference of such agents between rooms. Before each study the room was cleaned and disinfected with a bactericide.

Access was limited to authorised personnel who were required to shower and change into clean protective clothing. Where practicable, materials and equipment entered the facility through an autoclave or a chamber in which their external surfaces were treated with a bactericide.

Alarms were activated if there was any failure of the ventilation system, or if temperature limits were exceeded.

A stand-by electricity supply was available to be automatically brought into operation should the public supply fail.

Animal accommodation

Rats were housed in TR 18 cages from Arrowmigh Biosciences, Hereford, England, or RB3 modified cages from North Kent Plastic Cages Ltd, Erith, Kent, England. The cages consisted of stainless steel (TR 18) or high density polypropylene (RB3) bodies with lids and floors of stainless steel grid and were suspended in batteries over trays covered with absorbent paper which was replaced twice weekly or

daily during pairing. Wood shavings used as bedding from late gestation onwards (Lignocel Type 3/4) were sterilised by autoclaving and changed at least twice a week. Cages, food hoppers and water bottles were changed at appropriate intervals.

The cages were distributed on the racking to equalise, as far as possible, environmental influences amongst the groups. At various stages of the study the maximum number of rats per cage was:

Stage	Number of rats		Cage type	Cage flooring (cage dimensions cm)
	M	F		
F0 generation				
Acclimatisation and treatment period for unmated adult males and females	Up to 4	Up to 4	TR18	Stainless steel grid (50.5×36×20)
Mating	1	1	RB3 modified	Stainless steel grid (39×22.5×19.5)
Gestation	-	1	RB3 modified	Stainless steel grid (39×22.5×19.5)
(up to GD 20)				
Littering (from GD 20 to PND 14-18)	-	1 + litter	RB3	Solid polypropylene (39×22.5×20)
Lactation (from PND 14-18 lactation until weaning)		1 + litter	TR18	Stainless steel grid (50.5×36×20)
F1 generation				
From weaning	Up to 4	Up to 4	TR18	Stainless steel grid (50.5×36×20)

Diet and water supply

A commercially available pelleted rodent diet, UAR VRF1 Certified, manufactured by Usine d'Alimentation Rationale, France and supplied by Charles River UK Limited, Margate, Kent, England, was freely available.

Tap water taken from the public supply (Essex and Suffolk Water Company, Chelmsford, Essex, England) was freely available via polycarbonate bottles fitted with sipper tubes.

Quality control of wood shavings, diet and water

Certificates of analysis were routinely received from the wood shavings bedding supplier. These specified the levels of a range of chemical and microbiological contaminants. At approximately six-month intervals bedding was analysed, by a laboratory independent of the supplier, for selected chlorinated pesticides, polychlorinated biphenyls, lead, copper and *Salmonella* spp.

Each batch of diet was routinely analysed by the supplier for various nutritional components and chemical and microbiological contaminants. The supplier's analytical certificates were scrutinised and approved before any batch of diet was released for use.

The quality of the water supply is governed by regulations published by the UK Department of the Environment. Certificates of analysis were routinely received from the supplier. At approximately

six-month intervals water was routinely sampled for analysis for selected chlorinated and organophosphorus pesticides, polychlorinated biphenyls, lead and cadmium contaminants; it was also examined for coliform bacteria.

Certificates of analysis for the batches of diet used on the study, and for the drinking water are presented in Addenda 1 and 2 (pages 255-261).

No other specific contaminants that were likely to have been present in the wood shavings, diet or water were analysed, as none that may have interfered with or prejudiced the outcome of the study was known.

Mating procedure

Females were paired on a one-to-one basis with stock males of the same strain. Each morning following pairing, the trays beneath the cages were checked for ejected copulation plugs and a vaginal smear was prepared from each female and examined for the presence of spermatozoa.

The day on which a sperm positive vaginal smear or at least three copulation plugs were found was designated GD 0.

Allocation to treatment groups

Females showing unequivocal evidence of mating were allocated to group and cage position in sequence, thus ensuring that animals mated on any one day were evenly distributed amongst the groups as far as possible.

Adult males and females which were not mated were allocated as follows for each sex: the animals were weighed and separated according to weight into bodyweight ranges (5 g blocks). Animals were then taken from each block and assigned in rotation to treatment group. Excess animals with grossly atypical bodyweights were discarded.

Each animal was assigned a number and identified by tail tattoo.

Composition and identity of treatment groups

The identity of treatment groups, duration of treatment and animal numbers was:

Group	Treatment	Dosage (mg/kg/day)#	Number of animals/sex	Animal numbers
1	Control	0	19F	1-9A, 10-19B
2	Dimethoate	0.1	19F	20-28A, 29-38B
3	Dimethoate	0.5	19F	39-47A, 48-57B
4	Dimethoate	3	19F	58-66A, 67-76B
5	Untreated	0	8F	77-84
6	Control	0	16F	85-92C, 93-100D
			16M	201-208C, 209-216D
7	Dimethoate	0.1	16F	101-108C, 109-116D
			16M	217-224C, 225-232D
8	Dimethoate	0.5	16F	117-124C, 125-132D
			16M	233-240C, 241-248D
9	Dimethoate	3	16F	133-140C, 141-148D
			16M	249-256C, 257-264D

Expressed in terms of the test substance as supplied.

F females; M Males

A Treated from GD 6 to GD 20 inclusive.

B Treated from GD 6 to PND 10.

C Treated for one day only
D Treated for 11 consecutive days

For Groups 1-4 inclusive: Nine adult females were dosed from GD 6 to GD 20 inclusive. Ten adult females were treated from GD 6 to PND 10. Offspring from 8 of these ten litters per group were treated from PND 11 to PND 21 inclusive. These litters were: Group 1: 10, 11, 12, 13, 14, 16, 17 and 19; Group 2: 29, 30, 31, 32, 33, 34, 35 and 36; Group 3: 48, 49, 50, 51, 52, 53, 54 and 55; Group 4: 67, 68, 69, 70, 72, 73, 74 and 76

Group 5: These adult females were undosed throughout the study. On PND 11, one male and one female offspring per litter were assigned to each of the following dose groups for treatment on PND 11: Control group; 0.1 mg/kg/day; 0.5 mg/kg/day; 3 mg/kg/day. The procedure for assigning pups to treatment groups was as follows: the male and female pup with the lowest within-litter identity numbers within each sex were assigned to the Control group; the male and female pup with the second lowest identity numbers were assigned to the 0.1 mg/kg/day group; the male and female pup with the third lowest identity numbers were assigned to the 0.5 mg/kg/day group and, the male and female pup with the highest identity numbers were assigned to the 3 mg/kg/day group.

Groups 6-9 inclusive: Eight naïve adult male and female rats were dosed for one day. The remaining naïve 8 males and 8 females in each group were dosed for 11 consecutive days.

TREATMENT

Test material

The dimethoate used on the study was from batch number 20522-00 supplied by the Sponsor. It was supplied as a white solid, 150 g of which was received at the Huntingdon Research Centre on 8 May 2000.

Before use the identity, strength, purity and composition, or other characteristics which appropriately defined the batch from which the test material for this study was drawn, were determined by the Sponsor. Stability of the test material and methods of synthesis, fabrication or derivation were documented by the Sponsor (a certificate of analysis is presented in Addendum 3, page 262-264).

The test material was stored frozen at approximately -20 °C.

Dosing Solutions

Fresh solutions were prepared each week. The highest required concentration (0.6 mg/ml) was prepared by adding the required amount of dimethoate to a suitable amount of the vehicle, water for formulation (water obtained by reverse osmosis). The solution was mixed using a magnetic stirrer. The lower concentrations of 0.1 and 0.02 mg/ml were then prepared by serial dilution. A bulk volume of each solution was made up each week and then divided into appropriate aliquots for administration to the different groups on the study as required.

Quality control of dosage form

Before the start of treatment, the suitability of the proposed mixing procedure was determined and, specimen 0.02 and 2 mg/ml solutions analysed to assess the stability of dimethoate solutions as part of the Developmental Neurotoxicity Study (Study CHV/069). Specimen solutions were shown to be stable for up to 2 days at ambient temperature or 15 days at 4 °C.

For solutions prepared for use during the first week of treatment and the first week of lactation, single samples (nominally 20 ml) were taken from each formulation; 2 assays were made from each sample for determination of achieved concentration of dimethoate. Results are presented in the Solution Chemistry Report - Addendum 4, page 265.

Administration and treatment

Doses were administered to adult males and females, mated dams and selected offspring by oral gavage once daily at approximately the same time each day at a dosage volume of 5 ml/kg/day.

Adult males and females were dosed for one day or eleven consecutive days as appropriate.

Mated dams were dosed from GD 6 to GD 20 or, PND 10.

Offspring from eight litters in each of Groups 1-4 were dosed from PND 11 to PND 21 inclusive.

Offspring from litters in group 5 were dosed on PND 11.

For adult males and females, individual dose volumes were calculated from the most recently recorded scheduled bodyweight.

For mated dams, individual dose volumes were calculated from the most recently recorded scheduled bodyweight up to and including GD 17; thereafter the dosage volume remained constant to PND 1. From PND 1 dose volumes were calculated, once again, from the most recently recorded bodyweight. Animals that were in parturition at the time of dosing were not dosed. Animals that had completed parturition and could be dosed (prior to day 1) were weighed and the weight recorded and dose volume adjusted and recorded. Where day 1 weighing was scheduled for later on the same day the earlier weight was accepted as the day 1 weight.

For offspring, individual dose volumes were calculated from the most recently recorded scheduled bodyweight.

Controls received the vehicle (water for formulation - water obtained by reverse osmosis) at the same volume dosage as treated groups.

For the adult males and females and mated dams, a daily record of the usage of each solution was maintained based on weights used. This balance was compared with the expected usage as a check of correct administration - no significant discrepancies were found.

In view of the small dose volumes for the offspring, no records of solution usage were maintained but records were kept of volumes dosed to individual offspring.

Solutions were stirred using a magnetic stirrer before and throughout the dosing procedure.

Adults or pups were generally dosed in ascending group order. However for logistical reasons, on days when timed dosing of adult animals or pups was required, these animals were generally dosed before those not requiring timed dosing.

SERIAL OBSERVATIONS (ADULT MALES AND FEMALES AND MATED DAMS)

General condition and clinical signs

During the acclimatisation period, observations of the animals and their cages were recorded at least once per day.

Following arrival at Huntingdon Life Sciences, all animals were visually inspected at least twice daily for evidence of reaction to treatment or ill-health. Each adult animal was subjected to a full physical examination daily on each day of the study until parturition (if applicable), and then weekly during lactation. Any deviations from normal were recorded at the time in respect of nature and severity, date and time of onset, duration and the progress of the observed condition, as appropriate.

In addition, detailed observations were performed, on each day of treatment, at the following times during the day:

Pre-dose observation;

As each animal was returned to its home cage;

At the end of dosing each group;

One to two hours after completion of dosing all groups;

As late as possible in the working day.

The complete set of observations were not performed where the protocol required animals to be killed at 2 or 3 hours after dosing.

Bodyweight

Adult males and females (Groups 6-9) were weighed on the day before treatment commenced and then daily to termination.

Mated females were weighed on GDs 0, 3, 6, 10, 14, 17 and 20, then daily until parturition. During lactation, females were weighed on PND 1, 4, 7, 11, 14, 17 and 21. For gestation, bodyweights are only presented up to GD 20; weights recorded between GD 20 and parturition are not presented (because weights were not recorded for all animals due to differences in the time of the start of parturition) but retained with the rest of the raw data for the study.

Parturition and the duration of gestation

From GD 20, the females in Groups 1-5 allocated to litter were checked three times daily for evidence of parturition. The females were permitted to deliver their young naturally and rear their own offspring until PND 21 (Groups 1-4) or Day 11 (Group 5). Approximate numbers of live and dead offspring were recorded during the parturition process. The time elapsing between the detection of mating and commencement of parturition was reported as the duration of gestation.

POST-NATAL OBSERVATIONS (F₁ OFFSPRING)**Observations/procedures at PND 1**

All offspring were examined at approximately 24 hours after birth (PND 1) and the following were recorded for each litter:

- Number of offspring (live and dead);
- Individual offspring bodyweights;
- Sex ratio;
- Observations on individual offspring.

Pups were uniquely identified within each litter on PND 1, by toe marking.

Signs

Offspring were examined on each day from PND 1 to PND 21 for general clinical signs.

On each day of dosing observations were made in association with treatment according to the following schedule:

- Pre-dose observation;
- As each pup was returned to its home cage;
- At the end of dosing each group;
- As late as possible in the working day.

Selected F₁ offspring were subjected to a full physical examination weekly from weaning to termination.

Mortality and litter size

Daily records were maintained of mortality and consequent changes in litter size. The offspring were given individual within-litter identification marks on PND 1 by toe tattoo. Litters were culled to 8 (4 males and 4 females when possible) on PND 4.

Sex ratio

The sex of the offspring was determined on PNDs 1, 4 and 21 (Groups 1-4) and on PNDs 1, 4, and 11 (Group 5).

Bodyweight

The live offspring in each litter were weighed individually on PNDs 1, 4, 7, 11, 14, 17, 21 and 28, then weekly until termination and on PND 60 (where appropriate).

Identification of F₁ animals from PND 21

All offspring were assigned a unique four-digit animal number with the first two digits indicating the dam identity and the last two digits the within-litter pup identity e.g. pup 7 from litter 1 was assigned the number 0107 and pup 13 from litter 71 was numbered as 7113.

F₁ offspring were identified with their four digit number by tail marking with a permanent marker between PNDs 21 and 28.

At 4 weeks of age animals were identified with their four digit number by tail tattoo.

TERMINAL PROCEDURES

Measurement of plasma, erythrocyte and brain cholinesterase activity

Samples were obtained as follows:

Group (s)	Day	Samples	Animals
Groups 1-4	GD 20	Blood and brain	8 Dams per Group and fetuses. Dams were killed 3 hours after dosing.
Groups 1-4	PND 4	Blood and brain	Up to two male and two female pups in each litter were killed 4 hours after dosing of the dam.
Group 5	PND 11	Blood and brain	All offspring in each litter were killed 2 hours after dosing.
Groups 6-9	day 1 of treatment	Blood and brain	8 males and 8 females per group were killed 2 hours after dosing.
Groups 1-4	PND 21	Blood and brain	One male and one female offspring in each litter (up to 8 litters per group) were killed 2 hours after dosing.
Groups 6-9	day 11 of treatment	Blood and brain	8 males and 8 females per group were killed 2 hours after dosing.
Groups 1-4	PND 60	Blood and brain	8 males and 8 females per group were killed.

Blood samples were collected from the retro-orbital sinus under light isoflurane anaesthesia (all adult animals and PND 21 pups) or umbilical cord (GD 20 fetuses). For the PND 4 and PND 11 pups blood samples were obtained following decapitation. Lithium heparin was used as the anticoagulant and the sample volume was circa 0.7 ml.

Blood samples were pooled for all fetuses within each litter, providing one sample per litter.

For the adult animals and PND 21 pups, blood samples were collected into appropriately labelled lithium heparin tubes. These tubes were then placed inside another plastic tube and these tubes were then cooled on water ice and taken to the Clinical Pathology Department for processing and centrifugation. The resultant plasma samples and red cell haemolysates were stored at -80 °C prior to shipment packed in dry ice to Huntingdon Research Centre for analysis. For the PND 4 and PND 11 pups, blood samples were collected into labelled heparinised microtainers (without gel).

For the GD 20 fetuses, blood samples were collected from individual fetuses into heparinised microhaematocrit tubes. For each litter, microhaematocrit tubes containing blood samples from all fetuses were placed in an appropriately labelled sterilin tube. The sterilin tubes were then cooled on water ice and taken to the Clinical Pathology Department for processing and centrifugation. For each litter, the contents of the microhaematocrit tubes from the fetuses were expelled into an appropriately labelled plastic tube and the pooled contents centrifuged. The resultant plasma samples and red cell haemolysates were stored at -80 °C prior to shipment packed in dry ice to Huntingdon Research Centre for analysis.

Samples were analysed for plasma cholinesterase and erythrocyte acetyl cholinesterase.

For the adult animals and fetuses on GD 20, following blood collection, brains were removed, weighed at necropsy and brain cholinesterase activity measured. Brains and brain weights were pooled for all fetuses in each litter.

For the male and female pups which were dosed in each litter, following blood collection, the brains were removed and weighed at necropsy and brain cholinesterase activity measured.

For the adult animals and pups, individual brains were wrapped in aluminium foil and then snap frozen in liquid nitrogen and then placed in appropriately labelled plastic bags. The bags were then stored in dry ice prior to transfer to storage at -80 °C.

For the GD 20 fetuses, each pool of brains was wrapped in aluminium foil and then snap frozen in liquid nitrogen and then placed in appropriately labelled plastic bags. The bags were then stored in dry ice prior to transfer to storage at -80 °C.

The method of analysis for Cholinesterase was a modified Ellman method (US EPA, Federal Register April 26, 1996; Vol 61: (82), 18593, Notice: Standard Operating Procedure for Measuring Cholinesterase in Laboratory Rats and Dogs Exposed to Non-reversible Cholinesterase Inhibitors).

The erythrocyte cholinesterase activity was measured using 6,6'-Dithiodinitrobenzoic Acid (DTNA) as the colour component on the Hitachi 911 chemistry analyser.

The plasma and brain cholinesterase activity was measured using DTNB as the colour component on the Hitachi 911 chemistry analyser.

Necropsy procedures

GD 20 - dams and fetuses

On GD 20, the females were killed immediately after blood sampling (if appropriate) by inhaled carbon dioxide for examination of their uterine contents. The brain was removed, weighed and snap frozen in liquid nitrogen as previously described. At the same time, the reproductive tract, complete with ovaries, was dissected out and the following recorded:

- a) Number of corpora lutea in each ovary (assessed before removal);
- b) Number of implantation sites;
- c) Number of resorption sites (classified as early or late);
- d) Number and distribution of fetuses in each uterine horn.

Fetuses were dissected from the uterus and sexed. Blood samples were then obtained from the umbilical cord as detailed in the section on cholinesterase analysis. Fetuses were then weighed and the brains dissected out and weighed as previously described in the section on cholinesterase analysis.

Fetuses were killed by chilling on a cool plate at approximately 0°C.

The dams were subjected to a detailed macroscopic necropsy. The animals were examined externally and internally for macroscopic abnormalities. Specimens of any abnormal tissues were retained in appropriate fixative.

Adult males and females on day 1 or day 11 of treatment or on PND 60

The animals were killed immediately after blood sampling by inhaled carbon dioxide. The brain was removed, weighed and snap frozen in liquid nitrogen as previously described.

The animals were subjected to a detailed macroscopic necropsy. The animals were examined externally and internally for macroscopic abnormalities. Specimens of any abnormal tissues were retained in appropriate fixative.

PND 4 and PND 11 offspring selected for blood and brain sampling and cholinesterase analysis

The pups were killed by decapitation. Following blood sampling the brain was removed, weighed and snap frozen in liquid nitrogen as previously described.

The animals were subjected to a detailed macroscopic necropsy. The animals were examined externally and internally for macroscopic abnormalities. Specimens of any abnormal tissues were retained in appropriate fixative.

PND 4 offspring not selected for blood/brain sampling and sporadic deaths in early neonates

Offspring culled on PND 4 and not selected for blood sampling were killed by intraperitoneal injection of barbiturate and discarded without further examination.

Sporadic deaths in early neonates were also discarded without further examination.

PND 21 - offspring selected for blood and brain sampling

On PND 21, the animals were killed immediately after blood sampling by inhaled carbon dioxide. The brain was removed, weighed and snap frozen in liquid nitrogen as previously described.

The animals were subjected to a detailed macroscopic necropsy. The animals were examined externally and internally for macroscopic abnormalities. Specimens of any abnormal tissues were retained in appropriate fixative.

Dams allocated to litter

On or shortly after PND 21 (Groups 1-4) or, on PND 11 (Group 5), the animals were killed by inhaled carbon dioxide. The animals were subjected to a detailed macroscopic necropsy. The animals were examined externally and internally for macroscopic abnormalities. Specimens of any abnormal tissues were retained in appropriate fixative. The number of uterine implantation sites was recorded for the adult females.

Excess offspring at weaning

The protocol required that excess offspring were killed by inhaled carbon dioxide and discarded without further examination. Excess weanlings were in fact subjected to macroscopic necropsy examination; this deviation from the requirements of the study protocol was considered to have no impact on the integrity of the study and the necropsy findings are reported.

Eight offspring allocated to kill on PND 61 - five offspring allocated to gravity perfusion and neuropathology

Five offspring were killed on PND 61 by receiving an intraperitoneal injection of a lethal dose of barbiturate. The heart was exposed to permit *in situ* gravity perfusion with fixative (glutaraldehyde and paraformaldehyde from a reservoir approximately 90 cm above the work bench) via the left ventricle. Fixation was completed by immersion in glutaraldehyde and paraformaldehyde.

Following perfusion, animals were subjected to a macroscopic necropsy examination. Specimens of any abnormal tissues were retained in fixative. The brain was removed and subsequently embedded in paraffin wax.

Samples of sciatic and tibial nerves were taken. After primary fixation in glutaraldehyde and paraformaldehyde, the tissues were washed in phosphate buffer followed by secondary fixation in 1% osmium tetroxide.

Sections of brain and nerves were prepared, stained and examined by light microscopy as follows:

Tissue/sections	Areas sectioned and examined by light microscopy
Brain 4-5 μ m sections stained with haematoxylin and eosin	Coronal sections: Olfactory lobes Forebrain Cerebrum, hippocampus, thalamus, hypothalamus Cerebrum, tectum, tegmentum Medulla oblongata Mid-sagittal sections: Cerebellum, Pons
Sciatic and tibial nerves 2 μ m sections stained with toluidine blue	
Sciatic nerve	Longitudinal and transverse sections at the sciatic notch and mid-thigh
Tibial nerve	Longitudinal and transverse sections of calf muscle branch(es)
	Longitudinal and transverse sections at the knee

Some samples of sciatic nerve were stretched on card in order to see if this improved the presentation of cross-sections of nerve at microscopic examination.

The three animals not used for perfusion were killed by inhaled carbon dioxide and discarded without further examination.

TREATMENT OF DATA

For presentation purposes the values shown in appendices may be rounded. For calculation of group mean values unrounded values may have been used, therefore, it may not always be possible to calculate these values exactly by using values presented in the appendices.

Group values

Data were expressed as group means with standard deviations (SD), where appropriate, calculated according to the formula:

$$SD = \sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}}$$

where x = individual or litter mean values

\bar{x} = group mean value

n = sample size

In the tabulated data the abbreviation SD - for standard deviation - has been commonly used.

In the text sections of the report day x of gestation and day x of lactation/age are identified as GD x and PND x respectively. For labelling of figures, tables and appendices, day of gestation, day of lactation and day of age have been used.

Presentation of bodyweight, brain weight, cholinesterase activity and necropsy data for individual pups

Where this data is presented in relevant appendices of this report individual pups are identified using their unique four digit identity.

Bodyweight

For the adult males and females in Groups 6-9, group mean values and SD have been calculated. Group mean weight changes were calculated and plotted graphically with respect to day 1 of treatment.

For the adult females, group mean values and SD have been calculated for the bodyweights during gestation and lactation. Group mean weight changes were calculated and plotted graphically with respect to GD 6 and PND 1. For gestation, group mean values have been calculated for females with live young at GD 20 or a live litter. Group mean values during lactation have been calculated using data for females with live young on PND 21 (Groups 1-4) or PND 11 (Group 5).

For all the offspring, litter mean and SD values have been calculated for males and females for each litter up to PND 11. Group mean values have been calculated from the individual litter values. Group mean offspring weight change was calculated relative to PND 1.

For the undosed offspring, group mean and SD values have been calculated for males and females for each litter up to PND 21. Group mean values have been calculated from the individual values. Group mean offspring weight change was calculated relative to PND 11.

For the dosed offspring, group mean and SD values have been calculated for males and females for each litter up to PND 60. Group mean values have been calculated from the individual values. Group mean offspring weight change was calculated relative to PND 11.

Gestation index

For each group the gestation index was calculated as :

$$\text{Gestation index} = \frac{\text{Number of live litters born}}{\text{Number pregnant}} \times 100$$

Gestation length

Gestation length was calculated as the number of gestation days up to and including the day on/upon which offspring were first observed, with day 1 being the day of mating for calculation purposes. Where parturition had started overnight, this value was adjusted by subtracting half of one day.

Litter data at GD 20

Litter data group mean values and SD (where appropriate) were calculated for numbers of corpora lutea, implantations, resorptions (early, late and total) and live young (male, female and total) at GD 20. The group mean sex ratio (percentage of males) was also calculated.

Pre-natal losses were considered separately for the pre- and post-implantation phases.

a) Pre-implantation loss

Pre-implantation loss was calculated from the formula:

$$\frac{(\text{Number of corpora lutea} - \text{Number of implantations})}{\text{Number of corpora lutea}} \times 100$$

b) Post-implantation loss

Post-implantation loss was calculated from the formula:

$$\frac{(\text{Number of implantations} - \text{Number of live fetuses})}{\text{Number of implantations}} \times 100$$

Group values were calculated using litter mean values. The number of implantations was substituted for the corpora lutea count in calculating pre-implantation loss where the number of implantations exceeded the corpora lutea count.

Group mean fetal and litter weights

Group mean fetal weights and SD were calculated for each group as:

$$\frac{\text{Total of individual litter mean fetal weights}}{\text{Number of litters}}$$

Mean fetal weights and SD were also calculated for each sex.

Group mean litter weights and SD were calculated for each group as:

$$\frac{\text{Total of individual litter weights}}{\text{Number of litters}}$$

Litter size after birth and survival indices

The following survival indices for each litter was calculated:

$$\text{Post-implantation survival index} = \frac{\text{Total number of offspring born}}{\text{Total number of implantation sites}} \times 100$$

$$\text{Live birth index} = \frac{\text{Number of live offspring on PND 1}}{\text{Total number of offspring born}} \times 100$$

$$\text{Viability index} = \frac{\text{Number of live offspring on PND 4\#}}{\text{Number of live offspring on PND 1}} \times 100$$

Before culling

$$\text{Lactation index} = \frac{\text{Number of live offspring on PND 7 or 11}}{\text{Number of live offspring after culling on PND 4}} \times 100$$

Brain weights

GD 20 dams and fetuses

Group mean values and SD were calculated for absolute weights (dams) and also for pooled fetal weights. For pooled fetal weights, means and SD were calculated from individual litter mean values.

Brain weights for adult animals on days 1 or 11 of treatment, and offspring on PNDs 4, 11, 21 and 60

Group mean values and SD were calculated from individual absolute values.

Plasma, erythrocyte and brain cholinesterase activity

Group mean values and SD were calculated from individual animal or pooled fetal values as appropriate.

Statistical evaluation

The Study Protocol indicated that appropriate statistical tests would be performed as required.

Significance tests, employing analysis of variance followed by an inter-group comparison with the control, were performed on the following parameters, and results are presented in relevant tables of this report:

Bodyweight change of adult males and females; bodyweight change of females during lactation; dam and fetal brain weights at GD 20; brain weights for male and female offspring at PND 21; plasma, erythrocyte and brain cholinesterase activity.

Dependent on the heterogeneity of variance between treatment groups, parametric tests (analysis of variance, Snedecor and Cochran 1967) followed by Williams' test (Williams' 1971/2) or non-parametric tests, (Kruskal-Wallis, Hollander and Wolfe 1973) followed by Shirley's test (Shirley 1977) were used to analyse these data, as appropriate. For litter data the basic sample unit was generally the litter and, due to preponderance of non-normal distributions, non-parametric analyses were routinely used. Where 75% or more of the values for a given variable are the same, a Fisher's exact test (Fisher, 1950) was used

All significant (i.e. $p < 0.05$) inter-group differences from the control are reported only where supported by a significant analysis of variance (i.e. $p < 0.05$).

RESULTS

Solution chemistry (Addendum 4 - pages 265-277)

The mean concentrations for dimethoate in test solutions analysed during the study were within $\pm 2\%$ of nominal values confirming accurate preparation.

GENERAL TOXICITY FINDINGS

Young adult males and females - Groups 6 to 9

Clinical signs and mortality (Appendix 1 – page 111)

There were no general clinical signs which were considered to be related to treatment.

No post-dosing signs were observed and there were no mortalities.

Bodyweight and bodyweight change (Figures 1 to 4, Tables 1 to 4 - Appendices 2 and 3, pages 43-46, 64-67, 112-119)

There was no conclusive effect of treatment on bodyweight or bodyweight gain of males or females.

Treatment of males at 3 and 0.1 mg/kg/day was associated with a slight reduction in bodyweight gain throughout the treatment period, although this was not statistically significant. There was no effect of treatment on males at 0.5 mg/kg/day. In females, treatment at all dosages was associated with a slight, but not statistically significant, reduction in bodyweight gain in the early treatment period.

Brain weights (Tables 5 and 6, Appendices 4 and 5 - pages 68 and 69, 120 and 121)

There was no effect of treatment on absolute brain weight of males or females on Days 1 or 11 of treatment.

Necropsy findings (Appendix 6 - pages 122 - 125)

Necropsy of male and female animals on Days 1 or 11 of treatment showed no findings which were considered to be treatment related.

Parent females and offspring

Clinical signs and mortality-parent females (Appendix 7 - pages 126 - 130)

There were no general clinical signs which were considered to be related to treatment. Signs were generally restricted to staining of the coat and hairloss which were seen in animals at a similar frequency throughout all groups.

No post-dosing signs were observed and there were no mortalities.

Bodyweight and bodyweight change during gestation and lactation (Figures 5 to 8, Tables 7 to 10, Appendices 8 and 9 – pages 47-50, 70-73, 131-140)

There was no effect of treatment on bodyweight or bodyweight gain during gestation; group mean values for all treated groups and the untreated group were similar to the control throughout.

There was no obvious adverse effect of treatment on bodyweight or bodyweight change during lactation. At 3 mg/kg/day, the magnitude of the expected weight loss during PNDs 17 to 21 was slightly less than the control, resulting in a slightly higher (although not statistically significant) overall bodyweight gain from PND 1 to 21.

Gestation length and gestation index (Figure 9, Table 11, Appendix 10 – pages 51, 74 and 141)

There was no effect of treatment on gestation length or gestation index.

Litter data on GD 20 (Tables 12 and 13, Appendices 12 and 13 – pages 75 and 76, 147-152)

All females allocated to the interim kill at GD 20 were confirmed to be pregnant with live young.

There was no adverse effect of treatment upon the mean numbers of corpora lutea, implantations or the growth and survival of the fetuses; sex ratio, expressed as percentage males was similar for all groups. Group mean litter weights and overall fetal weights in treated groups were comparable to the Control.

Necropsy findings for adult females (Appendix 11 – pages 142-146)

Macroscopic examination of females at necropsy on GD 20, and on PND 21 for those females which were allowed to litter, revealed no findings which were considered to be related to treatment.

Brain weights for dams and fetuses on GD 20 (Table 14, Appendix 14 – pages 77 and 153 and 154)

There was no definite effect of treatment on brain weights of dams on GD 20. Group mean values at 3 and 0.5 mg/kg/day were slightly, although not statistically significantly lower than the control.

Brain weights for fetuses on GD 20 were unaffected by treatment; group mean values for all treated groups were similar to the control.

Litter data up to PND 11 (Figures 10 to 13, Tables 15 to 21, Appendices 15 to 19 and 24 – pages 52-55, 78-84, 155-179, 196-197)

All females allocated to the littering phase of the study were pregnant and gave birth to live young.

The number of implantation sites, total litter size at PND 1 and survival of the offspring through to PND 11 of age was unaffected by treatment; group mean values for all treated groups were similar to the control throughout.

Sex ratio of the offspring, expressed as percentage males was comparable in all groups.

Offspring survival was excellent. Offspring in a few litters showed clinical findings such as small in size, hairloss or bruising of skin but there was no relationship to treatment.

Group mean bodyweight and bodyweight gain for both male and female offspring from PND 1 through to PND 11 of age were unaffected by treatment; values for all treated groups were similar to the control throughout.

Clinical signs and mortalities for dosed male and female offspring (Appendices 24 and 29 – pages 196 and 197 and 208-222)

There were no general clinical signs which were considered to be related to treatment. A few animals showed minor signs such as brown staining of the coat or hairloss but there was no relationship to treatment.

There was no effect of direct treatment on the survival of offspring. There was one mortality among dosed offspring and this occurred in the control group: Female 1408 was killed for humane reasons on PND 12 due to a dome shaped head. Necropsy of this animal revealed that the skin of the head had detached from the cranium and resulted in a pocket filled with air. The thoracic cavity contained a large amount of white gelatinous material with a small amount of clotted blood adjacent to the diaphragm.

No post-dosing signs were observed.

Bodyweight and bodyweight change for dosed and undosed male and female offspring (Figures 14 to 21, Tables 22 to 29, Appendices 20 to 23 – pages 56-63, 85-92, 180-195)

There was no effect of direct treatment on bodyweight or bodyweight gain of male or female offspring. Bodyweight up to PND 11 was similar for all groups, and bodyweight gain from the start of treatment and up to PND 60 in all treated groups remained similar to the control throughout. Comparison with undosed offspring in Groups 1 to 4 up to PND 21 confirmed this, as overall bodyweight gain for both treated and untreated offspring was comparable throughout.

Brain weights for offspring on PNDs 4, 11, 21 and 60 (Tables 30 to 33, Appendices 25 to 28 – pages 93-96, 198-207)

There was no effect of treatment on absolute brain weight for male and female offspring at PND 4, 11, 21 or 60 of age; group mean values for all treated groups were similar to the control.

Necropsy findings for offspring on PNDs 4, 11, 21 and 60 (Appendix 29 – pages 208-222)

Necropsy of offspring at PNDs 4, 11, 21 and 60 of age revealed no findings which were considered to be related to treatment.

EFFECTS ON PLASMA, ERYTHROCYTE AND BRAIN CHOLINESTERASE ACTIVITY

Naïve Adult males and females - Groups 6 to 9 (Tables 34 and 35, Appendices 30 and 31 – pages 97-100, 223-230)

Treatment with one dose of 3 mg/kg/day dimethoate was associated with decreases in plasma, RBC and brain cholinesterase activity among males (19%, 17% and 12% lower respectively) and among females (13%, 27% and 14% lower respectively); differences attained significance for all parameters in males and RBC and brain cholinesterase activity among females. There was also a marginal but significant decrease in brain cholinesterase activity among males at 0.5 mg/kg/day (4% lower than in controls) but this marginal difference was considered to be of no biological importance.

Treatment of adult males with eleven consecutive doses of 3 mg/kg/day dimethoate was associated with marked and statistically significant decreases in plasma, RBC and brain cholinesterase activity (37%, 58% and 47% lower than in controls respectively). At 0.5 mg/kg/day, plasma, RBC and brain cholinesterase activity were slightly lower than in controls (12%, 17% and 10% lower respectively) with only the difference for brain cholinesterase activity attaining significance; these differences were considered to be of equivocal biological significance.

Treatment of adult females with eleven doses of 3 mg/kg/day was associated with marked and significant decreases in RBC and brain cholinesterase activity (63% and 58% lower than in controls respectively); plasma activity was also decreased compared with controls (21% lower) but the difference did not attain significance. There was also a marginal decrease in RBC and brain cholinesterase activity at 0.5 mg/kg/day (7% and 13% lower than in controls) with the difference for brain cholinesterase activity attaining significance: these differences were considered to be of equivocal biological significance.

Dams and fetuses on GD 20 (Tables 36 and 37, Appendices 32 and 33 – pages 101 and 102, 231-234)

There was a marked and significant decrease in levels of plasma, RBC and brain cholinesterase activity among dams on GD 20 in the 3 mg/kg/day group compared with controls (44%, 58% and 60% lower than in controls respectively). At 0.5 mg/kg/day, plasma, RBC and brain cholinesterase activity were slightly lower than in Controls (14%, 13% and 10% respectively) but the difference only attained significance for cholinesterase activity in the brain; these differences were considered to be of equivocal biological significance.

There was a marked and significant decrease in levels of plasma, RBC and brain cholinesterase activity among fetuses on GD 20 in the 3 mg/kg/day group compared with controls (43%, 31% and 33% lower than in controls respectively). There were also marginal but significant decreases in brain cholinesterase activity among fetuses in the 0.5 and 0.1 mg/kg/day groups (10% and 12% lower than in controls) but these marginal, non dosage-related differences were considered to be of doubtful biological significance.

Offspring on PND 4 (Table 38, Appendix 34 – pages 103 and 104, 235-242)

Among male offspring in the 3 mg/kg/day group there were slight but significant decreases in RBC and brain cholinesterase activity compared with controls (17% and 13% lower respectively). There was also a marginal but significant decrease in brain cholinesterase activity among males at 0.5 mg/kg/day (8% lower than in controls). These differences probably represent a small residual effect of treatment.

Among female offspring in the 3 mg/kg/day group there were slight decreases in plasma and RBC and brain cholinesterase activity compared with controls (10%, 14% and 7% lower than in controls

respectively) with only the difference for plasma cholinesterase activity attaining significance. There was also a marginal but significant decrease in plasma cholinesterase activity among males at 0.5 mg/kg/day (8% lower than in controls). These differences probably represent a small residual effect of treatment.

Offspring on PND 11 (Table 39, Appendix 35 – pages 105 and 106, 243-246)

Treatment of male offspring with one dose of 3 mg/kg/day was associated with slight but significant decreases in plasma and brain cholinesterase activity compared with controls (19% and 17% lower respectively). There was also a marginal decrease in plasma and brain cholinesterase activity among males at 0.5 mg/kg/day (9% and 5% lower than in controls) with the difference for brain cholinesterase activity attaining significance: these marginal differences were considered to be of equivocal biological significance.

Among female offspring dosed once with 3 mg/kg/day there were slight decreases in plasma, RBC and brain cholinesterase activity compared with controls (18%, 26% and 18% lower than in controls respectively) with the difference for brain cholinesterase activity attaining significance.

Offspring on PND 21 (Table 40, Appendix 36 – pages 107 and 108, 247-250)

Treatment of male offspring with eleven consecutive doses of 3 mg/kg/day dimethoate was associated with marked and statistically significant decreases in plasma, RBC and brain cholinesterase activity (39%, 59% and 45% lower than in controls respectively). There was also a marginal decrease in plasma, RBC and brain cholinesterase activity among males at 0.5 mg/kg/day (6%, 9% and 13% lower than in controls) with the difference for brain cholinesterase activity attaining significance: these marginal differences were considered to be of equivocal biological significance. At 0.1 mg/kg/day brain cholinesterase activity was marginally but significantly lower than in controls (4% lower) but this marginal difference was considered to be of no biological importance.

Treatment of female offspring with 3 mg/kg/day was associated with marked and significant decreases in plasma, RBC and brain cholinesterase activity (38%, 65% and 42% lower than in controls respectively). Treatment of females at 0.5 mg/kg/day was associated with a slight but significant decrease in RBC and brain cholinesterase activity (23% and 12% lower than in controls).

Offspring on PND 60 (Table 41, Appendix 37 – pages 109 and 110, 251-254)

There were no biologically significant effects on cholinesterase activity of males or females. Earlier treatment at 3 and 0.5 mg/kg/day was associated with a marginal but significant decrease in brain cholinesterase in female offspring (4% lower than in control) but this was considered to be of no biological importance.

Brain histopathology – five offspring allocated to gravity perfusion and neuropathology on PND 61 (Addendum 5 – pages 278-280)

Examination of the sections produced showed there to be excellent fixation of the tissues with a low level of fixation artefact. In one animal however, blood was present in the capillaries and vessels of the brain sections, indicating inadequate flushing of the animal prior to perfusion of fixative. Another of the animals showed occasional vessels with blood present. There was no noticeable difference in the quality of the fixation of the material for these animals however, and it was considered not to have adversely affected the evaluation of the tissues.

Histopathological examination revealed no significant lesions in the brains or peripheral nerves of the five animals. Comparison of the sciatic nerves which had been stretched on card with those which had not been stretched did not show any consistent differences in the presentation of the nerves.

DISCUSSION

The decreases in cholinesterase activity detected among fetuses and offspring indicate that there has been an effect of treatment.

Percentage decreases in cholinesterase activity among naïve adult animals and offspring on PND 21 were generally greatest for RBC cholinesterase activity. In contrast, the greatest decrease in the GD 20 fetuses was recorded for plasma cholinesterase activity.

Percentage decreases in RBC cholinesterase activity of naïve adult and PND 21 males were similar to those recorded in the respective females.

A comparison of the magnitude of the decreases in cholinesterase activity detected for dams and fetuses on GD 20 reveal that the percentage decrease in plasma cholinesterase activity is similar for dams and fetuses (about 44% lower than in controls). In contrast, the decreases for erythrocyte and brain cholinesterase activity were more marked for the dams compared with the fetuses.

The magnitude of the decreases in cholinesterase activity detected for naïve adult males and females treated at 3 mg/kg/day for 1 day or 11 consecutive days were comparable with those detected for male and female pre-weaning offspring treated at 3 mg/kg/day for 1 day or 11 consecutive days.

CONCLUSION

Within the context of this study it is concluded that the no-observed-adverse-effect-level (NOAEL) for decreased cholinesterase activity in gestating dams and their fetuses is 0.5 mg/kg/day. The magnitude and progression of decreases in cholinesterase activity in juveniles and young adults is comparable and the NOAEL for juvenile and young rats is concluded to be 0.1 mg/kg/day.

Complete recovery of cholinesterase activity was demonstrated at PND 60, 39 days after the end of repeat dosing.

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FIGURE 1

Bodyweight – group mean values for adult males

Group	:	6	7	8	9
Compound	:	Control	-----	Dimethoate-----	-----
Dosage (mg/kg/day)	:	0	0.1	0.5	3

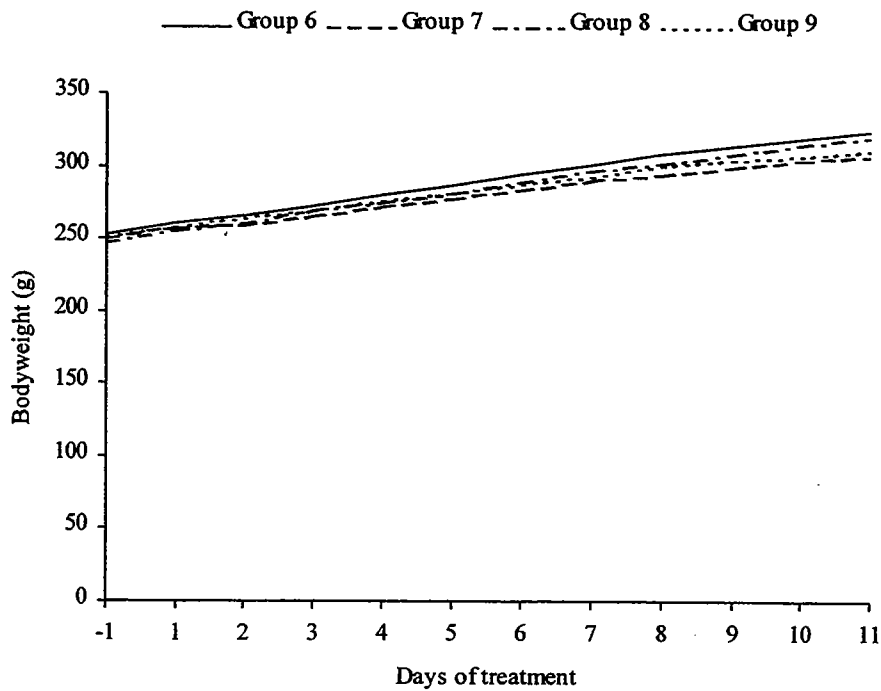


FIGURE 2

Bodyweight change - group mean values for adult males

Group	:	6	7	8	9
Compound	:	Control	-----	Dimethoate-----	
Dosage (mg/kg/day)	:	0	0.1	0.5	3

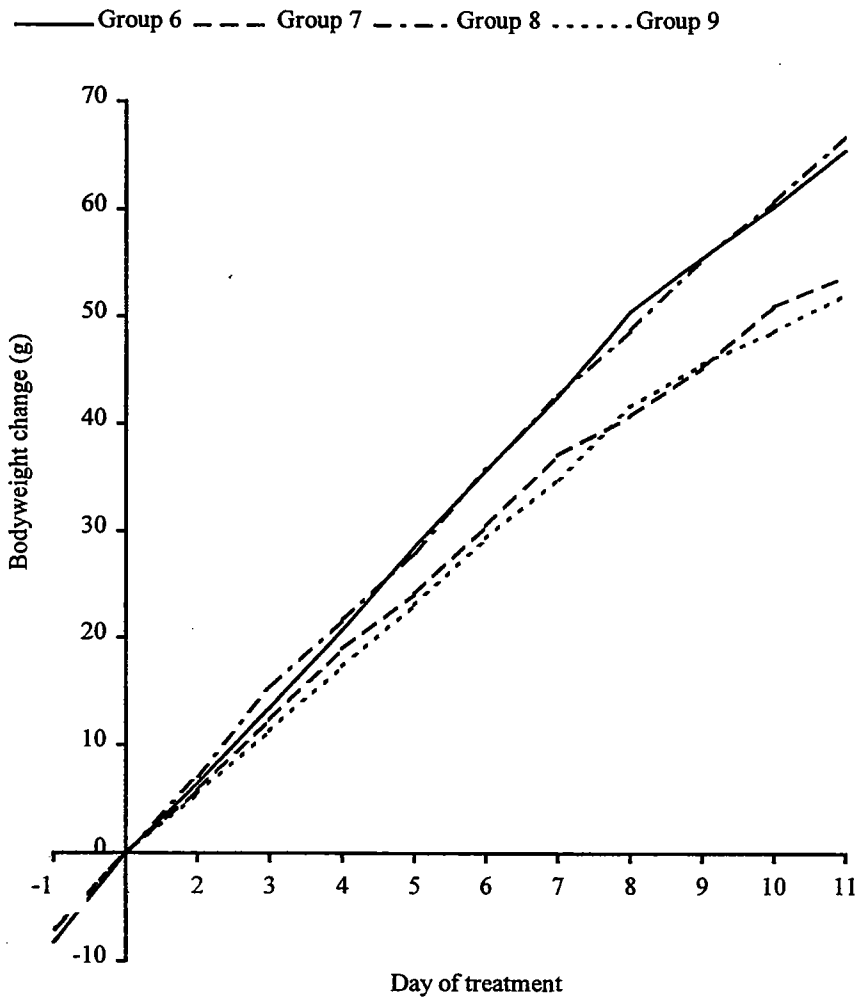


FIGURE 3

Bodyweight – group mean values for adult females

Group	:	6	7	8	9
Compound	:	Control	-----	Dimethoate-----	-----
Dosage (mg/kg/day)	:	0	0.1	0.5	3

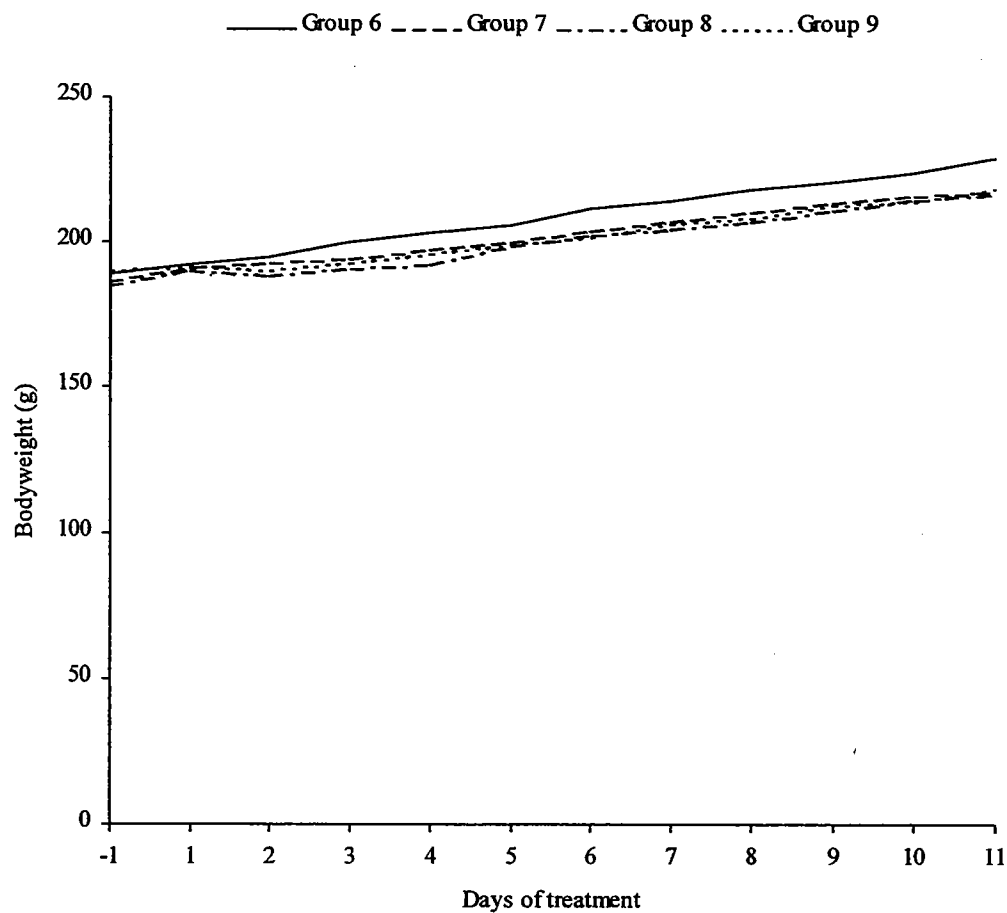


FIGURE 4

Bodyweight change - group mean values for adult females

Group	:	6	7	8	9
Compound	:	Control	-----	Dimethoate-----	
Dosage (mg/kg/day)	:	0	0.1	0.5	3

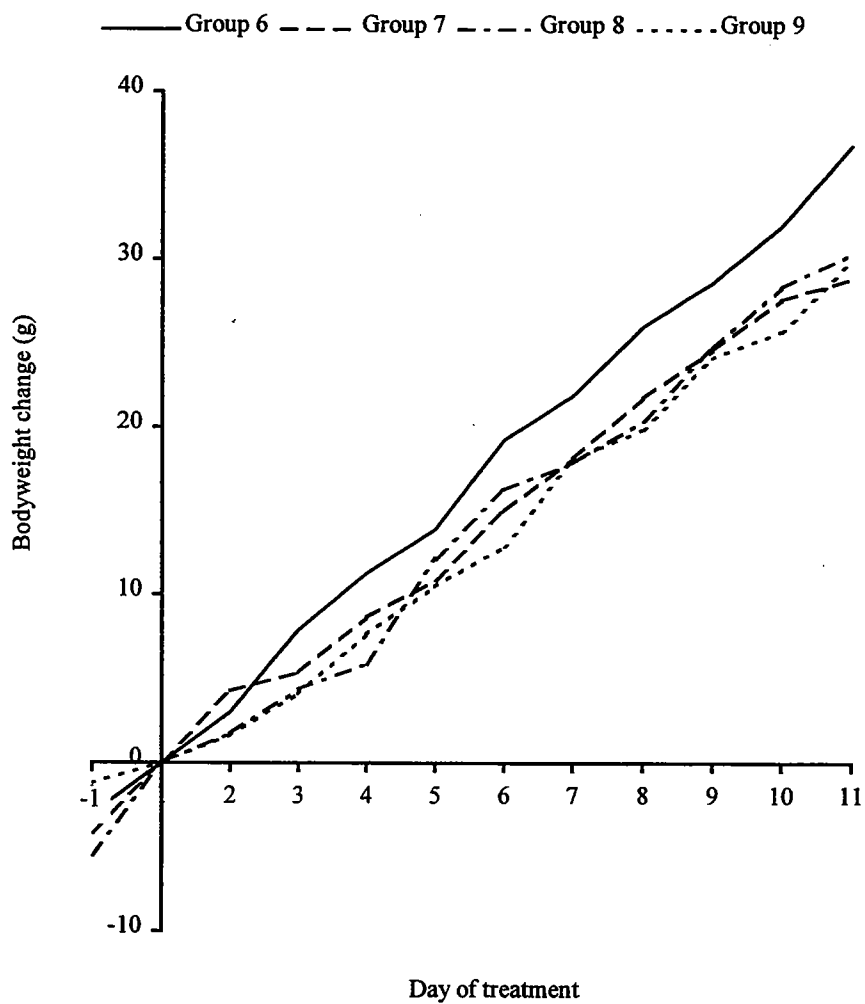


FIGURE 5

Bodyweight – group mean values for dams during gestation

Group	:	1	2	3	4	5
Compound	:	Control	-----	Dimethoate-----		Untreated
Dosage (mg/kg/day)	:	0	0.1	0.5	3	0

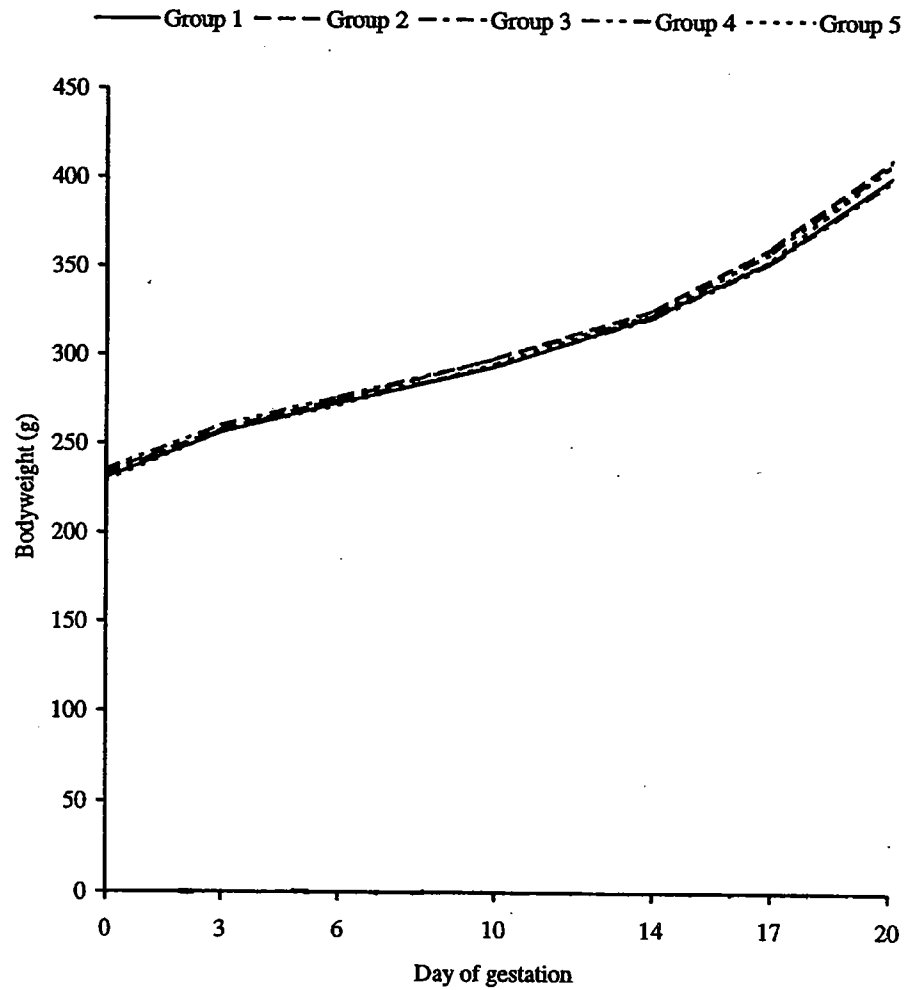


FIGURE 6

Bodyweight change – group mean values for dams during gestation

Group	:	1	2	3	4	5
Compound	:	Control	-----	Dimethoate-----		Untreated
Dosage (mg/kg/day)	:	0	0.1	0.5	3	0

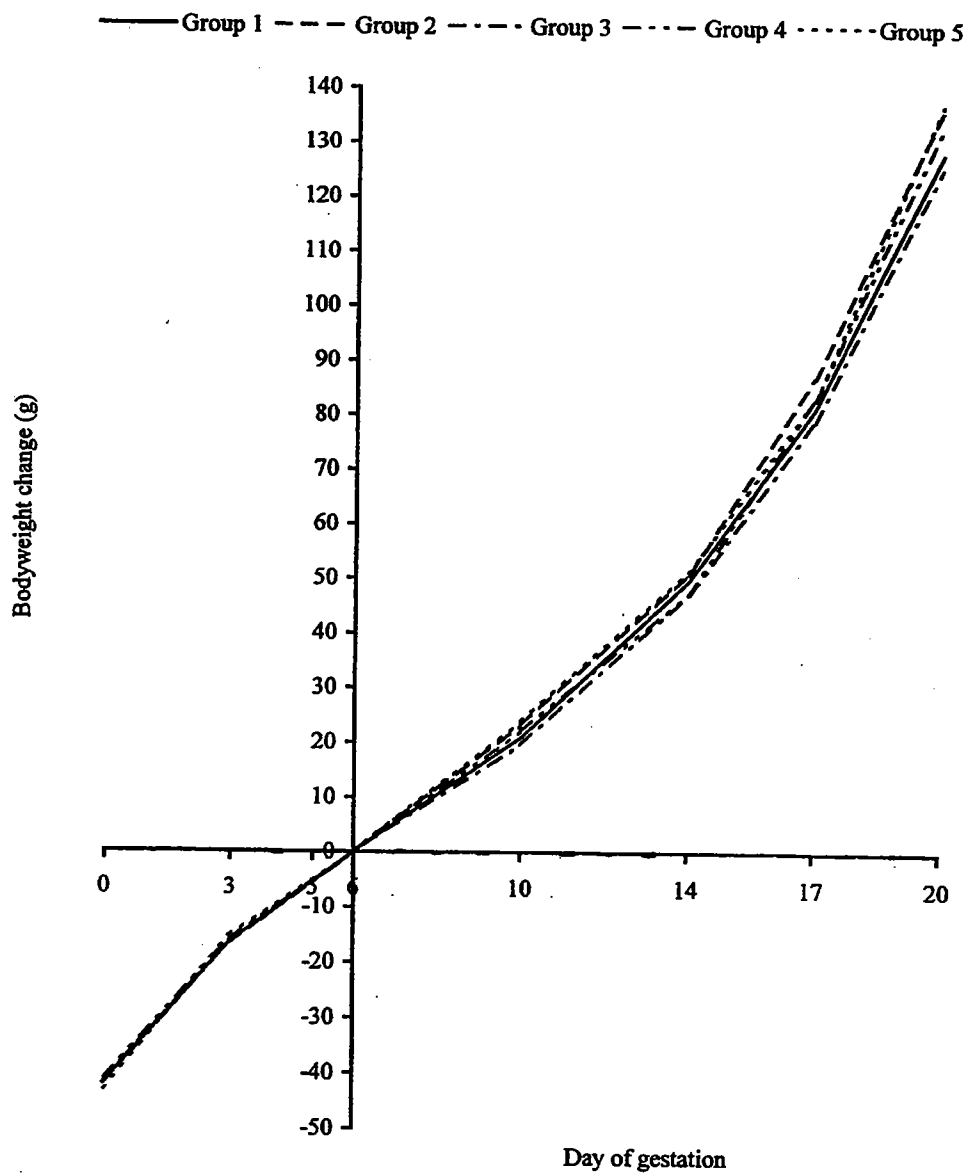


FIGURE 7

Bodyweight – group mean values for dams during lactation

Group	:	1	2	3	4	5
Compound	:	Control	-----	Dimethoate-----		Untreated
Dosage (mg/kg/day)	:	0	0.1	0.5	3	0

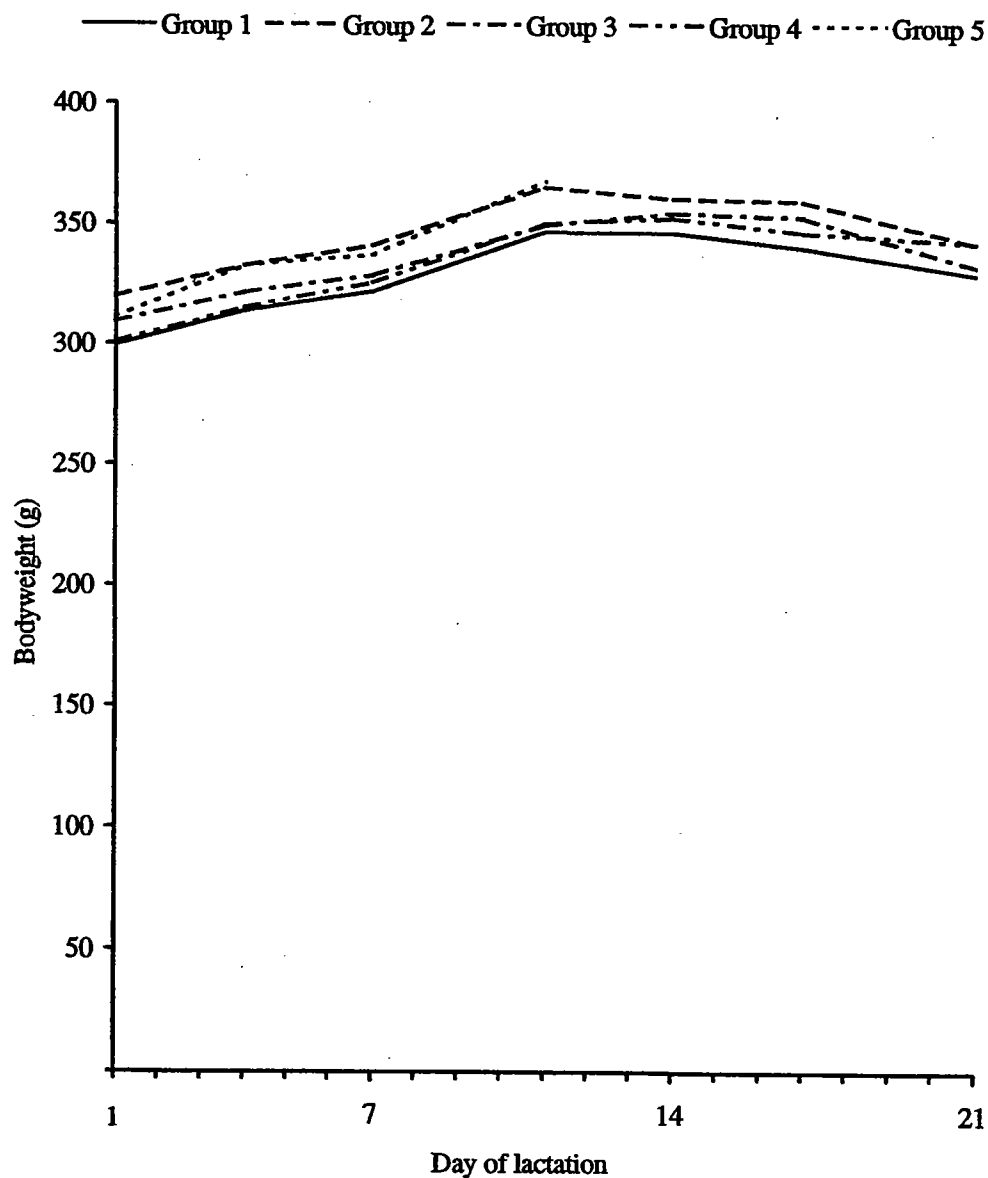


FIGURE 8

Bodyweight change – group mean values for dams during lactation

Group	:	1	2	3	4	5
Compound	:	Control	-----	Dimethoate-----		Untreated
Dosage (mg/kg/day)	:	0	0.1	0.5	3	0

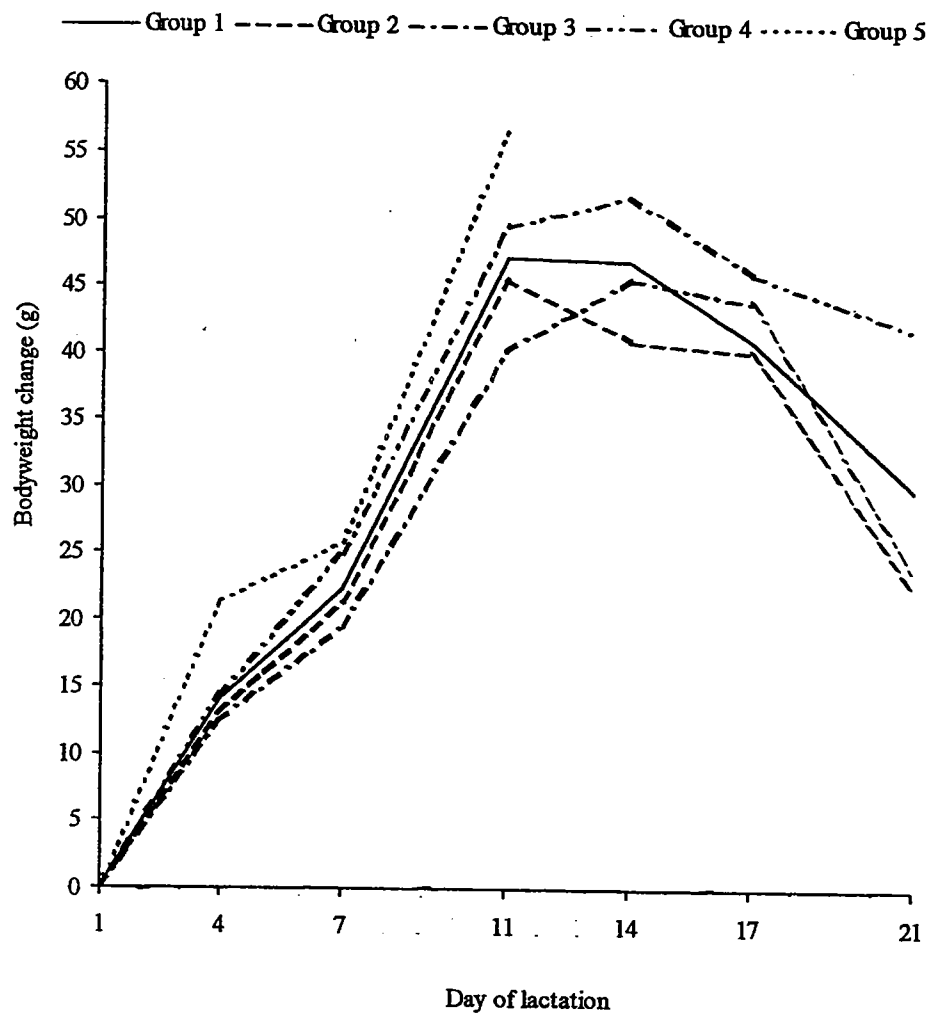


FIGURE 9

Gestation length – group distribution

Group	:	1	2	3	4	5
Compound	:	Control	-----	Dimethoate-----		Untreated
Dosage (mg/kg/day)	:	0	0.1	0.5	3	0

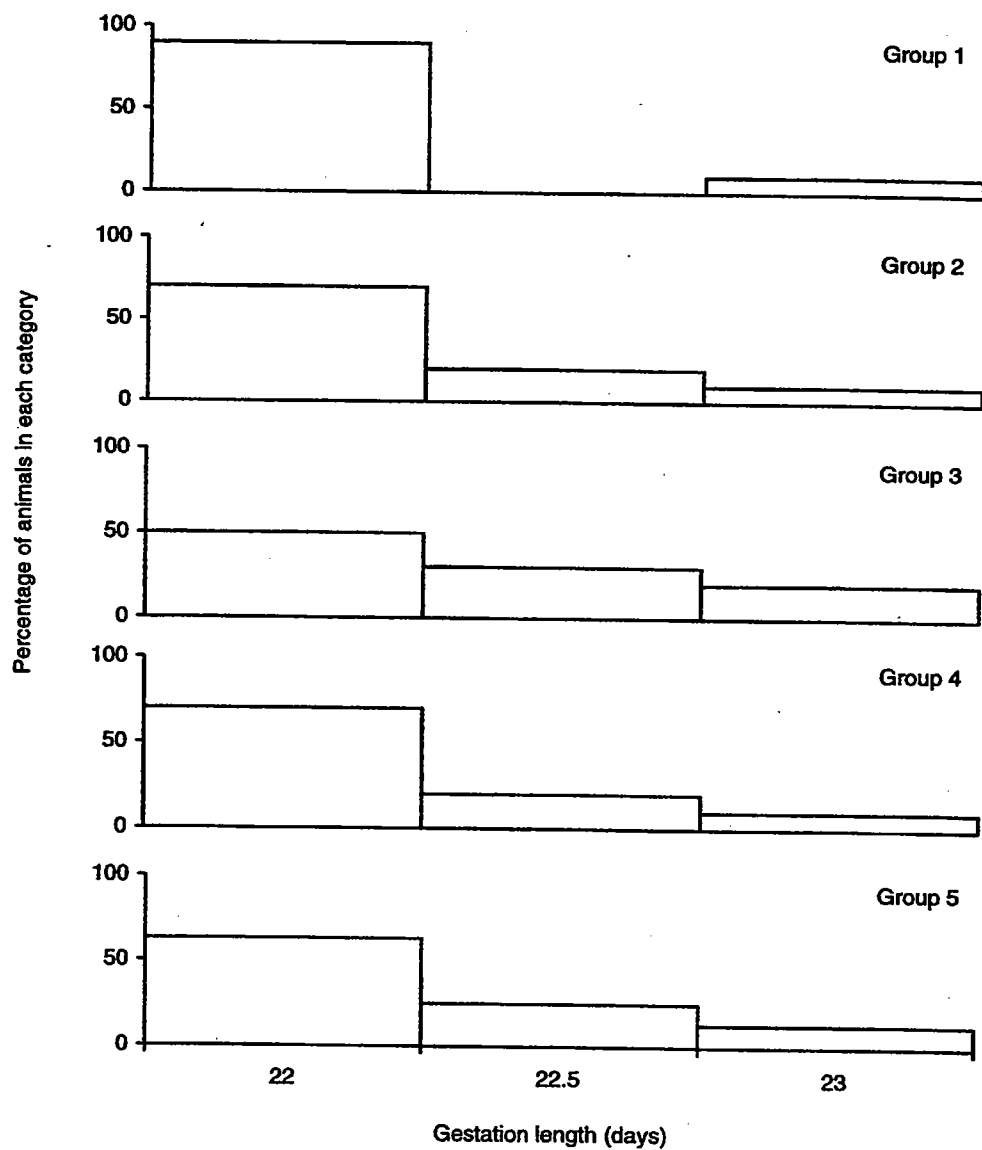


FIGURE 10

Bodyweight up to Day 11 of age – group mean values for male offspring

Group	:	1	2	3	4	5
Compound	:	Control	----- Dimethoate -----			Untreated
Dosage (mg/kg/day)	:	0	0.1	0.5	3	0

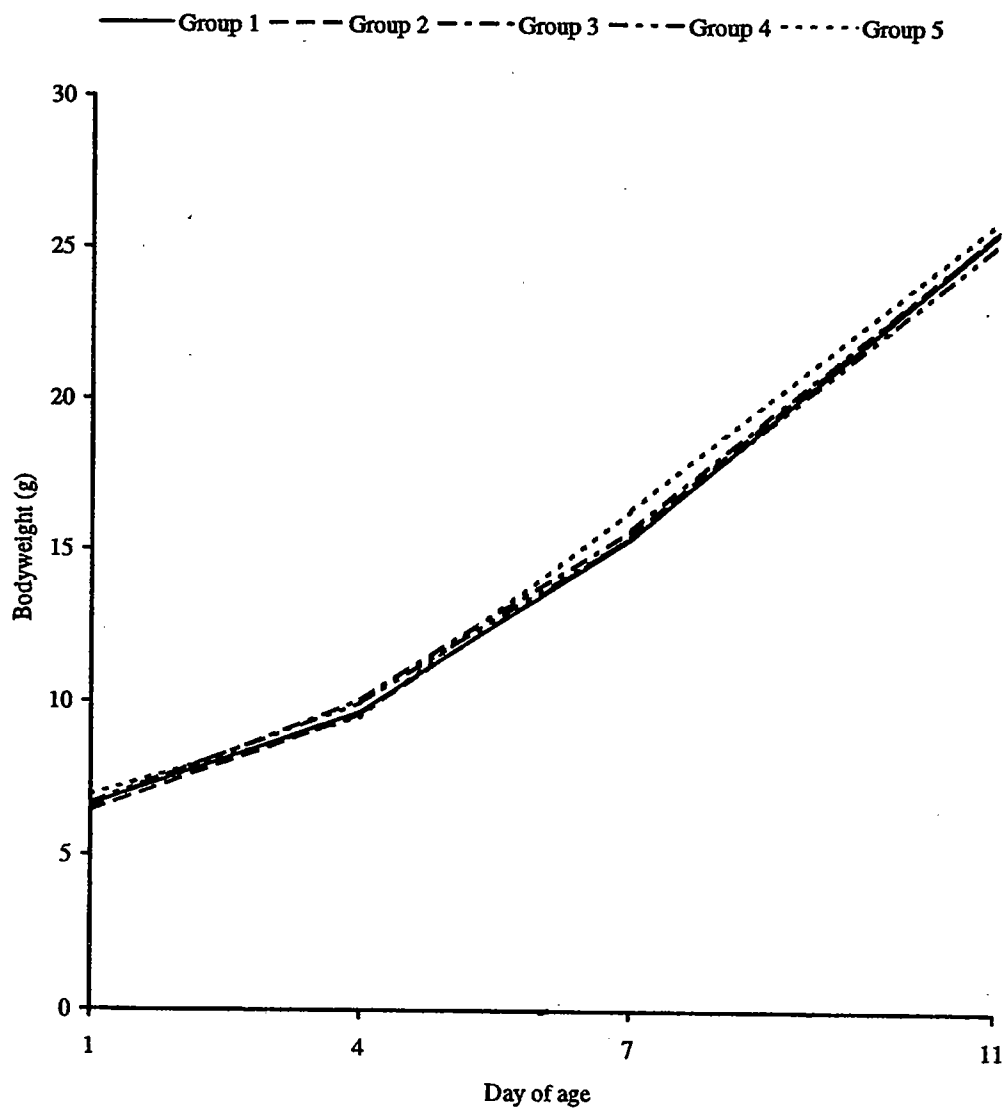


FIGURE 11

Bodyweight change up to Day 11 of age – group mean values for male offspring

Group	:	1	2	3	4
Compound	:	Control	-----	Dimethoate-----	
Dosage (mg/kg/day)	:	0	0.1	0.5	3

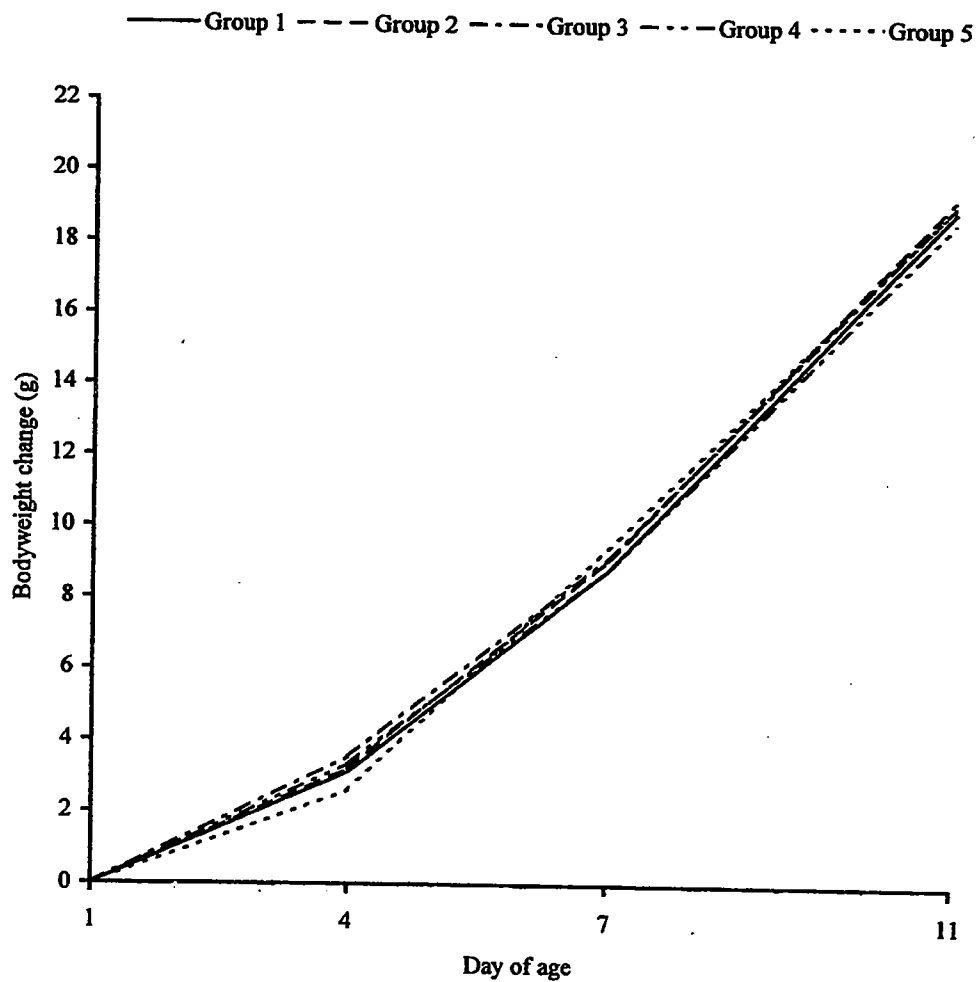


FIGURE 12

Bodyweight up to Day 11 of age – group mean values for female offspring

Group	:	1	2	3	4	5
Compound	:	Control	-----	Dimethoate-----		Untreated
Dosage (mg/kg/day)	:	0	0.1	0.5	3	0

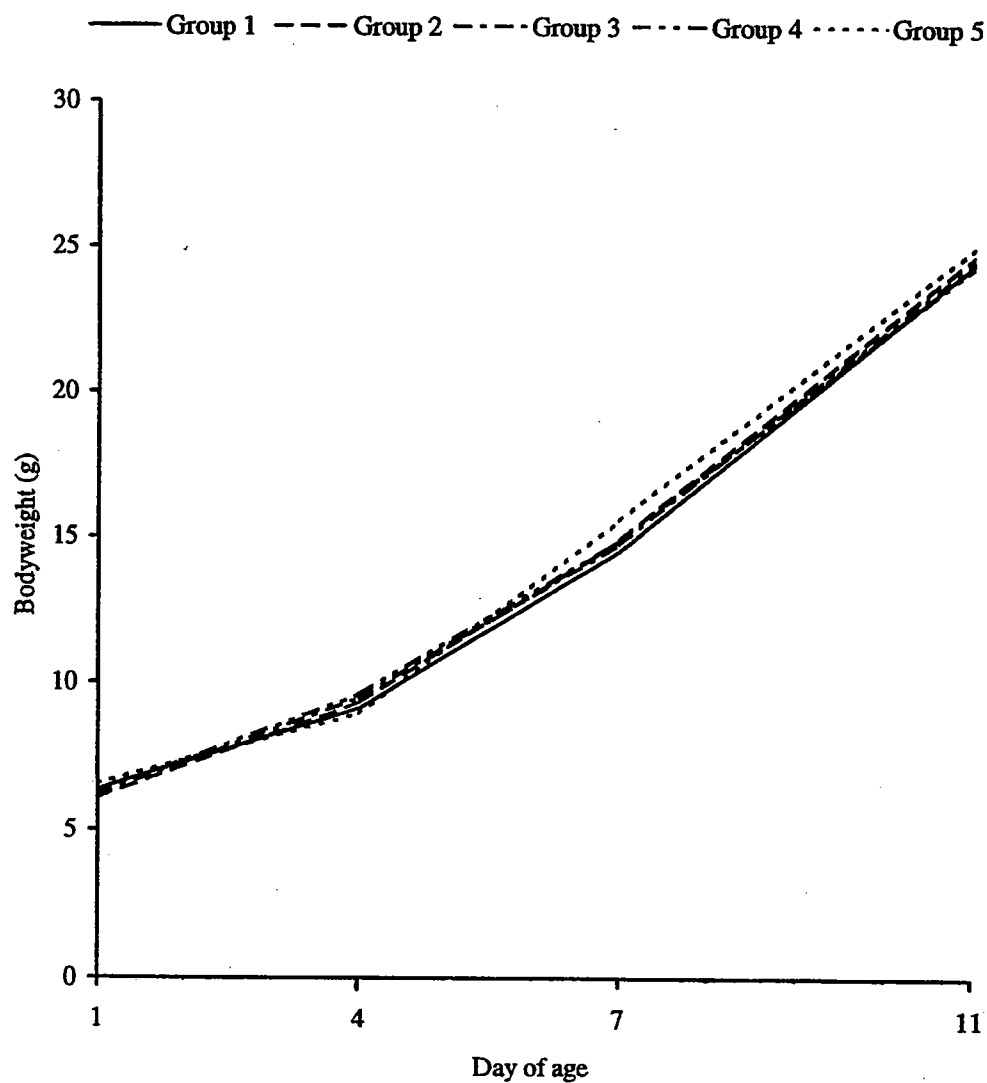


FIGURE 13

Bodyweight change up to Day 11 of age - group mean values for female offspring

Group	:	1	2	3	4
Compound	:	Control	-----	Dimethoate-----	
Dosage (mg/kg/day)	:	0	0.1	0.5	3

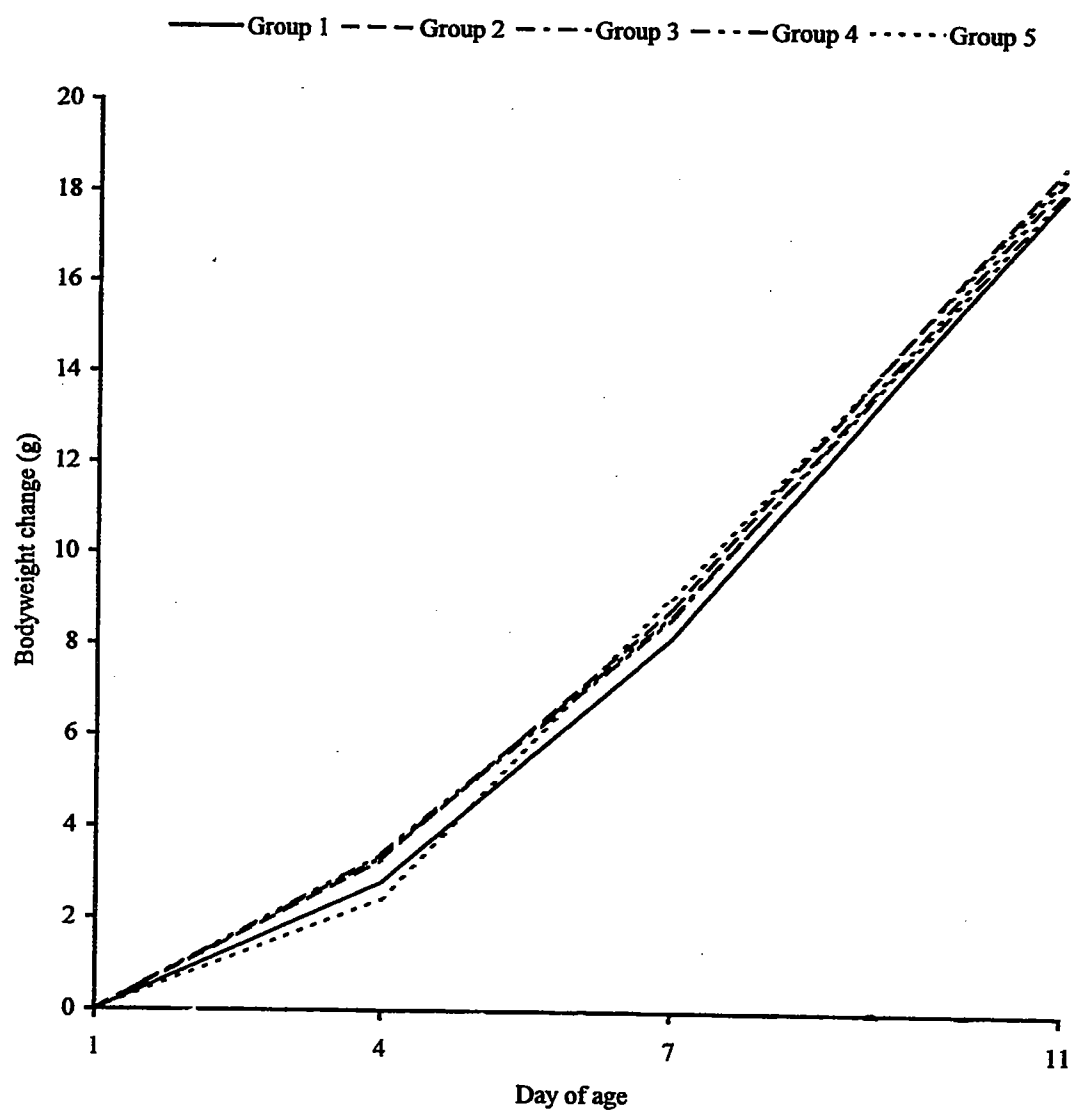


FIGURE 14

Bodyweight – group mean values for dosed male offspring

Group	:	1	2	3	4
Compound	:	Control	-----	Dimethoate-----	
Dosage (mg/kg/day)	:	0	0.1	0.5	3

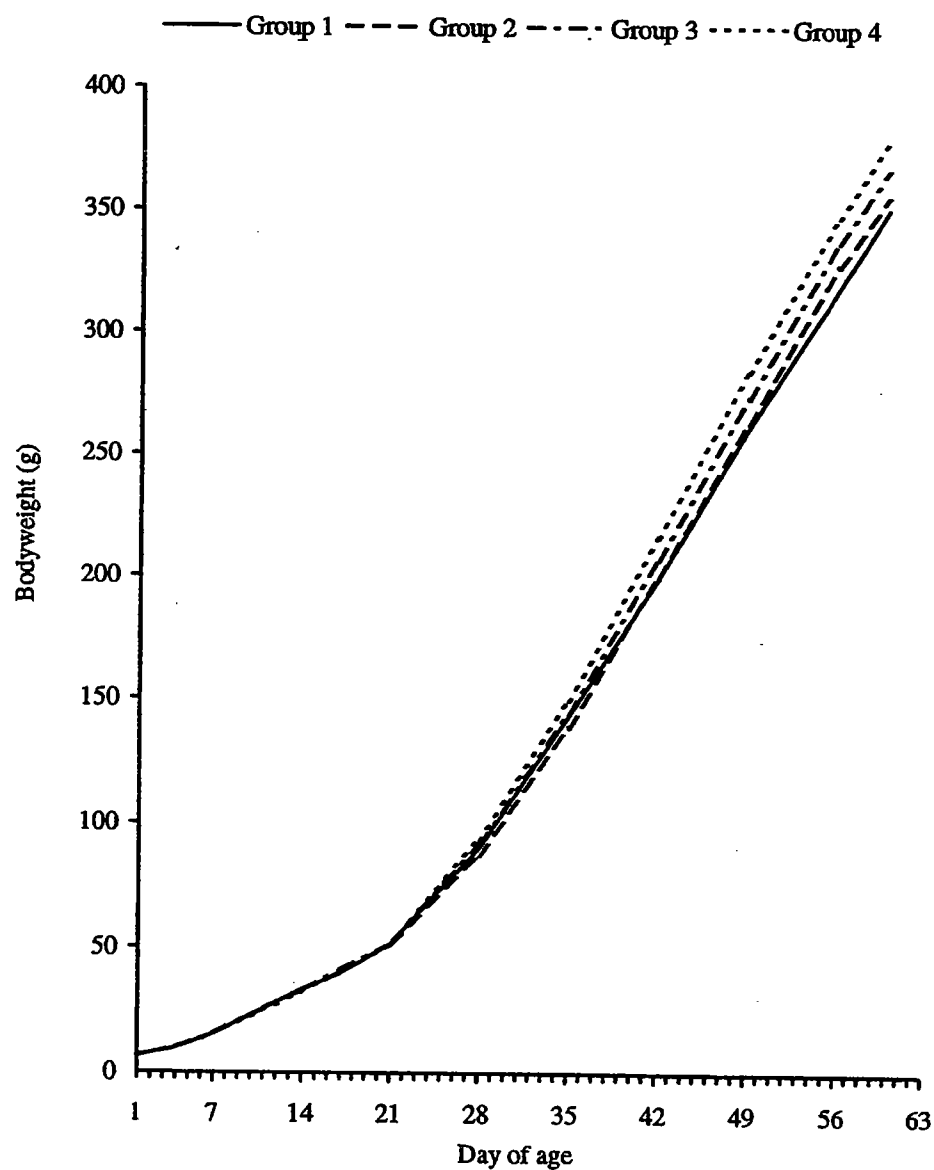


FIGURE 15

Bodyweight change – group mean values for dosed male offspring

Group	:	1	2	3	4
Compound	:	Control	-----	Dimethoate-----	
Dosage (mg/kg/day)	:	0	0.1	0.5	3

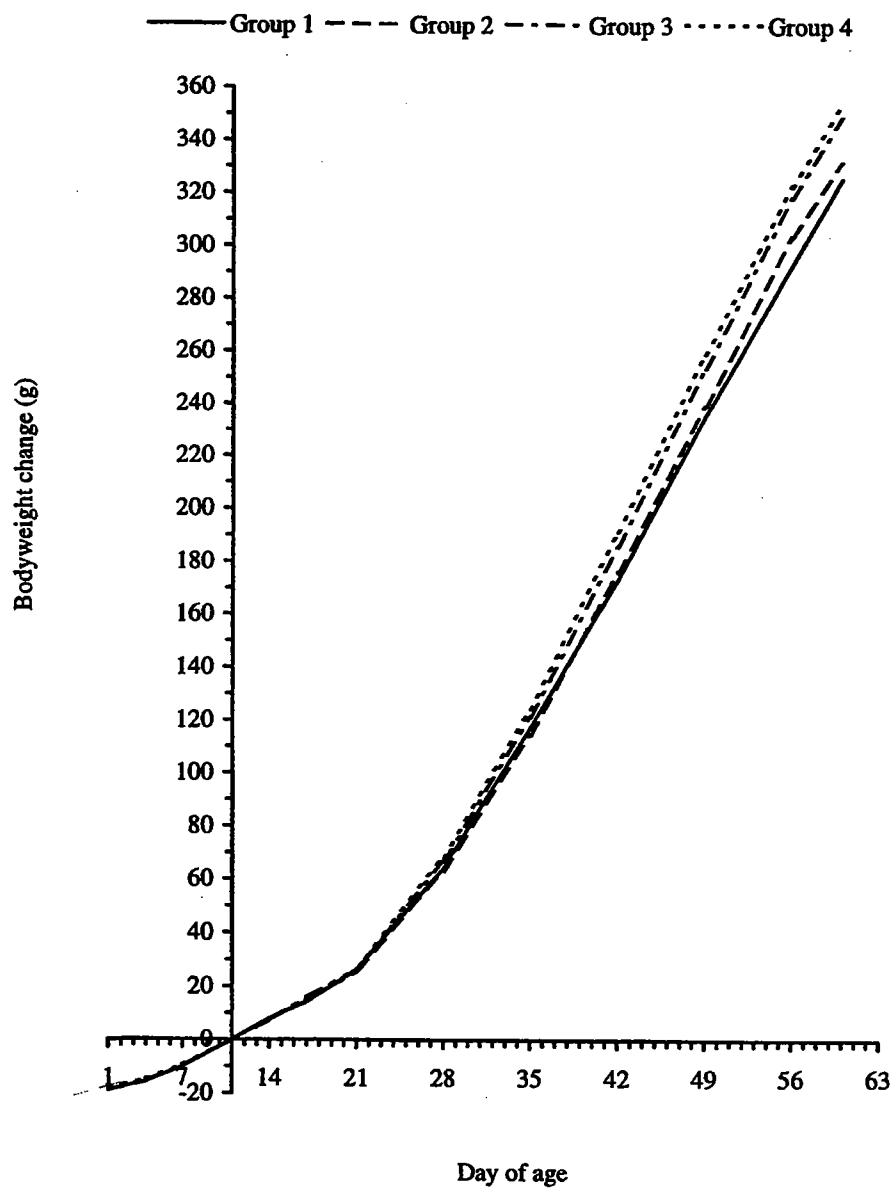


FIGURE 16

Bodyweight – group mean values for undosed male offspring

Group	:	1	2	3	4
Compound	:	Control	-----	Dimethoate-----	
Dosage (mg/kg/day)	:	0	0.1	0.5	3

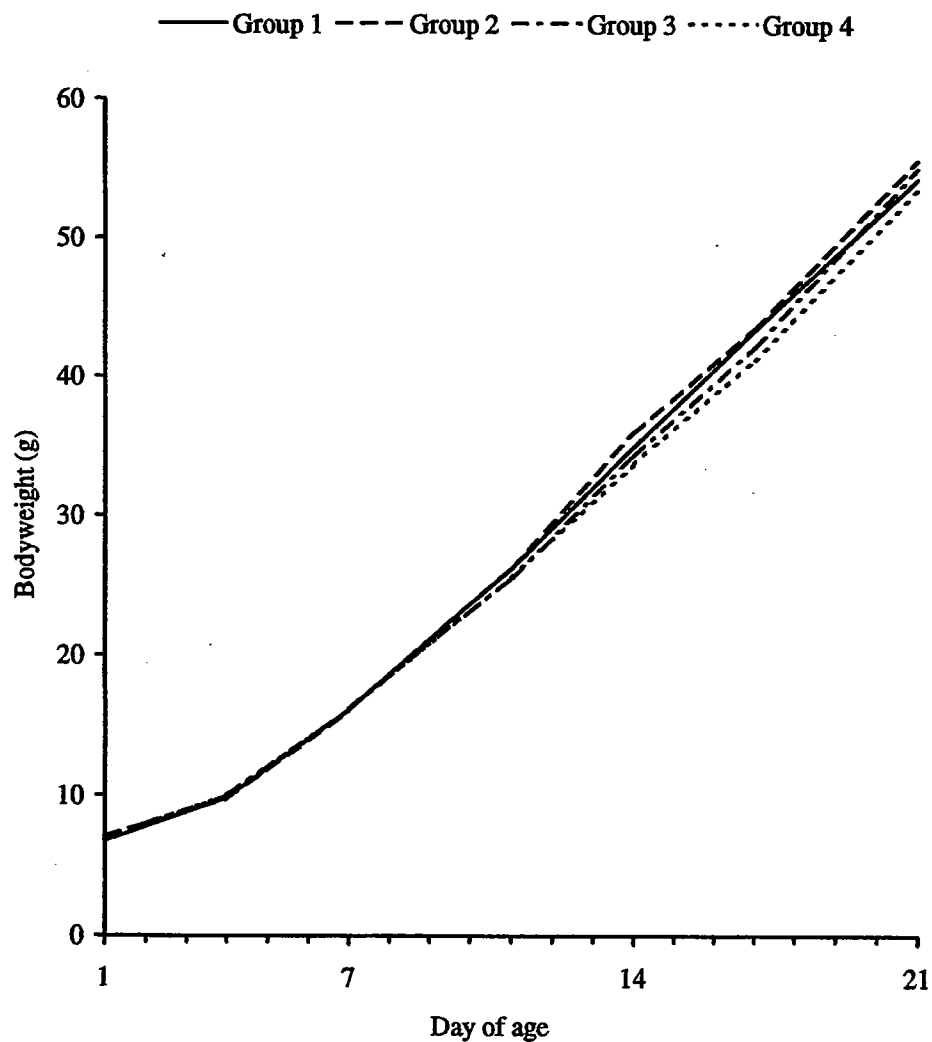


FIGURE 17

Bodyweight change – group mean values for undosed male offspring

Group	:	1	2	3	4
Compound	:	Control	-----	Dimethoate-----	
Dosage (mg/kg/day)	:	0	0.1	0.5	3

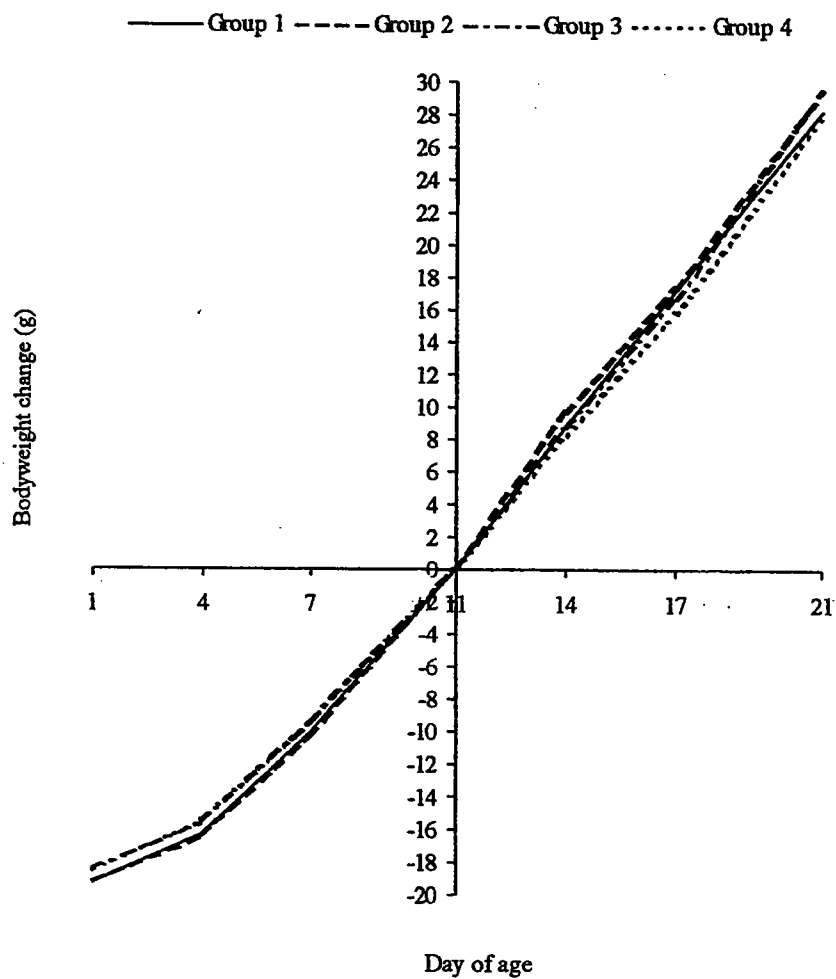


FIGURE 18

Bodyweight – group mean values for dosed female offspring

Group	:	1	2	3	4
Compound	:	Control	-----	Dimethoate-----	
Dosage (mg/kg/day)	:	0	0.1	0.5	3

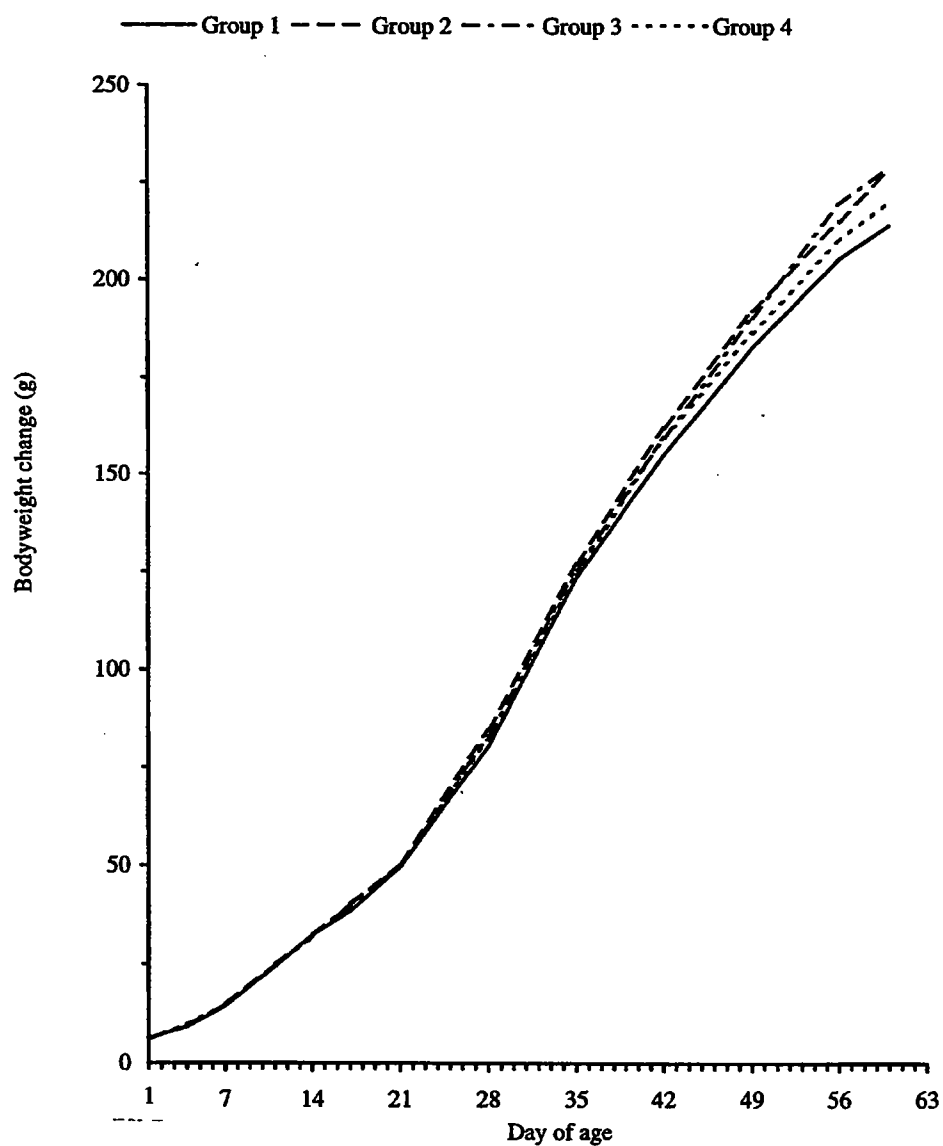


FIGURE 19

Bodyweight change – group mean values for dosed female offspring

Group	:	1	2	3	4
Compound	:	Control	-----	Dimethoate-----	
Dosage (mg/kg/day)	:	0	0.1	0.5	3

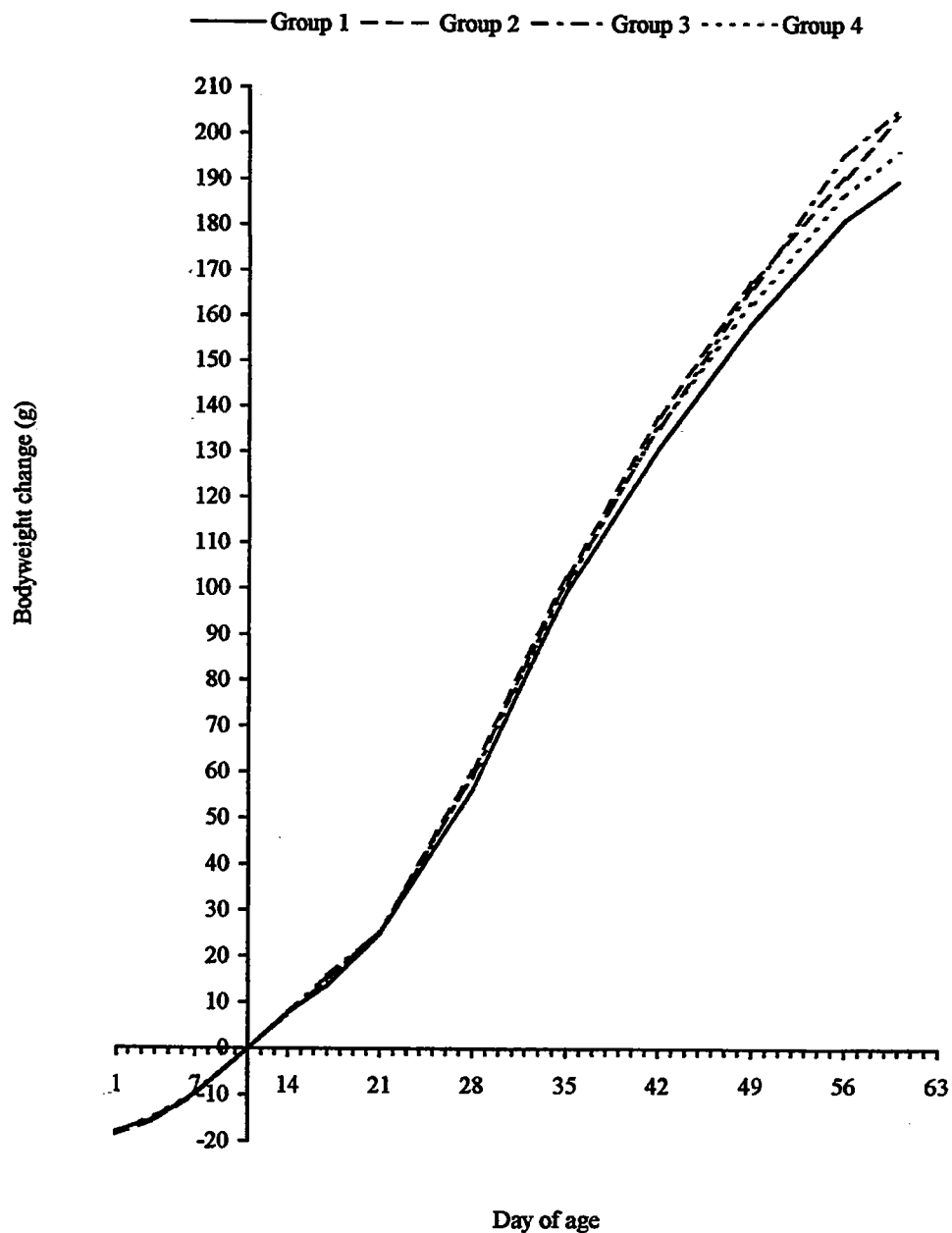


FIGURE 20

Bodyweight – group mean values for undosed female offspring

Group	:	1	2	3	4
Compound	:	Control	-----	Dimethoate-----	
Dosage (mg/kg/day)	:	0	0.1	0.5	3

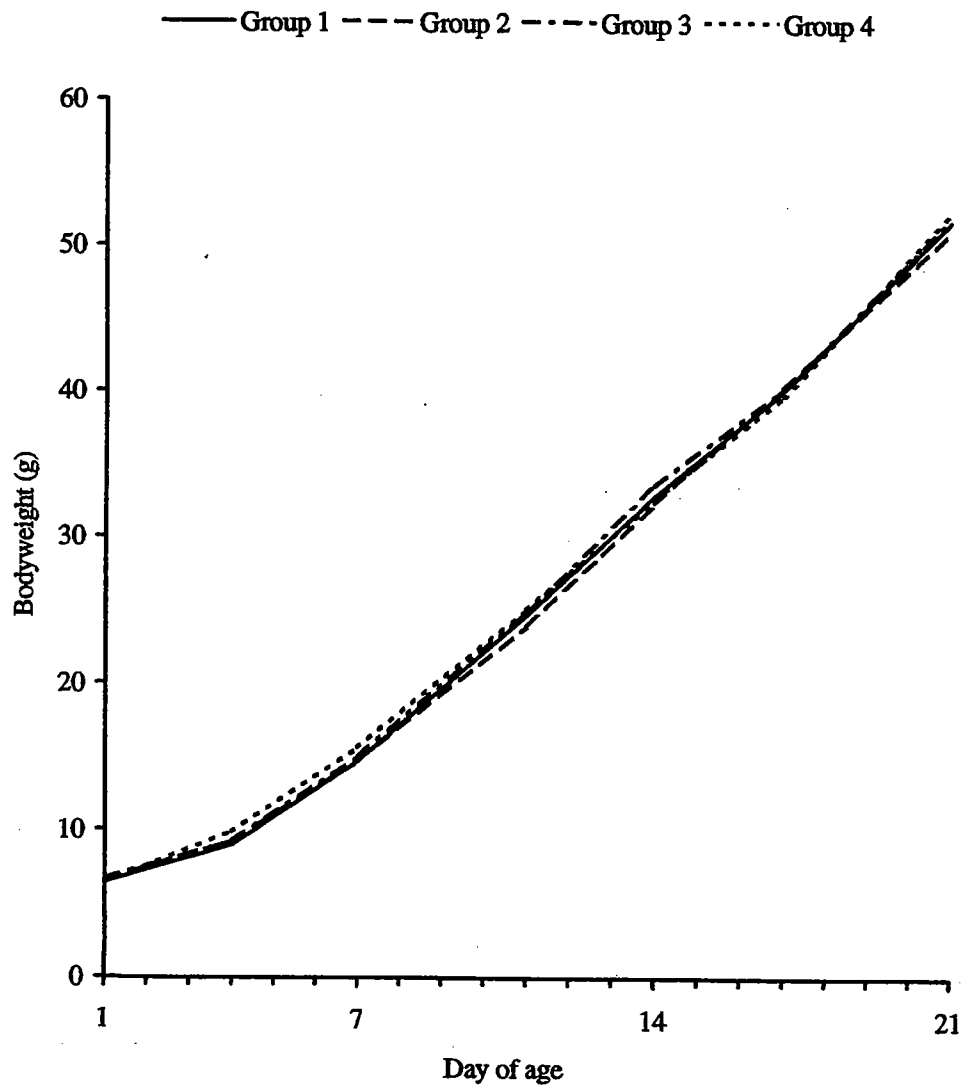


FIGURE 21

Bodyweight change – group mean values for undosed female offspring

Group	:	1	2	3	4
Compound	:	Control	-----	Dimethoate-----	-----
Dosage (mg/kg/day)	:	0	0.1	0.5	3

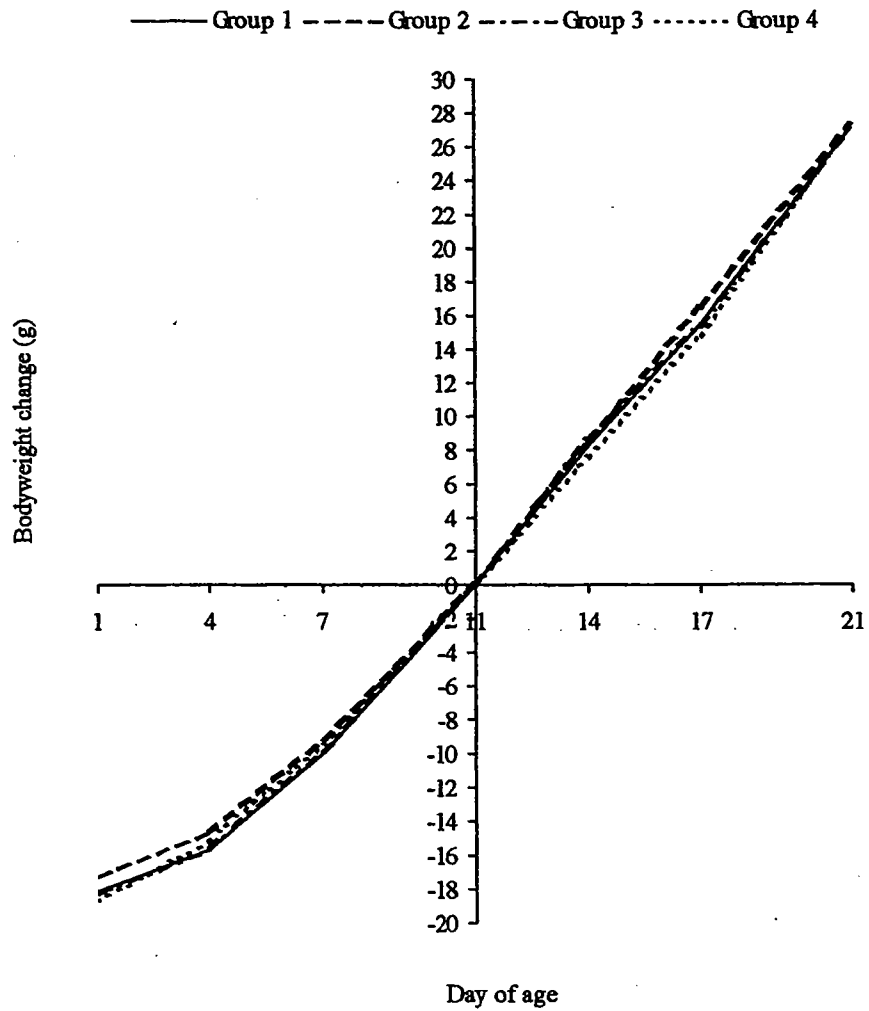


TABLE 1

Bodyweight - group mean values (g) for adult males

Group : 6 7 8 9
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Day of treatment											
		-1	1	2	3	4	5	6	7	8	9	10	11
6	Mean	253	261	265	272	279	287	294	301	309	314	319	324
	SD	17	17	22	23	24	23	23	24	25	26	26	30
	n	16	16	8	8	8	8	8	8	8	8	8	8
7	Mean	250	258	260	266	273	278	284	291	295	299	305	308
	SD	18	18	23	23	23	22	24	24	24	24	25	23
	n	16	16	8	8	8	8	8	8	8	8	8	8
8	Mean	248	255	261	269	275	282	290	296	303	309	315	321
	SD	17	18	23	21	23	24	25	24	25	27	27	27
	n	16	16	8	8	8	8	8	8	8	8	8	8
9	Mean	251	258	264	270	276	282	288	294	300	304	307	311
	SD	18	19	21	21	21	22	24	24	25	24	24	24
	n	16	16	8	8	8	8	8	8	8	8	8	8

SD Standard deviation.

TABLE 2

Bodyweight change - group mean values (g) for adult males

Group : 6 7 8 9
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Days of treatment										
		-1-1	1-2	1-3	1-4	1-5	1-6	1-7	1-8	1-9	1-10	1-11
6	Mean	8	7	14	21	29	36	43	51	56	60	66
	SD	3	3	5	6	6	7	8	10	12	12	16
	n	16	8	8	8	8	8	8	8	8	8	8
7	Mean	8	6	12	19	24	31	37	41	45	51	54
	SD	2	2	3	3	4	5	5	5	6	6	7
	n	16	8	8	8	8	8	8	8	8	8	8
8	Mean	7	7	15	22	28	36	43	49	55	61	67
	SD	3	2	3	3	7	8	8	8	12	14	13
	n	16	8	8	8	8	8	8	8	8	8	8
9	Mean	7	6	11	17	23	29	35	42	46	49	52
	SD	3	3	6	6	8	10	10	15	14	13	15
	n	16	8	8	8	8	8	8	8	8	8	8

SD Standard deviation.

No statistical significance ($p > 0.05$).

TABLE 3

Bodyweight - group mean values (g) for adult females

Group : 6 7 8 9
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Day of treatment											
		-1	1	2	3	4	5	6	7	8	9	10	11
6	Mean	189	192	195	200	204	206	212	214	218	221	224	229
	SD	11	11	14	13	16	16	17	18	18	18	20	18
	n	16	16	8	8	8	8	8	8	8	8	8	8
7	Mean	187	191	193	194	198	200	204	207	211	214	217	218
	SD	10	11	13	12	13	16	14	14	14	16	17	15
	n	16	16	8	8	8	8	8	8	8	8	8	8
8	Mean	185	191	188	191	193	199	203	205	207	211	215	217
	SD	9	10	13	12	12	13	13	11	10	12	13	12
	n	16	16	8	8	8	8	8	8	8	8	8	8
9	Mean	190	192	191	193	197	199	202	207	209	213	215	219
	SD	11	12	12	12	15	15	13	14	17	16	15	14
	n	16	16	8	8	8	8	8	8	8	8	8	8

SD Standard deviation.

TABLE 4

Bodyweight change - group mean values (g) for adult females

Group : 6 7 8 9
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Days of treatment										
		-1-1	1-2	1-3	1-4	1-5	1-6	1-7	1-8	1-9	1-10	1-11
6	Mean	3	3	8	11	14	19	22	26	29	32	37
	SD	4	5	4	6	4	7	7	8	7	10	9
	n	16	8	8	8	8	8	8	8	8	8	8
7	Mean	4	4	5	9	11	15	18	22	25	28	29
	SD	4	4	7	5	6	8	10	8	10	10	12
	n	16	8	8	8	8	8	8	8	8	8	8
8	Mean	6	2	4	6	12	16	18	20	25	28	30
	SD	3	1	1	2	3	3	4	6	6	6	6
	n	16	8	8	8	8	8	8	8	8	8	8
9	Mean	1	2	4	8	11	13	18	20	24	26	30
	SD	4	3	5	6	4	5	8	8	5	6	7
	n	16	8	8	8	8	8	8	8	8	8	8

SD Standard deviation.

No statistical significance ($p > 0.05$).

TABLE 5

Brain weights - group mean values (g) for adult males and females on Day 1 of treatment

Group : 6 7 8 9
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Male brain weight	Female brain weight
6	Mean	1.860	1.718
	SD	0.039	0.053
	n	8	8
7	Mean	1.863	1.755
	SD	0.077	0.063
	n	8	8
8	Mean	1.886	1.756
	SD	0.088	0.072
	n	8	8
9	Mean	1.848	1.772
	SD	0.086	0.069
	n	8	8

SD Standard deviation.

TABLE 6

Brain weights - group mean values (g) for adult males and females on Day 11 of treatment

Group	:	6	7	8	9
Compound	:	Control	-----	Dimethoate-----	
Dosage (mg/kg/day)	:	0	0.1	0.5	3

Group		Male brain weight	Female brain weight
6	Mean	1.929	1.802
	SD	0.132	0.035
	n	8	8
7	Mean	1.936	1.819
	SD	0.120	0.065
	n	8	8
8	Mean	1.922	1.837
	SD	0.129	0.065
	n	8	8
9	Mean	1.919	1.822
	SD	0.114	0.078
	n	8	8

SD Standard deviation.

TABLE 7

Bodyweight – group mean values (g) during gestation

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Day of gestation						
		0	3	6	10	14	17	20
1	Mean	231	257	273	294	323	354	401
	SD	9	10	12	16	17	20	24
	n	19	19	19	19	19	19	19
2	Mean	233	258	275	298	326	362	411
	SD	10	13	16	21	25	30	33
	n	19	19	19	19	19	19	19
3	Mean	232	258	275	294	322	354	400
	SD	12	14	16	16	20	23	27
	n	19	19	19	19	19	19	19
4	Mean	235	260	276	299	324	359	409
	SD	13	14	18	21	22	25	29
	n	19	19	19	19	19	19	19
5#	Mean	229	257	272	296	324	355	409
	SD	9	11	15	19	22	23	27
	n	8	8	8	8	8	8	8

SD Standard deviation.

Untreated females.

TABLE 8

Bodyweight change - group mean values (g) during gestation

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Days of gestation					
		0-6	3-6	6-10	6-14	6-17	6-20
1	Mean	42	17	21	50	81	128
	SD	7	4	5	8	10	14
	n	19	19	19	19	19	19
2	Mean	42	17	23	51	87	136
	SD	9	5	6	11	16	20
	n	19	19	19	19	19	19
3	Mean	42	17	20	47	79	126
	SD	8	4	4	8	10	16
	n	19	19	19	19	19	19
4	Mean	41	16	22	47	83	133
	SD	8	7	5	8	11	17
	n	19	19	19	19	19	19
5#	Mean	43	15	24	52	83	137
	SD	8	6	6	9	10	15
	n	8	8	8	8	8	8

SD Standard deviation.

Untreated females.

TABLE 9

Bodyweight – group mean values (g) during lactation

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Days of lactation						
		1	4	7	11	14	17	21
1	Mean	300	314	322	347	346	341	329
	SD	15	13	15	10	16	22	14
	n	10	10	10	10	10	10	10
2	Mean	320	333	341	365	361	360	342
	SD	32	29	29	32	30	35	29
	n	10	10	10	10	10	10	10
3	Mean	309	321	329	349	355	353	333
	SD	21	22	21	26	27	23	18
	n	10	10	10	10	10	10	10
4	Mean	301	315	326	350	353	347	343
	SD	23	22	19	18	23	23	22
	n	10	10	10	10	10	10	10
5#	Mean	311	332	337	368			
	SD	27	30	28	27			
	n	8	8	8	8			

SD Standard deviation.

Untreated females killed on Day 11 of lactation.

TABLE 10

Bodyweight change - group mean values (g) during lactation

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Days of lactation					
		1-4	1-7	1-11	1-14	1-17	1-21
1	Mean	14	22	47	47	41	30
	SD	7	13	14	16	17	11
	n	10	10	10	10	10	10
2	Mean	13	21	46	41	40	22
	SD	9	10	12	12	16	19
	n	10	10	10	10	10	10
3	Mean	12	19	40	46	44	24
	SD	11	12	20	17	17	17
	n	10	10	10	10	10	10
4	Mean	14	25	50	52	46	42
	SD	7	8	14	13	13	14
	n	10	10	10	10	10	10
5#	Mean	21	26	57			
	SD	11	11	13			
	n	8	8	8			

SD Standard deviation.

Untreated females killed on Day 11 of lactation.

No statistical significance ($p > 0.05$) in Groups 2, 3 and 4; Group 5 untreated females not included in statistical analysis.

TABLE 11

Gestation length and gestation index – group values

Group : 1 2 3 4 5
 Compound : Control ----- Dimethoate----- Untreated
 Dosage (mg/kg/day) : 0 0.1 0.5 3 0

Group	Number of pregnant animals		Gestation length (days)			Number of live litters born	Gestation index (%)
			22	22.5	23		
1	10	n (%)	9 (90)	0	1 (10)	10	100
2	10	n (%)	7 (70)	2 (20)	1 (10)	10	100
3	10	n (%)	5 (50)	3 (30)	2 (20)	10	100
4	10	n (%)	7 (70)	2 (20)	1 (10)	10	100
5	8	n (%)	5 (63)	2 (25)	1 (13)	8	100

n Numbers of animals in category.

TABLE 12

Litter data - group mean values at Day 20 of gestation

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Corpora Lutea	Implantations	Resorptions			Live young			Sex ratio (% M)	Implantation loss (%)	
				Early	Late	Total	Male	Female	Total		Pre-	Post-
1	Mean	16.3	14.8	0.6	0.0	0.6	7.1	7.1	14.2	50.1	8.3	4.1
	SD	1.9	1.9				2.0	1.9	2.4			
	n	9	9	9	9	9	9	9	9	9	9	9
2	Mean	16.1	16.0	1.1	1.4	2.6	6.4	7.0	13.4	52.7	2.1	16.1
	SD	1.4	1.7				2.6	3.2	4.7			
	n	9	9	9	9	9	9	9	9	9	9	9
3	Mean	16.3	15.3	0.8	0.0	0.8	7.0	7.6	14.6	48.4	6.5	5.2
	SD	1.7	1.3				1.9	2.4	1.6			
	n	9	9	9	9	9	9	9	9	9	9	9
4	Mean	16.6	16.2	0.9	0.0	0.9	7.2	8.1	15.3	48.1	3.1	5.7
	SD	1.6	1.3				1.6	2.9	1.9			
	n	9	9	9	9	9	9	9	9	9	9	9

SD Standard deviation.

TABLE 13

Fetal and litter weights - group mean values (g) on Day 20 of gestation

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Litter Weight	Fetal weights		
			Males	Females	Overall
1	Mean	55.30	3.96	3.82	3.89
	SD	9.46	0.25	0.17	0.19
	n	9	9	9	9
2	Mean	53.45	4.03	3.92	3.94
	SD	18.61	0.19	0.16	0.18
	n	9	9	8	9
3	Mean	57.06	4.06	3.81	3.93
	SD	5.92	0.33	0.27	0.31
	n	9	9	9	9
4	Mean	62.02	4.21	3.92	4.06
	SD	6.60	0.22	0.20	0.23
	n	9	9	9	9

SD Standard deviation.

TABLE 14

Brain weights - group mean values (g) for dams and fetuses on Day 20 of gestation

Group	:	1	2	3	4
Compound	:	Control	----- Dimethoate-----		
Dosage (mg/kg/day)	:	0	0.1	0.5	3

Group		Brain weight	
		Dam	Fetuses
1	Mean	1.903	0.163
	SD	0.11	0.01
	n	8	8
2	Mean	1.934	0.161
	SD	0.08	0.01
	n	8	8
3	Mean	1.837	0.164
	SD	0.04	0.01
	n	8	8
4	Mean	1.816	0.167
	SD	0.22	0.01
	n	8	8

SD Standard deviation.

No statistical significance ($p < 0.05$).

TABLE 15

Litter size up to Day 11 of age - group mean values

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Implants	Total litter Size Day 1	Live litter size on Day				
				Before cull		After cull		
				1	4	4	7	11
1	Mean	14.6	13.3	13.3	13.3	8.0	8.0	8.0
	SD	0.8	1.4	1.4	1.4	0.0	0.0	0.0
	n	10	10	10	10	10	10	10
2	Mean	16.0	14.2	14.2	14.0	8.0	8.0	8.0
	SD	1.6	1.9	1.9	2.0	0.0	0.0	0.0
	n	10	10	10	10	10	10	10
3	Mean	14.6	13.3	13.3	13.1	7.7	7.7	7.7
	SD	3.1	3.8	3.8	3.8	0.9	0.9	0.9
	n	10	10	10	10	10	10	10
4	Mean	14.0	13.7	13.7	13.6	7.9	7.9	7.8
	SD	2.6	2.8	2.8	2.8	0.3	0.3	0.4
	n	10	10	10	10	10	10	10
5#	Mean	16.4	15.3	15.3	15.3	8.0	8.0	8.0
	SD	1.4	1.5	1.5	1.5	0.0	0.0	0.0
	n	8	8	8	8	8	8	8

SD Standard deviation.

Untreated females.

TABLE 16

Offspring survival indices up to Day 11 of age – group mean values

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Post- implantation survival index (%)	Live birth index (%)	Viability index (%)	Lactation index (%)	
					Day 7	Day 11
1	Mean	91.1	100.0	100.0	100.0	100.0
	n	10	10	10	10	10
2	Mean	88.8	100.0	98.5	100.0	100.0
	n	10	10	10	10	10
3	Mean	89.6	100.0	98.5	100.0	100.0
	n	10	10	10	10	10
4	Mean	97.5	100.0	99.3	100.0	98.8
	n	10	10	10	10	10
5#	Mean	93.2	100.0	100.0	100.0	100.0
	n	8	8	8	8	8

Untreated females.

TABLE 17

Sex ratio up to Day 11 of age – group mean values

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Total Day			Live (before cull)						Live (after cull)								
		1			Day 1			Day 4			Day 4			Day 7			Day 11		
		M	F	%M	M	F	%M	M	F	%M	M	F	%M	M	F	%M	M	F	%M
1	Mean	7.7	5.6	58.1	7.7	5.6	58.1	7.7	5.6	58.1	4.1	3.9	51.3	4.1	3.9	51.3	4.1	3.9	51.3
	SD	1.4	1.6	10.6	1.4	1.6	10.6	1.4	1.6	10.6	0.3	0.3	4.0	0.3	0.3	4.0	0.3	0.3	4.0
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
2	Mean	6.2	8.0	44.4	6.2	8.0	44.4	6.1	7.9	44.6	4.0	4.0	50.0	4.0	4.0	50.0	4.0	4.0	50.0
	SD	2.3	2.9	18.4	2.3	2.9	18.4	2.2	3.0	18.7	0.9	0.9	11.8	0.9	0.9	11.8	0.9	0.9	11.8
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
3	Mean	7.2	6.1	54.3	7.2	6.1	54.3	7.0	6.1	53.8	3.8	3.9	49.8	3.8	3.9	49.8	3.8	3.9	49.8
	SD	2.9	2.8	15.2	2.9	2.8	15.2	2.8	2.8	14.8	0.4	0.7	5.3	0.4	0.7	5.3	0.4	0.7	5.3
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
4	Mean	6.6	7.1	47.0	6.6	7.1	47.0	6.5	7.1	46.7	3.8	4.1	47.7	3.8	4.1	47.7	3.7	4.1	47.1
	SD	2.3	2.0	15.9	2.3	2.0	15.9	2.3	2.0	15.8	1.0	0.7	12.4	1.0	0.7	12.4	0.9	0.7	11.8
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
5#	Mean	7.6	7.6	49.0	7.6	7.6	49.0	7.6	7.6	49.0	4.0	4.0	50.0	4.0	4.0	50.0	4.0	4.0	50.0
	SD	3.1	2.1	15.8	3.1	2.1	15.8	3.1	2.1	15.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	n	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8

SD Standard deviation.

Untreated females.

M Males.

F Females.

TABLE 18

Bodyweight up to Day 11 of age - group mean values (g) for male offspring

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Before cull		After cull		
		Day 1	Day 4	Day 4	Day 7	Day 11
1	Mean	6.6	9.7	9.7	15.4	25.6
	SD	0.6	1.1	1.0	1.9	2.2
	n	10	10	10	10	10
2	Mean	6.4	9.6	9.6	15.5	25.7
	SD	0.5	0.8	0.7	1.1	1.8
	n	10	10	10	10	10
3	Mean	6.6	10.0	10.1	15.7	25.6
	SD	1.0	1.1	1.0	1.5	1.5
	n	10	10	10	10	10
4	Mean	6.7	9.9	10.0	15.5	25.2
	SD	1.0	1.3	1.3	2.0	3.0
	n	10	10	10	10	10
5#	Mean	6.9	9.3	9.5	16.4	26.0
	SD	0.6	1.0	1.0	1.3	1.6
	n	8	8	8	8	8

SD Standard deviation.

Offspring from untreated females.

TABLE 19

Bodyweight change up to Day 11 of age - group mean values (g) for male offspring

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Weight change during days of age		
		1-4 @	1-7	1-11
1	Mean	3.1	8.8	18.9
	SD	0.7	1.4	1.7
	n	10	10	10
2	Mean	3.2	9.1	19.2
	SD	0.7	1.1	1.6
	n	10	10	10
3	Mean	3.5	9.1	19.1
	SD	0.6	0.8	1.2
	n	10	10	10
4	Mean	3.3	8.8	18.6
	SD	0.7	1.5	2.3
	n	10	10	10
5#	Mean	2.6	9.4	19.1
	SD	0.7	1.2	1.4
	n	8	8	8

SD Standard deviation.
 # Offspring from untreated females.
 @ Days 1-4 after cull.

TABLE 20

Bodyweight up to Day 11 of age - group mean values (g) for female offspring

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Before cull		After cull		
		Day 1	Day 4	Day 4	Day 7	Day 11
1	Mean	6.4	9.2	9.1	14.5	24.4
	SD	0.6	1.0	1.1	1.6	2.4
	n	10	10	10	10	10
2	Mean	6.1	9.2	9.4	14.9	24.7
	SD	0.3	0.8	0.7	0.8	1.2
	n	10	10	10	10	10
3	Mean	6.2	9.4	9.5	14.8	24.5
	SD	1.0	1.2	1.1	1.6	1.8
	n	10	10	10	10	10
4	Mean	6.2	9.6	9.6	14.9	24.4
	SD	1.0	1.5	1.5	2.0	3.1
	n	10	10	10	10	10
5#	Mean	6.5	8.9	9.0	15.6	25.0
	SD	0.6	0.8	0.9	1.4	2.2
	n	8	8	8	8	8

SD Standard deviation.

Offspring from untreated females.

TABLE 21

Bodyweight change up to Day 11 of age - group mean values (g) for female offspring

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Weight change during days of age		
		1-4 @	1-7	1-11
1	Mean	2.8	8.2	18.1
	SD	0.9	1.3	2.0
	n	10	10	10
2	Mean	3.3	8.8	18.6
	SD	0.8	0.9	1.2
	n	10	10	10
3	Mean	3.3	8.6	18.4
	SD	0.6	0.9	1.3
	n	10	10	10
4	Mean	3.4	8.7	18.1
	SD	0.8	1.3	2.3
	n	10	10	10
5#	Mean	2.4	9.1	18.5
	SD	0.9	1.4	2.1
	n	8	8	8

SD Standard deviation.
 # Offspring from untreated females.
 @ Days 1-4 after cull.

TABLE 22

Bodyweight - group mean values (g) for dosed male offspring

Group	:	1	2	3	4
Compound	:	Control	----- Dimethoate -----		
Dosage (mg/kg/day)	:	0	0.1	0.5	3

Group		Bodyweight on Day of age												
		1	4	7	11	14	17	21	28	35	42	49	56	60
1	Mean	6.6	9.7	15.2	25.4	33.4	39.6	51.7	90.0	143.1	199.0	261.2	318.4	351.2
	SD	0.7	1.1	2.0	2.5	2.8	3.4	5.5	9.4	17.3	23.2	29.8	36.7	38.2
	n	33	33	33	33	33	33	33	9	9	9	9	9	8
2	Mean	6.3	9.7	15.4	25.5	32.8	40.3	51.3	87.0	138.6	200.2	263.4	327.8	355.9
	SD	0.7	1.0	1.7	2.6	3.7	4.5	6.4	14.3	19.5	24.5	28.2	33.4	39.3
	n	32	32	32	32	32	32	32	9	9	9	9	9	8
3	Mean	6.4	10.0	15.5	25.5	33.1	41.3	51.6	91.4	144.3	207.1	273.7	337.7	366.9
	SD	1.0	1.2	1.7	1.7	2.0	2.7	3.1	7.6	13.6	18.7	22.9	25.8	28.5
	n	30	30	30	26	25	26	27	9	9	9	9	9	8
4	Mean	6.4	9.7	15.0	24.6	32.2	40.1	51.5	93.5	149.5	216.6	284.5	347.9	379.1
	SD	0.9	1.3	2.0	3.0	3.2	3.7	5.3	10.5	15.3	16.7	22.8	24.7	31.2
	n	29	29	29	29	29	29	29	9	9	9	9	9	8

SD Standard deviation.

TABLE 23

Bodyweight change - group mean values (g) for dosed male offspring

Group	:	1	2	3	4
Compound	:	Control	----- Dimethoate -----		
Dosage (mg/kg/day)	:	0	0.1	0.5	3

Group		Bodyweight change during days											
		1-11	4-11	7-11	11-14	11-17	11-21	11-28	11-35	11-42	11-49	11-56	11-60
1	Mean	18.8	15.7	10.2	8.0	14.2	26.3	64.4	117.5	173.4	235.6	292.8	325.7
	SD	2.1	1.8	1.2	1.1	1.5	3.3	6.7	14.7	21.4	27.7	34.5	35.7
	n	33	33	33	33	33	33	9	9	9	9	9	8
2	Mean	19.2	15.8	10.1	7.3	14.8	25.7	63.1	114.6	176.2	239.4	303.8	331.8
	SD	2.1	2.1	1.6	2.2	2.8	4.4	10.4	15.8	21.1	25.1	30.7	36.6
	n	32	32	32	32	32	32	9	9	9	9	9	8
3	Mean	19.0	15.4	10.1	7.6	15.8	26.2	66.9	121.4	185.9	253.8	318.6	348.8
	SD	1.2	1.2	1.0	1.2	2.4	2.6	5.8	10.1	12.3	14.4	16.2	20.0
	n	26	26	26	25	26	26	8	8	8	8	8	7
4	Mean	18.2	14.9	9.7	7.6	15.5	26.9	68.4	124.4	191.5	259.5	322.9	353.9
	SD	2.5	2.1	1.5	0.6	1.7	2.8	7.5	12.5	14.4	20.5	22.8	29.1
	n	29	29	29	29	29	29	9	9	9	9	9	8

SD Standard deviation.

TABLE 24

Bodyweight - group mean values (g) for undosed male offspring

Group	:	1	2	3	4
Compound	:	Control	----- Dimethoate -----		
Dosage (mg/kg/day)	:	0	0.1	0.5	3

Group		Bodyweight on Day of age						
		1	4	7	11	14	17	21
1	Mean	6.8	9.8	16.1	26.1	35.0	43.4	54.3
	SD	0.5	0.6	1.1	1.3	1.9	2.1	2.5
	n	8	8	8	8	8	8	8
2	Mean	7.1	9.7	16.0	26.3	35.9	43.6	55.6
	SD	0.6	0.7	1.4	2.4	2.0	2.4	4.1
	n	8	8	8	8	8	8	8
3	Mean	7.1	9.9	16.1	25.5	34.3	42.2	55.0
	SD	0.4	0.8	1.6	2.7	3.8	4.4	5.2
	n	8	8	8	8	8	8	8
4	Mean	7.0	10.0	16.1	25.5	33.8	41.3	53.5
	SD	0.7	0.6	1.0	0.5	1.4	2.0	2.0
	n	8	8	8	8	8	8	8

SD Standard deviation.

TABLE 25

Bodyweight change - group mean values (g) for undosed male offspring

Group	:	1	2	3	4
Compound	:	Control	-----	Dimethoate	-----
Dosage (mg/kg/day)	:	0	0.1	0.5	3

Group		Bodyweight change during days					
		1-11	4-11	7-11	11-14	11-17	11-21
1	Mean	19.4	16.4	10.1	8.8	17.2	28.2
	SD	1.2	1.2	0.8	1.0	1.5	2.2
	n	8	8	8	8	8	8
2	Mean	19.2	16.6	10.3	9.7	17.3	29.3
	SD	2.1	2.0	1.3	1.4	2.5	3.3
	n	8	8	8	8	8	8
3	Mean	18.5	15.6	9.4	8.7	16.7	29.4
	SD	2.4	2.2	1.3	2.3	2.2	3.0
	n	8	8	8	8	8	8
4	Mean	18.5	15.6	9.5	8.2	15.8	27.9
	SD	0.9	0.6	0.9	1.2	1.7	1.9
	n	8	8	8	8	8	8

SD Standard deviation.

TABLE 26

Bodyweight - group mean values (g) for dosed female offspring

Group	:	1	2	3	4
Compound	:	Control	-----	Dimethoate	-----
Dosage (mg/kg/day)	:	0	0.1	0.5	3

Group		Bodyweight on Day of age												
		1	4	7	11	14	17	21	28	35	42	49	56	60
1	Mean	6.4	9.2	14.5	24.4	32.4	38.3	49.6	80.2	123.5	155.1	182.8	205.4	214.1
	SD	0.8	1.3	2.0	2.9	3.0	3.8	5.1	6.0	9.2	10.8	12.5	13.2	15.8
	n	31	31	31	31	30	30	30	9	9	9	9	9	8
2	Mean	6.2	9.3	14.9	24.9	32.3	39.7	50.5	84.4	127.0	162.1	192.2	215.0	228.3
	SD	0.5	0.8	1.1	1.5	1.9	2.4	3.9	6.2	10.0	12.4	16.9	21.6	19.3
	n	32	32	32	32	32	32	32	9	9	9	9	9	8
3	Mean	6.0	9.5	14.6	24.4	32.1	40.1	49.8	82.8	125.6	159.6	190.4	219.5	229.2
	SD	1.0	1.3	1.9	2.5	2.2	2.3	4.3	3.1	4.8	9.8	14.7	23.9	18.8
	n	31	31	31	31	31	31	31	9	9	9	9	9	8
4	Mean	6.3	9.7	14.9	24.4	31.9	39.3	50.1	81.9	124.4	159.5	186.5	210.1	220.2
	SD	1.2	1.9	2.4	3.6	3.9	3.6	6.2	8.3	10.3	10.1	12.5	15.1	17.9
	n	33	33	33	33	33	33	33	9	9	9	9	9	8

SD Standard deviation.

TABLE 27

Bodyweight change – group mean values (g) for dosed female offspring

Group	:	1	2	3	4
Compound	:	Control	----- Dimethoate -----		
Dosage (mg/kg/day)	:	0	0.1	0.5	3

Group		Bodyweight change during days											
		1-11	4-11	7-11	11-14	11-17	11-21	11-28	11-35	11-42	11-49	11-56	11-60
1	Mean	18.0	15.2	9.9	7.8	13.7	25.0	56.1	99.4	131.0	158.7	181.3	189.9
	SD	2.4	2.1	1.5	1.0	1.8	2.7	4.9	8.5	10.6	12.5	13.3	16.1
	n	31	31	31	30	30	30	9	9	9	9	9	8
2	Mean	18.7	15.6	9.9	7.4	14.8	25.6	60.2	102.8	137.9	168.0	190.8	204.0
	SD	1.4	1.2	0.9	1.1	1.7	2.9	5.4	9.5	11.9	16.5	21.5	19.6
	n	32	32	32	32	32	32	9	9	9	9	9	8
3	Mean	18.4	14.9	9.8	7.7	15.7	25.4	59.0	101.7	135.7	166.6	195.6	205.7
	SD	2.0	1.7	1.3	2.0	2.5	4.2	5.1	6.6	11.6	16.5	26.2	19.6
	n	31	31	31	31	31	31	9	9	9	9	9	8
4	Mean	18.1	14.7	9.5	7.5	14.9	25.7	58.7	101.1	136.2	163.3	186.9	196.6
	SD	2.6	2.0	1.5	0.7	1.2	3.1	5.2	8.0	8.8	11.6	14.6	17.0
	n	33	33	33	33	33	33	9	9	9	9	9	8

SD Standard deviation.

TABLE 28

Bodyweight - group mean values (g) for undosed female offspring

Group	:	1	2	3	4
Compound	:	Control	-----	Dimethoate	-----
Dosage (mg/kg/day)	:	0	0.1	0.5	3

Group		Bodyweight on Day of age						
		1	4	7	11	14	17	21
1	Mean	6.4	8.9	14.6	24.6	32.8	40.1	51.7
	SD	0.4	0.7	0.6	1.6	1.7	2.5	3.3
	n	8	8	8	8	8	8	8
2	Mean	6.6	9.2	14.7	23.9	32.4	40.3	51.0
	SD	0.5	0.8	1.2	1.8	2.1	3.1	5.3
	n	8	8	8	8	8	8	8
3	Mean	6.7	9.2	15.0	24.9	33.5	40.2	51.8
	SD	0.5	0.6	1.1	2.6	4.4	4.0	5.9
	n	8	8	8	8	8	8	8
4	Mean	6.4	9.9	15.6	25.0	32.6	39.7	52.2
	SD	0.4	0.7	0.9	1.2	1.7	2.4	2.5
	n	8	8	8	8	8	8	8

SD Standard deviation.

TABLE 29

Bodyweight change - group mean values (g) for undosed female offspring

Group	:	1	2	3	4
Compound	:	Control	----- Dimethoate -----		
Dosage (mg/kg/day)	:	0	0.1	0.5	3

Group		Bodyweight change during days					
		1-11	4-11	7-11	11-14	11-17	11-21
1	Mean	18.2	15.7	10.0	8.2	15.5	27.1
	SD	1.6	1.2	1.3	1.3	2.0	2.2
	n	8	8	8	8	8	8
2	Mean	17.3	14.7	9.2	8.5	16.4	27.2
	SD	1.7	1.7	1.5	0.6	1.4	3.6
	n	8	8	8	8	8	8
3	Mean	18.2	15.6	9.9	8.7	15.4	27.0
	SD	2.1	2.2	1.6	2.5	1.8	4.0
	n	8	8	8	8	8	8
4	Mean	18.6	15.2	9.4	7.6	14.7	27.2
	SD	1.0	0.8	0.8	1.0	2.2	2.1
	n	8	8	8	8	8	8

SD Standard deviation.

TABLE 30

Brain weights - group mean values (g) for male and female offspring at Day 4 of age

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Brain weight	
		Male	Female
1	Mean	0.402	0.387
	SD	0.049	0.026
	n	19	13
2	Mean	0.400	0.368
	SD	0.027	0.031
	n	15	16
3	Mean	0.404	0.381
	SD	0.039	0.046
	n	14	12
4	Mean	0.399	0.386
	SD	0.031	0.024
	n	17	16

SD Standard deviation.

TABLE 31

Brain weights - group mean values (g) for dosed male and female offspring in Group 5 at Day 11 of age

Treatment		Brain weight	
		Male	Female
Control	Mean	1.055	1.049
	SD	0.065	0.066
	n	8	8
0.1 mg/kg/day	Mean	1.057	1.035
	SD	0.054	0.069
	n	8	8
0.5 mg/kg/day	Mean	1.065	1.015
	SD	0.082	0.060
	n	8	8
3 mg/kg/day	Mean	1.054	1.041
	SD	0.081	0.045
	n	8	8

SD Standard deviation.

TABLE 32

Brain weights - group mean values (g) for dosed male and female offspring at Day 21 of age

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Brain weight	
		Male	Female
1	Mean	1.502	1.459
	SD	0.050	0.070
	n	8	8
2	Mean	1.484	1.467
	SD	0.077	0.059
	n	8	8
3	Mean	1.479	1.453
	SD	0.055	0.039
	n	8	8
4	Mean	1.458	1.436
	SD	0.040	0.063
	n	8	8

SD Standard deviation.

No statistical significance ($p > 0.05$).

TABLE 33

Brain weights - group mean values (g) for male and female offspring at Day 60 of age

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Brain weight	
		Male	Female
1	Mean	2.013	1.833
	SD	0.081	0.064
	n	8	8
2	Mean	1.972	1.886
	SD	0.101	0.044
	n	8	8
3	Mean	1.944	1.859
	SD	0.076	0.056
	n	8	8
4	Mean	1.992	1.816
	SD	0.053	0.076
	n	8	8

SD Standard deviation.

TABLE 34

Plasma, erythrocyte and brain cholinesterase activity – group mean values for adult males on Day 1 of treatment

Group : 6 7 8 9
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
6	Mean	375	1122	13794
	SD	48.5	225.8	247
	n	8	8	8
7	Mean	387 (-3)	1247 (-11)	13544 (2)
	SD	74.9	203.3	802.4
	n	8	8	8
8	Mean	364 (3)	1131 (-1)	13294 ^a (4)
	SD	63.8	67.8	241.2
	n	8	8	8
9	Mean	305 ^a (19)	928 ^a (17)	12131 ^b (12)
	SD	39.7	112.2	1096.4
	n	8	8	8

CHE Cholinesterase.

SD Standard deviation.

() Percentage inhibition of CHE compared with controls.

Statistically significant when compared with Control; a – p<0.05, b – p<0.01.

TABLE 34 - continued

Plasma, erythrocyte and brain cholinesterase activity – group mean values for adult females on Day 1 of treatment

Group : 6 7 8 9
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
6	Mean	688	1209	14150
	SD	131.9	167.9	554.8
	n	8	8	8
7	Mean	657 (5)	1128 (7)	13625 (4)
	SD	137.3	108.9	444.8
	n	8	8	8
8	Mean	729 (-6)	1106 (9)	13850 (2)
	SD	81.8	89.4	687.1
	n	8	8	8
9	Mean	602 (12)	881 ^b (27)	12106 ^b (14)
	SD	130.5	87.4	826.5
	n	8	8	8

CHE Cholinesterase.

SD Standard deviation.

() Percentage inhibition of CHE compared with controls.

Statistically significant when compared with Control; b – p<0.01.

TABLE 35

Plasma, erythrocyte and brain cholinesterase activity - group mean values for adult males on Day 11 of treatment

Group : 6 7 8 9
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
6	Mean SD n	343 32.5 8	1094 159.7 8	14100 529.2 8
7	Mean SD n	327 (5) 44.2 8	1169 (-7) 435.4 8	13988 (1) 661.6 8
8	Mean SD n	302 (12) 35.5 8	903 (17) 164.4 8	12700 ^a (10) 547.7 8
9	Mean SD n	215 ^b (37) 56.6 8	456 ^b (58) 239.7 8	7469 ^b (47) 2484.4 8

CHE Cholinesterase.

SD Standard deviation.

() Percentage inhibition of CHE compared with controls.

Statistically significant when compared with Control; a - $p < 0.05$, b - $p < 0.01$.

TABLE 35 - continued

Plasma, erythrocyte and brain cholinesterase activity – group mean values for adult females on Day 11 of treatment

Group : 6 7 8 9
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
6	Mean	790	1019	14869
	SD	119.1	140.6	1399.7
	n	8	8	8
7	Mean	949 (-20)	991 (3)	13913 (7)
	SD	324.3	101.7	446.2
	n	8	8	8
8	Mean	770 (3)	950 (7)	12881 ^b (13)
	SD	122.7	82.4	845.1
	n	8	8	8
9	Mean	624 (21)	375 ^b (63)	6188 ^b (58)
	SD	164	122.5	1077.6
	n	8	8	8

CHE Cholinesterase.

SD Standard deviation.

() Percentage inhibition of CHE compared with Controls.

Statistically significant when compared with Control; b – p<0.01.

TABLE 36

Plasma, erythrocyte and brain cholinesterase activity - group mean values for dams on Day 20 of gestation

Group	:	1	2	3	4
Compound	:	Control	-----	Dimethoate-----	
Dosage (mg/kg/day)	:	0	0.1	0.5	3

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1	Mean SD n	1381 168.8 8	1669 179.7 8	12838 1372.6 8
2	Mean SD n	1216 (12) 240.6 8	1563 (6) 224 8	13044 (-2) 530.1 8
3	Mean SD n	1184 (14) 241.9 8	1459 (13) 277.7 8	11563 ^a (10) 299.7 8
4	Mean SD n	776 ^b (44) 258 8	709 ^b (58) 103.5 8	5094 ^b (60) 1080.8 8

CHE Cholinesterase.

SD Standard deviation.

() Percentage inhibition of CHE compared with controls.

Statistically significant when compared with Control; a - $p < 0.05$, b - $p < 0.01$.

TABLE 37

Plasma, erythrocyte and brain cholinesterase activity - group mean values for male and female fetuses on Day 20 of gestation

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1	Mean	258	1213	1781
	SD	21.5	79.1	175.1
	n	8	8	8
2	Mean	257 (0)	1225 (-1)	1569 ^a (12)
	SD	26.4	98.2	173.1
	n	7	8	8
3	Mean	239 (7)	1181 (3)	1600 ^a (10)
	SD	28.3	172	136.3
	n	8	8	8
4	Mean	147 ^b (43)	834 ^b (31)	1188 ^b (33)
	SD	23.5	182.7	164.2
	n	8	8	8

CHE Cholinesterase.

SD Standard deviation.

() Percentage inhibition of CHE compared with controls.

Statistically significant when compared with Control; a – $p < 0.05$, b – $p < 0.01$.

TABLE 38

Plasma, erythrocyte and brain cholinesterase activity - group mean values for male offspring on Day 4 of age

Group	:	1	2	3	4
Compound	:	Control	-----	Dimethoate-----	
Dosage (mg/kg/day)	:	0	0.1	0.5	3

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1	Mean	612	1291	3137
	SD	63.8	226.1	322.2
	n	19	19	19
2	Mean	607 (1)	1403 (-9)	2817 ^a (10)
	SD	61.6	203.9	434.1
	n	15	15	15
3	Mean	588 (4)	1254 (3)	2889 ^a (8)
	SD	51.4	201.6	215
	n	14	14	14
4	Mean	566 (8)	1071 ^b (17)	2744 ^b (13)
	SD	55	156.9	334.9
	n	16	17	17

CHE Cholinesterase.

SD Standard deviation.

() Percentage inhibition of CHE compared with controls.

Statistically significant when compared with Control; a - $p < 0.05$, b - $p < 0.01$.

TABLE 38 - continued

Plasma, erythrocyte and brain cholinesterase activity - group mean values for female offspring on Day 4 of age

Group : 1 2 3 4

Compound : Control ----- Dimethoate-----

Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1	Mean	640	1260	2823
	SD	49	350.1	310
	n	13	13	13
2	Mean	605 (5)	1261 (0)	2941 (-4)
	SD	49.5	230.2	253.1
	n	15	16	16
3	Mean	591 ^a (8)	1352 (-7)	2650 (6)
	SD	41.2	272.5	286.8
	n	12	12	12
4	Mean	576 ^b (10)	1088 (14)	2638 (7)
	SD	49.2	287.1	268.6
	n	15	16	16

CHE Cholinesterase.

SD Standard deviation.

() Percentage inhibition of CHE compared with controls.

Statistically significant when compared with Control; a - $p < 0.05$, b - $p < 0.01$.

TABLE 39

Plasma, erythrocyte and brain cholinesterase activity - group mean values for dosed male offspring on Day 11 of age

Group	:	1	2	3	4
Compound	:	Control	-----	Dimethoate-----	
Dosage (mg/kg/day)	:	0	0.1	0.5	3

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1	Mean	756	1663	6475
	SD	112.6	279.3	243.5
	n	8	8	8
2	Mean	748 (1)	1634 (2)	6363 (2)
	SD	63.3	335.7	235.7
	n	8	8	8
3	Mean	688 (9)	1597 (4)	6144 ^a (5)
	SD	49.4	192.5	360
	n	8	8	8
4	Mean	614 ^b (19)	1544 (7)	5375 ^b (17)
	SD	75.8	524.4	290.3
	n	8	8	8

CHE Cholinesterase.

SD Standard deviation.

() Percentage inhibition of CHE compared with controls.

Statistically significant when compared with Control; a – p<0.05, b – p<0.01.

TABLE 39 - continued

Plasma, erythrocyte and brain cholinesterase activity - group mean values for dosed female offspring on Day 11 of age

Group	:	1	2	3	4
Compound	:	Control	----- Dimethoate -----		
Dosage (mg/kg/day)	:	0	0.1	0.5	3

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1	Mean	742	1997	6256
	SD	110.1	620.4	195.4
	n	8	8	8
2	Mean	700 (6)	1647 (18)	6350 (-2)
	SD	120.2	290.5	338.1
	n	8	8	8
3	Mean	720 (3)	1894 (5)	6125 (2)
	SD	78.7	394.8	297.6
	n	8	8	8
4	Mean	609 (18)	1475 (26)	5144 ^b (18)
	SD	92.6	245.7	532.1
	n	8	8	8

CHE Cholinesterase.

SD Standard deviation.

() Percentage inhibition of CHE compared with controls.

Statistically significant when compared with Control; b - p<0.01.

TABLE 40

Plasma, erythrocyte and brain cholinesterase activity - group mean values for dosed male offspring on Day 21 of age

Group	:	1	2	3	4
Compound	:	Control	-----	Dimethoate-----	
Dosage (mg/kg/day)	:	0	0.1	0.5	3

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1	Mean	506	1638	10375
	SD	79.1	454.3	207
	n	8	8	8
2	Mean	535 (-6)	1659 (-1)	9944 ^a (4)
	SD	67.8	393.9	331.1
	n	8	8	8
3	Mean	478 (6)	1494 (9)	9044 ^b (13)
	SD	29.3	325.6	339.6
	n	8	8	8
4	Mean	307 ^b (39)	669 ^b (59)	5675 ^b (45)
	SD	64.5	160.8	551
	n	8	8	8

CHE Cholinesterase.

SD Standard deviation.

() Percentage inhibition of CHE compared with controls.

Statistically significant when compared with Control; a – p<0.05, b – p<0.01.

TABLE 40 - continued

Plasma, erythrocyte and brain cholinesterase activity - group mean values for dosed female offspring on Day 21 of age

Group	:	1	2	3	4
Compound	:	Control	-----	Dimethoate-----	
Dosage (mg/kg/day)	:	0	0.1	0.5	3

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1	Mean	487	1900	10275
	SD	70.4	587.4	376.1
	n	8	8	8
2	Mean	507 (-4)	1619 (15)	9906 (4)
	SD	70.2	296	313.3
	n	8	8	8
3	Mean	463 (5)	1466 ^a (23)	9019 ^b (12)
	SD	54.1	254.2	247.8
	n	8	8	8
4	Mean	304 ^b (38)	663 ^b (65)	5956 ^b (42)
	SD	52.6	205.3	964.9
	n	8	8	8

CHE Cholinesterase.

SD Standard deviation.

() Percentage inhibition of CHE compared with controls.

Statistically significant when compared with Control; a - $p < 0.05$, b - $p < 0.01$.

TABLE 41

Plasma, erythrocyte and brain cholinesterase activity - group mean values for male offspring on Day 60 of age

Group	:	1	2	3	4
Compound	:	Control	-----	Dimethoate-----	
Dosage (mg/kg/day)	:	0	0.1	0.5	3

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1	Mean	373	1075	13000
	SD	80.4	85.6	449.6
	n	8	8	8
2	Mean	369 (1)	1100 (-2)	13100 (-1)
	SD	43.3	68.1	410.6
	n	8	8	8
3	Mean	340 (9)	1100 (-2)	12988 (0)
	SD	36.8	73.2	422.4
	n	8	8	8
4	Mean	337 (10)	1038 (3)	13044 (0)
	SD	33	124.6	756.1
	n	8	8	8

CHE Cholinesterase.

SD Standard deviation.

() Percentage inhibition of CHE compared with controls.

No statistical significance ($p > 0.05$).

TABLE 41 - continued

Plasma, erythrocyte and brain cholinesterase activity - group mean values for female offspring on Day 60 of age

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1	Mean	907	1109	13275
	SD	200.2	149.4	276.5
	n	8	8	8
2	Mean	915 (-1)	1119 (-1)	12950 (2)
	SD	197.9	93.3	317.4
	n	8	8	8
3	Mean	945 (-4)	991 (11)	12738 ^a (4)
	SD	231.9	109.3	243.1
	n	8	8	8
4	Mean	846 (7)	1044 (6)	12744 ^a (4)
	SD	189.2	107.5	585.8
	n	8	8	8

CHE Cholinesterase.

SD Standard deviation.

() Percentage inhibition of CHE compared with controls.

Statistically significant when compared with Control; a - p<0.05.

APPENDIX 1

Clinical signs - individual observations for adult males and females #

Group	:	6	7
Compound	:	Control --	Dimethoate --
Dosage (mg/kg/day)	:	0	0.1

Group	Animal number	Signs observed on Days
6	210M	Hairloss on dorsal body surface on Days 8 - 11 of treatment.
7	229M	Encrustation on lower jaw on Days 2 and 3 of treatment.

Only animals with signs are presented.

APPENDIX 2

Bodyweight – individual values (g) for adult males

Group 6: Control

Animal number	Day of treatment											
	-1	1	2	3	4	5	6	7	8	9	10	11
201 #	245	254										
202 #	268	273										
203 #	259	270										
204 #	253	258										
205 #	241	248										
206 #	233	245										
207 #	252	267										
208 #	281	293										
209	268	276	281	296	302	305	313	321	326	332	340	350
210	227	233	241	249	255	264	271	279	286	295	300	303
211	244	251	256	265	267	278	285	289	299	304	309	312
212	237	246	250	257	267	278	286	294	301	309	316	320
213	280	284	294	301	307	311	318	326	330	336	337	340
214	233	238	240	242	253	259	263	270	276	274	280	281
215	272	281	292	298	311	321	329	336	351	357	361	375
216	252	260	267	269	273	281	289	294	304	306	308	312

Animals killed on Day 1 of treatment.

APPENDIX 2 - continued

Bodyweight – individual values (g) for adult males

Group 7: 0.1 mg/kg/day

Animal number	Day of treatment											
	-1	1	2	3	4	5	6	7	8	9	10	11
217 #	245	250										
218 #	267	270										
219 #	258	267										
220 #	256	260										
221 #	244	254										
222 #	238	247										
223 #	251	260										
224 #	277	289										
225	229	236	243	246	253	256	261	270	274	279	283	287
226	231	239	243	254	256	263	267	276	279	281	287	291
227	232	240	243	251	258	263	268	274	278	281	287	293
228	223	231	238	243	252	259	261	266	269	277	281	287
229	245	255	262	269	275	282	293	297	302	304	309	310
230	264	273	279	281	287	291	298	302	307	308	318	315
231	286	296	305	311	318	322	330	336	338	346	350	351
232	251	260	264	274	283	287	296	306	309	316	323	326

Animals killed on Day 1 of treatment.

APPENDIX 2 - continued

Bodyweight – individual values (g) for adult males

Group 8: 0.5 mg/kg/day

Animal number	Day of treatment											
	-1	1	2	3	4	5	6	7	8	9	10	11
233 #	244	250										
234 #	229	233										
235 #	253	260										
236 #	254	261										
237 #	274	284										
238 #	251	262										
239 #	246	254										
240 #	237	248										
241	224	226	231	245	248	256	264	272	278	285	291	296
242	233	242	250	257	263	268	279	283	285	292	295	302
243	232	235	240	247	250	249	252	259	266	265	268	277
244	240	247	255	266	272	277	287	297	300	311	317	323
245	238	245	253	262	268	280	286	296	303	314	325	327
246	280	284	294	300	309	318	322	326	332	334	337	342
247	274	281	285	290	300	306	318	324	333	343	347	353
248	260	270	279	286	293	300	308	314	323	329	336	345

Animals killed on Day 1 of treatment.

APPENDIX 2 - continued

Bodyweight – individual values (g) for adult males

Group 9: 3 mg/kg/day

Animal number	Day of treatment											
	-1	1	2	3	4	5	6	7	8	9	10	11
249 #	249	253										
250 #	271	280										
251 #	232	240										
252 #	248	251										
253 #	282	290										
254 #	246	254										
255 #	237	249										
256 #	236	242										
257	238	246	258	269	278	286	296	303	320	322	323	330
258	221	226	233	240	244	251	254	262	267	274	274	283
259	232	238	242	245	250	256	258	263	268	270	277	280
260	262	264	269	275	278	284	287	291	300	304	303	305
261	280	291	297	304	309	315	323	329	332	337	337	346
262	263	270	273	280	286	290	298	301	310	316	323	320
263	250	258	260	263	270	269	278	284	284	291	294	292
264	270	276	281	284	293	303	310	315	321	320	327	330

Animals killed on Day 1 of treatment.

APPENDIX 3

Bodyweight – individual values (g) for adult females

Group 6: Control

Animal number	Day of treatment											
	-1	1	2	3	4	5	6	7	8	9	10	11
85 #	194	189										
86 #	185	186										
87 #	196	201										
88 #	191	192										
89 #	201	204										
90 #	182	190										
91 #	191	200										
92 #	170	174										
93	178	177	182	189	190	190	194	200	208	205	210	218
94	170	177	180	185	178	186	191	191	190	196	197	202
95	188	190	197	197	208	206	211	218	226	224	228	238
96	191	194	185	196	199	202	202	205	215	217	212	220
97	206	210	216	216	225	230	233	236	240	244	249	248
98	210	209	216	222	226	226	239	242	242	244	252	255
99	181	185	189	196	196	197	204	202	203	205	210	214
100	191	196	197	200	206	212	218	219	222	232	236	237

Animals killed on Day 1 of treatment.

APPENDIX 3 - continued

Bodyweight – individual values (g) for adult females

Group 7: 0.1 mg/kg/day

Animal number	Day of treatment											
	-1	1	2	3	4	5	6	7	8	9	10	11
101 #	181	185										
102 #	197	204										
103 #	196	194										
104 #	184	192										
105 #	176	186										
106 #	192	197										
107 #	187	192										
108 #	178	192										
109	203	204	201	202	206	210	208	209	220	221	222	222
110	172	174	180	185	186	188	192	200	203	207	207	209
111	190	190	197	200	203	198	206	212	213	214	220	221
112	180	181	187	192	194	196	203	210	210	214	216	222
113	171	168	176	178	178	177	187	189	192	192	196	203
114	199	206	213	214	216	227	231	233	232	242	247	245
115	197	200	204	201	209	213	213	214	221	223	228	226
116	184	189	188	183	189	190	192	190	195	196	197	195

Animals killed on Day 1 of treatment.

APPENDIX 3 - continued

Bodyweight – individual values (g) for adult females

Group 8: 0.5 mg/kg/day

Animal number	Day of treatment											
	-1	1	2	3	4	5	6	7	8	9	10	11
117 #	186	196										
118 #	186	189										
119 #	190	198										
120 #	186	191										
121 #	190	196										
122 #	179	184										
123 #	198	203										
124 #	191	201										
125	186	193	196	197	198	200	205	203	203	204	209	209
126	191	194	197	199	200	206	212	215	212	220	226	226
127	178	178	180	182	182	186	192	200	200	203	206	211
128	169	174	176	179	179	186	191	194	193	200	207	206
129	182	184	184	186	189	197	203	200	210	212	215	215
130	191	194	194	199	204	209	207	208	216	219	217	222
131	194	206	209	210	210	221	226	224	224	233	240	240
132	166	170	171	176	178	185	187	192	198	200	200	207

Animals killed on Day 1 of treatment.

APPENDIX 3 - continued

Bodyweight – individual values (g) for adult females

Group 9: 3 mg/kg/day

Animal number	Day of treatment											
	-1	1	2	3	4	5	6	7	8	9	10	11
133 #	189	185										
134 #	182	184										
135 #	199	202										
136 #	182	180										
137 #	200	198										
138 #	190	193										
139 #	203	209										
140 #	204	204										
141	198	192	200	201	206	203	212	219	219	217	226	231
142	189	196	197	204	208	207	203	218	219	221	219	226
143	189	185	186	187	191	196	195	204	207	208	208	213
144	179	182	180	187	191	196	194	204	204	209	210	218
145	193	200	198	196	202	209	208	204	213	223	222	220
146	178	183	184	182	178	187	193	190	186	197	201	203
147	167	166	171	176	177	174	183	189	186	189	191	198
148	204	207	208	211	219	223	226	227	236	240	240	241

Animals killed on Day 1 of treatment.

APPENDIX 4

Brain weights – individual values (g) for adult males and females on Day 1 of treatment

Group : 6 7 8 9
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Sex	Animal number	Brain weight	Sex	Animal number	Brain weight
6	M	201	1.799	F	85	1.703
		202	1.893		86	1.652
		203	1.915		87	1.705
		204	1.824		88	1.758
		205	1.885		89	1.798
		206	1.852		90	1.752
		207	1.872		91	1.732
		208	1.836		92	1.643
7	M	217	1.879	F	101	1.869
		218	1.759		102	1.767
		219	1.925		103	1.754
		220	1.802		104	1.794
		221	1.864		105	1.670
		222	1.785		106	1.709
		223	1.987		107	1.700
		224	1.906		108	1.773
8	M	233	1.944	F	117	1.759
		234	1.855		118	1.672
		235	1.758		119	1.674
		236	1.804		120	1.685
		237	2.037		121	1.804
		238	1.882		122	1.866
		239	1.943		123	1.801
		240	1.864		124	1.783
9	M	249	1.800	F	133	1.803
		250	1.996		134	1.750
		251	1.857		135	1.887
		252	1.794		136	1.662
		253	1.894		137	1.729
		254	1.836		138	1.808
		255	1.900		139	1.729
		256	1.708		140	1.810

M Males
 F Females

APPENDIX 5

Brain weights – individual values (g) for adult males and females on Day 11 of treatment

Group : 6 7 8 9
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Sex	Animal number	Brain weight	Sex	Animal number	Brain weight
6	M	209	2.175	F	93	1.804
		210	1.928		94	1.780
		211	1.872		95	1.772
		212	1.778		96	1.776
		213	1.928		97	1.873
		214	1.802		98	1.824
		215	1.885		99	1.774
		216	2.064		100	1.815
7	M	225	1.725	F	109	1.814
		226	1.892		110	1.872
		227	1.906		111	1.810
		228	1.952		112	1.806
		229	2.025		113	1.862
		230	1.843		114	1.821
		231	2.072		115	1.889
		232	2.070		116	1.680
8	M	241	1.895	F	125	1.852
		242	2.031		126	1.833
		243	1.825		127	1.800
		244	1.762		128	1.865
		245	1.891		129	1.872
		246	1.871		130	1.944
		247	2.179		131	1.811
		248	1.922		132	1.721
9	M	257	1.876	F	141	1.942
		258	1.953		142	1.760
		259	1.781		143	1.901
		260	1.957		144	1.817
		261	1.989		145	1.719
		262	1.986		146	1.787
		263	1.736		147	1.769
		264	2.074		148	1.877

M Males
 F Females

APPENDIX 6

Necropsy findings – individual observations for adult males and females on Day 1 of treatment

Group : 6 7 8 9
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Male number	Necropsy observations	Female number	Necropsy observations
6	201	Renal cavitation in right kidney	85	NAD
	202	NAD	86	NAD
	203	NAD	87	NAD
	204	NAD	88	NAD
	205	NAD	89	NAD
	206	NAD	90	NAD
	207	NAD	91	NAD
	208	NAD	92	NAD
7	217	NAD	101	NAD
	218	NAD	102	NAD
	219	NAD	103	NAD
	220	NAD	104	NAD
	221	NAD	105	NAD
	222	NAD	106	NAD
	223	NAD	107	NAD
	224	NAD	108	NAD

NAD No abnormalities detected.

APPENDIX 6 - continued

Necropsy findings – individual observations for adult males and females on Day 1 of treatment

Group : 6 7 8 9
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Male number	Necropsy observations	Female number	Necropsy observations
8	233	NAD	117	NAD
	234	NAD	118	NAD
	235	NAD	119	NAD
	236	NAD	120	NAD
	237	NAD	121	NAD
	238	NAD	122	NAD
	239	NAD	123	NAD
	240	NAD	124	NAD
9	249	NAD	133	NAD
	250	NAD	134	NAD
	251	NAD	135	NAD
	252	NAD	136	NAD
	253	NAD	137	NAD
	254	NAD	138	NAD
	255	NAD	139	NAD
	256	NAD	140	NAD

NAD No abnormalities detected.

APPENDIX 6 - continued

Necropsy findings – individual observations for adult males and females on Day 11 of treatment

Group : 6 7 8 9
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Male number	Necropsy observations	Female number	Necropsy observations
6	209	NAD	93	NAD
	210	NAD	94	NAD
	211	NAD	95	NAD
	212	NAD	96	NAD
	213	NAD	97	NAD
	214	NAD	98	NAD
	215	NAD	99	NAD
	216	NAD	100	NAD
7	225	NAD	109	NAD
	226	NAD	110	NAD
	227	Right testis blue and flaccid, right epididymis small.	111	NAD
	228	NAD	112	NAD
	229	NAD	113	NAD
	230	NAD	114	NAD
	231	NAD	115	NAD
	232	NAD	116	NAD

NAD No abnormalities detected.

APPENDIX 6 - continued

Necropsy findings – individual observations for adult males and females on Day 11 of treatment

Group : 6 7 8 9
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Male number	Necropsy observations	Female number	Necropsy observations
8	241	NAD	125	NAD
	242	NAD	126	NAD
	243	NAD	127	NAD
	244	NAD	128	NAD
	245	NAD	129	NAD
	246	NAD	130	NAD
	247	NAD	131	NAD
	248	NAD	132	NAD
9	257	NAD	141	NAD
	258	NAD	142	NAD
	259	NAD	143	NAD
	260	NAD	144	NAD
	261	NAD	145	NAD
	262	NAD	146	NAD
	263	NAD	147	NAD
	264	Thymus and mandibular lymph nodes haemorrhagic.	148	NAD

NAD No abnormalities detected.

APPENDIX 7

Clinical signs - individual observations during gestation and lactation

Group 1: Control

Animal number	Signs observed on Days
1A	-
2A	-
3A	Hairloss on upper ventral thorax on Days 8-15 of gestation.
4A	Brown staining on upper dorsal thorax on Days 2-6 of gestation. Brown staining on head on Days 7-20, and on pinnae on Days 13-20 of gestation.
5A	Brown staining on upper dorsal thorax and yellow staining on sacral region on Days 2-9 of gestation. Brown staining on head and pinnae on Days 10-17 of gestation.
6A	Brown staining on upper dorsal thorax on Days 1-20 of gestation. Yellow staining on sacral region on Days 1-2 and 10-14 of gestation. Brown staining on head on Days 10-14 and 20 of gestation.
7A	-
8A	Hairloss on dorsal body surface on Days 3-4 of gestation. Brown staining on dorsal body surface and upper dorsal thorax on Day 4 of gestation. Brown staining on forelimbs on Days 6-10 of gestation.
9A	Hairloss on forelimbs on Days 0-20 of gestation. Brown staining on head on Days 3-20, and on pinnae on Days 4-20 of gestation.
10B	Brown staining on upper dorsal thorax on Days 2-17 of gestation. Brown staining on pinnae on Days 8-17 of gestation.
11B	Brown staining on upper dorsal thorax on Days 2-6 of gestation and Days 20-21 of lactation. Hairloss on forelimbs on Days 13-21 of lactation, and encrustations on forelimbs on Day 13 of lactation.
12B	Brown staining on upper dorsal thorax on Days 6 and 8-15 of gestation, and on Days 13-20 of lactation. Brown staining on head on Day 6 of gestation.
13B	-
14B	Brown staining on upper dorsal thorax on Days 1-3 of gestation. Yellow staining on sacral region on Days 1-2 of gestation. Brown staining on head on Days 12-14 of gestation.
15B	Brown staining on head and upper dorsal thorax on Day 5 of gestation. Hairloss on forelimbs on Days 5-16 and 20-21 of gestation, and Days 1-21 of lactation.
16B	Brown staining on upper dorsal thorax on Days 1-21 of gestation and Days 1-5 and 19-21 of lactation. Brown staining on head on Days 5-21 of gestation and Days 1-5 of lactation. Yellow staining on sacral region on Days 1-2 of gestation. Hairloss on forelimbs on Days 9-21 of gestation and 1-21 of lactation.
17B	Brown staining on upper dorsal thorax on Day 5 of gestation. Hairloss on dorsal body surface on Days 5 and 10-14 of gestation and on upper dorsal thorax on Days 6-9 of gestation. Hairloss on forelimbs on Day 5 of lactation and on right forelimb on Day 12 of lactation.
18B	Brown staining on upper dorsal thorax on Days 1-5 and 9-12 of gestation. Brown staining on pinnae on Days 5-16 of gestation. Brown staining on head on Days 13-16 of gestation and on muzzle on Day 17 of gestation. Yellow staining on sacral region on Days 1-2 of gestation.
19B	Brown staining on head on Days 0-7 of gestation and on pinnae on Days 4-9 of gestation. Brown staining on upper dorsal thorax on Days 18-21 of lactation.

A Treated from Day 6 to Day 20 after mating.

B Treated from Day 6 after mating to Day 10 of lactation (where litter survived to Day 10).

APPENDIX 7 - continued

Clinical signs - individual observations during gestation and lactation

Group 2: 0.1 mg/kg/day

Animal number	Signs observed on Days
20A	Hairloss on dorsal body surface on Days 7 and 12-16 of gestation. Hairloss on upper dorsal thorax on Days 8-11 of gestation.
21A	-
22A	-
23A	Brown staining on head on Days 2-6 of gestation.
24A	Yellow staining on sacral region on Days 2-3 of gestation. Brown staining on upper dorsal thorax on Days 4-6 of gestation. Brown staining on head on Days 6-7 of gestation.
25A	Brown staining on dorsal body surface on Days 1-6 of gestation, and on upper dorsal thorax on Days 1-2 of gestation. Brown staining on head on Days 3-6 of gestation. Yellow staining on sacral region on Days 1-2 and 9-11 of gestation. Hairloss on head on Days 4-11 of gestation. Hairloss on dorsal body surface on Days 5-11 of gestation.
26A	-
27A	Brown staining on head on Days 3-20 of gestation, and on pinnae on Days 4-20 of gestation.
28A	-
29B	Brown staining on muzzle on Days 11-13 of gestation and Days 13-21 of lactation. Brown staining on head on Days 14-17 of gestation. Brown staining on right forelimb on Days 13-21 of lactation. Hairloss on hindlimbs on Days 11-13 of gestation. Hairloss on dorsal body surface on Days 14-21 of gestation. Hairloss on ventral body surface on Days 20-21 of gestation and Days 1-21 of lactation.
30B	Partially absent left pinnae from Day 6 of gestation onwards. Hairloss on dorsal body surface on Days 7-13 of gestation. Brown staining on head on Days 15-17 of gestation. Brown staining on upper dorsal thorax on Days 20-21 of lactation.
31B	Brown staining on upper dorsal thorax on Days 2-17 of gestation.
32B	Hairloss on dorsal body surface on Days 1-13 of gestation. Hairloss on head on Days 6-13 of gestation. Prominent upper ventral thorax on Days 6-7 of gestation. Brown staining on head on Days 15-17 of gestation.
33B	-
34B	Brown staining on upper dorsal thorax on Days 5-11 of gestation and Days 19-21 of lactation. Brown staining on head on Days 5-16 of gestation and Day 21 of lactation. Brown staining on dorsal body surface on Days 12-20 of gestation. Brown staining around left orbital region on day 21 of lactation.
35B	Yellow staining on sacral region on Days 1-2 of gestation. Hairloss on dorsal body surface on Days 4-11 of gestation.
36B	Brown staining on upper dorsal thorax on Day 5 of gestation.
37B	Hairloss on left forelimb on Days 6-7 of gestation and on both forelimbs on Days 8-14 of gestation. Brown staining on head on Days 12-16 of gestation. Brown staining on upper dorsal thorax on Day 21 of lactation.
38B	-

A Treated from Day 6 to Day 20 after mating.

B Treated from Day 6 after mating to Day 10 of lactation (where litter survived to Day 10).

APPENDIX 7 - continued

Clinical signs - individual observations during gestation and lactation

Group 3: 0.5 mg/kg/day

Animal number	Signs observed on Days
39A	Hairloss on dorsal body surface on Day 7 of gestation.
40A	Brown staining on upper dorsal thorax on Days 2-5 of gestation. Brown staining on head on Days 6-17 of gestation, and on pinnae on Days 6-7 of gestation. Yellow staining on sacral region on Days 8-11 of gestation. Hairloss on dorsal body surface on Days 2-6 of gestation, and on upper dorsal thorax on Days 7-12 of gestation. Black colouration to first digit (outermost) on left forelimb on Days 10 and 11 of gestation.
41A	Hairloss on dorsal body surface on Days 6 and 9-15 of gestation, and on upper dorsal thorax on Days 7-8 of gestation. Brown staining on muzzle on Days 7-8 of gestation, and on pinnae on Days 9-20 of gestation.
42A	Hairloss on upper dorsal thorax on Day 7 of gestation.
43A	Brown staining on upper dorsal thorax on Days 1-18 of gestation. Brown staining on head on Days 5-18 of gestation.
44A	Brown staining on pinnae on Days 5-20 of gestation, and on head on Days 6-20 of gestation.
45A	Brown staining on head on Days 1-20 of gestation, and on muzzle on Days 6-17 of gestation. Brown staining on dorsal body surface on Days 3-19 of gestation. Yellow staining on sacral region on Days 1-2 of gestation.
46A	-
47A	-
48B	-
49B	Brown staining on upper dorsal thorax on Days 2-9 of gestation and Day 21 of lactation. Brown staining on head on Days 2-9 of gestation and on pinnae on Days 6-9 and 12-21 of gestation. Brown staining on muzzle on Days 10-17 of gestation. Yellow staining on sacral region on Days 2 and 6-9 of gestation.
50B	Brown staining on head and pinnae on Days 6-17 of gestation. Brown staining on muzzle and forelimbs on Day 7 of gestation. Yellow staining on sacral region on Day 8 of gestation.
51B	-
52B	Yellow staining on sacral region on Day 1 of gestation. Brown staining on upper dorsal thorax on Days 2-5 of gestation. Brown staining on head on Days 12-17 of gestation.
53B	Brown staining on head on Days 4-12 of gestation and Day 21 of lactation.
54B	-
55B	Brown staining on head on Days 4-16 of gestation. Hairloss on dorsal body surface on Days 5 and 8-14 of gestation and on upper dorsal thorax on Days 6-7 of gestation.
56B	Brown staining on upper dorsal thorax on Days 1-4 of gestation. Brown staining on head on Days 5 and 12-14 of gestation.
57B	-

A Treated from Day 6 to Day 20 after mating.

B Treated from Day 6 after mating to Day 10 of lactation (where litter survived to Day 10).

APPENDIX 7 - continued

Clinical signs - individual observations during gestation and lactation

Group 4: 3 mg/kg/day

Animal number	Signs observed on Days
58A	Yellow staining on sacral region on Day 3 of gestation.
59A	Brown staining on upper dorsal thorax on Days 4-6 of gestation. Hairloss on dorsal body surface on Days 6-10 of gestation, and on upper dorsal thorax on Days 7-8 of gestation.
60A	-
61A	Brown staining on head on Days 2-3 and 13-20 of gestation. Hairloss on forelimbs on Days 16-20 of gestation.
62A	Brown staining on upper dorsal thorax on Days 1-5 of gestation. Hairloss on dorsal body surface on Days 1-19 of gestation, and on upper dorsal thorax on Days 6-7 of gestation.
63A	-
64A	-
65A	Brown staining on head on Days 11-20 of gestation.
66A	Brown staining on head on Days 4-7 of gestation, on pinnae on Days 4-15 of gestation and on muzzle on Days 5-15 of gestation.
67B	Yellow staining on sacral region on Day 2 of gestation. Brown staining on head and pinnae on Days 6-9 of gestation, and on muzzle on Days 10-17 of gestation. Hairloss on upper dorsal thorax on Days 7-8 of gestation and on dorsal body surface on Days 9-10 of gestation.
68B	-
69B	Hairloss on dorsal body surface on Days 1-18 of gestation. Brown staining on upper dorsal thorax on Days 2-6 of gestation and Days 20-21 of lactation.
70B	Brown staining on upper dorsal thorax on Days 1-5 of gestation and Days 20-21 of lactation. Brown staining on head on Days 6-18 of gestation and on pinnae on Days 6-21 of gestation.
71B	Hairloss on forelimbs on Days 11-21 of lactation.
72B	-
73B	Brown staining on upper dorsal thorax on Days 1-3 and 5-6 of gestation. Hairloss on head on Days 7-11 of gestation.
74B	Brown staining on head on Days 13-17 of gestation and on pinnae on Days 15-17 of gestation.
75B	Yellow staining on sacral region on Days 1-2 of gestation. Limited use of left hindlimb on Days 3-6 of gestation and abnormal gait on Days 4-6 of gestation. Opaque left eye on Days 5-6, 8-21 of gestation and Days 1-21 of lactation. Brown staining on forelimbs on Day 21 of lactation.
76B	Hairloss on forelimbs on Days 4-21 of gestation and Days 1-21 of lactation. Hairloss on dorsal body surface on Days 9-21 of gestation and Days 1-21 of lactation. Hairloss on ventral body surface on Days 18-21 of gestation and Days 1-21 of lactation. Encrustations on forelimbs on Days 9-11 of gestation, and on dorsal body surface on Days 9-15 of gestation.

A Treated from Day 6 to Day 20 after mating.

B Treated from Day 6 after mating to Day 10 of lactation (where litter survived to Day 10).

8/19

APPENDIX 7 - continued

Clinical signs - individual observations during gestation and lactation

Group 5 (untreated)

Animal number	Signs observed on Days
77	Hairloss on dorsal body surface on Days 14-22 of gestation and Days 1-11 of lactation. Hairloss on ventral body surface on Days 20-22 of gestation and Days 1-11 of lactation.
78	-
79	Hairloss on dorsal body surface on Days 2-12 of gestation. Encrustations on upper dorsal thorax on Days 2-3 of gestation. Brown staining on upper dorsal thorax on Days 17-18 of gestation.
80	-
81	Hairloss on dorsal body surface on Days 6-11 of gestation. Brown staining on head on Days 7-15 of gestation.
82	Yellow staining on sacral region on Days 2-3 of gestation. Brown staining on head on Days 5-21 of gestation. Hairloss on dorsal body surface on Days 3-13 of gestation.
83	Brown staining on upper dorsal thorax on Days 2-7 of gestation. Brown staining on pinnae on Days 6-7 and 13-17 of gestation, and on head on Days 13-17 of gestation.
84	Brown staining on head on Days 2-17 of gestation.

All Group 5 dams were killed on Day 11 of lactation.

APPENDIX 8

Bodyweight - individual values (g) for dams after mating

Group 1: Control

Animal number	Day after mating									
	0	3	6	10	14	17	20			
1	222	256	274	292	325	351	387			
2	222	257	274	303	336	367	405			
3	240	268	287	313	342	376	428			
4	233	261	276	302	337	366	418			
5	230	257	282	306	330	366	416			
6	238	269	286	315	344	389	442			
7	234	253	267	283	318	350	391			
8	251	275	296	320	351	383	444			
9	249	280	301	320	350	385	431			
10	234	258	266	290	313	346	387			
11	222	253	269	292	325	362	413			
12	218	242	258	275	300	330	371			
13	217	242	255	271	294	323	363			
14	229	249	270	291	312	339	388			
15	236	256	272	296	322	352	407			
16	231	251	265	275	300	326	372			
17	224	252	263	273	303	338	378			
18	225	247	262	281	315	341	396			
19	231	248	265	287	312	342	387			

APPENDIX 8 - continued

Bodyweight - individual values (g) for dams after mating

Group 2 : 0.1 mg/kg/day

Animal number	Day after mating									
	0	3	6	10	14	17	20			
20	232	255	271	291	315	353	406			
21	248	279	300	333	370	409	472			
22	238	264	284	313	340	386	411			
23	242	270	284	312	343	393	440			
24	224	243	256	279	302	336	394			
25	257	282	304	332	370	411	466			
26	242	267	284	304	334	367	408			
27	221	241	253	268	287	318	365			
28	231	248	265	284	308	336	381			
29	235	264	275	296	314	343	388			
30	226	248	256	277	306	327	365			
31	224	254	271	297	324	357	416			
32	219	246	258	279	302	335	380			
33	240	280	303	339	377	422	477			
34	236	258	285	303	334	369	418			
35	230	249	263	280	304	339	392			
36	224	242	260	279	307	347	403			
37	224	254	276	306	334	376	436			
38	243	265	279	297	323	356	398			

APPENDIX 8 - continued

Bodyweight - individual values (g) for dams after mating

Group 3: 0.5 mg/kg/day

Animal number	Day after mating									
	0	3	6	10	14	17	20			
39	221	248	268	291	311	352	397			
40	246	279	293	313	350	385	439			
41	233	270	291	312	348	378	433			
42	222	240	251	270	296	324	372			
43	256	282	300	318	350	385	416			
44	240	261	274	287	312	349	392			
45	243	271	296	317	348	372	428			
46	240	256	272	288	309	340	384			
47	226	247	265	287	307	340	386			
48	225	254	271	290	316	345	385			
49	227	249	262	278	305	315	345			
50	216	243	261	282	317	337	381			
51	218	243	256	287	319	352	411			
52	239	264	282	298	325	362	411			
53	232	254	267	286	309	340	386			
54	230	252	267	288	308	341	386			
55	228	256	277	298	325	362	412			
56	219	243	256	275	295	333	378			
57	255	284	308	326	363	406	460			

APPENDIX 8 - continued

Bodyweight - individual values (g) for dams after mating

Group 4 : 3 mg/kg/day

Animal number	Day after mating									
	0	3	6	10	14	17	20			
58	223	251	266	295	322	360	416			
59	241	271	290	321	360	404	471			
60	243	279	293	321	341	379	439			
61	230	251	270	296	317	353	403			
62	260	287	307	336	365	406	451			
63	221	265	264	276	308	352	408			
64	254	271	292	317	339	376	423			
65	251	270	295	316	343	383	438			
66	244	271	294	316	331	366	415			
67	228	249	260	276	302	335	387			
68	223	251	260	284	302	336	383			
69	218	247	257	281	306	331	381			
70	224	251	260	279	312	343	397			
71	233	256	278	294	318	352	400			
72	243	263	282	301	326	363	417			
73	227	253	268	286	312	348	385			
74	232	253	270	294	314	336	366			
75	221	230	240	260	280	319	361			
76	254	277	306	323	353	388	437			

APPENDIX 8 - continued

Bodyweight - individual values (g) for dams after mating

Group 5: untreated

Animal number	Day after mating									
	0	3	6	10	14	17	20			
77	230	264	286	322	349	386	446			
78	245	272	290	311	341	372	417			
79	236	264	273	302	330	352	413			
80	231	258	277	299	329	360	414			
81	218	238	251	266	286	320	374			
82	226	255	273	296	331	364	421			
83	226	256	276	302	326	364	424			
84	218	246	250	269	296	322	364			

APPENDIX 9

Bodyweight – individual values (g) for dams during lactation

Group 1: Control

Animal number	Day of lactation									
	1	4	7	11	14	17	21			
10	304	308	329	351	328	352	323			
11	313	328	324	354	344	326	333			
12	285	306	306	341	342	320	329			
13	294	300	297	330	333	308	309			
14	308	329	346	365	366	350	340			
15	313	321	333	343	363	372	346			
16	273	300	314	335	325	325	310			
17	283	299	307	349	346	327	332			
18	303	316	335	355	372	369	323			
19	320	329	327	344	345	357	349			

APPENDIX 9 - continued

Bodyweight – individual values (g) for dams during lactation

Group 2: 0.1 mg/kg/day

Animal number	Day of lactation									
	1	4	7	11	14	17	21			
29	303	318	341	359	354	338	323			
30	284	292	300	324	317	302	310			
31	321	344	345	382	384	362	349			
32	289	314	315	348	334	328	318			
33	382	390	400	413	417	411	387			
34	340	353	356	378	374	383	337			
35	293	305	313	323	331	335	311			
36	304	320	326	340	341	353	333			
37	357	350	357	407	378	386	362			
38	323	339	355	378	376	400	390			

APPENDIX 9 - continued

Bodyweight – individual values (g) for dams during lactation

Group 3: 0.5 mg/kg/day

Animal number	Day of lactation									
	1	4	7	11	14	17	21			
48	300	309	315	333	341	335	324			
49	298	298	309	321	324	319	311			
50	311	326	344	370	361	356	319			
51	294	330	336	379	376	370	340			
52	326	344	339	374	389	384	369			
53	295	300	317	329	327	333	323			
54	278	299	302	316	325	338	317			
55	329	338	350	367	368	376	352			
56	308	310	310	325	338	341	328			
57	352	360	363	379	398	378	343			

APPENDIX 9 - continued

Bodyweight – individual values (g) for dams during lactation

Group 4: 3 mg/kg/day

Animal number	Day of lactation									
	1	4	7	11	14	17	21			
67	292	303	306	329	327	312	312			
68	284	292	313	340	345	337	337			
69	284	309	317	346	345	331	333			
70	298	318	324	358	367	350	342			
71	308	328	340	364	379	374	368			
72	311	324	331	361	346	347	351			
73	290	311	319	352	340	345	334			
74	313	322	335	338	357	353	333			
75	272	281	304	328	321	328	326			
76	355	361	367	386	398	390	389			

APPENDIX 9 - continued

Bodyweight – individual values (g) for dams during lactation

Group 5: untreated #

Animal number	Day of lactation				
	1	4	7	11	
77	338	360	354	404	
78	319	345	337	366	
79	308	345	356	382	
80	338	343	362	380	
81	266	281	291	335	
82	319	354	356	386	
83	324	344	342	367	
84	276	287	296	322	

Females killed on Day 11 of lactation.

APPENDIX 10

Gestation length – individual values

Group : 1 2 3 4 5
 Compound : Control ----- Dimethoate ----- Untreated
 Dosage (mg/kg/day) : 0 0.1 0.5 3.0 0

Group 1			Group 2			Group 3			Group 4			Group 5		
Female Number	Gestation Length (days)	Female number	Gestation Length (days)	Female number	Gestation Length (days)	Female number	Gestation Length (days)	Female number	Gestation Length (days)	Female number	Gestation Length (days)	Female number	Gestation Length (days)	Female number
10	23	29	22	48	23	67	22	77	23	77	23	77	23	77
11	22	30	22	49	23	68	22	78	22.5	78	22.5	78	22.5	78
12	22	31	22	50	22.5	69	22	79	22	79	22	79	22	79
13	22	32	22	51	22	70	22	80	22	80	22.5	80	22.5	80
14	22	33	22.5	52	22	71	22.5	81	22.5	81	22	81	22	81
15	22	34	22	53	22	72	22	82	22	82	22	82	22	82
16	22	35	22	54	22	73	22	83	22	83	22	83	22	83
17	22	36	22	55	22	74	22	84	23	84	22	84	22	84
18	22	37	22.5	56	22.5	75	22.5		22	75	22		22	
19	22	38	23	57	22.5	76	22.5		22.5	76	22.5		22.5	

APPENDIX 11

Necropsy observations for adult females

Day 20 of gestation

Group	Animal number	Necropsy observations
1	1	NAD
	2	NAD
	3	NAD
	4	Brown staining on head and pinnae.
	5	NAD
	6	Brown staining on head and upper dorsal thorax.
	7	NAD
	8	NAD
	9	Brown staining on muzzle and pinnae. Hairloss on forelimbs.
2	20	NAD
	21	NAD
	22	NAD
	23	NAD
	24	NAD
	25	NAD
	26	NAD
	27	Brown staining on head and pinnae.
	28	NAD

NAD No abnormalities detected.

APPENDIX 11 - continued

Necropsy observations for adult females.

Day 20 of gestation.

Group	Animal number	Necropsy observations
3	39	NAD
	40	NAD
	41	NAD
	42	NAD
	43	NAD
	44	Brown staining on head and pinnae.
	45	Brown staining on head.
	46	NAD
	47	NAD
4	58	NAD
	59	NAD
	60	NAD
	61	Slight brown staining on head. Hairloss on head and forelimbs.
	62	NAD
	63	NAD
	64	NAD
	65	Slight brown staining on head.
	66	NAD

NAD No abnormalities detected.

APPENDIX 11 – continued

Necropsy observations for adult females.

Day 21 of lactation.

Group	Animal number	Necropsy observations
1	10	NAD
	11	Brown staining on head and upper dorsal surface. Hairloss on forelimbs.
	12	NAD
	13	NAD
	14	NAD
	15	Slight hairloss on forelimbs.
	16	Slight brown staining on head and upper dorsal thorax. Hairloss on forelimbs.
	17	NAD
	18	NAD
	19	Brown staining on head and upper dorsal thorax.
2	29	Hairloss on ventral surface. Brown staining on forelimbs and muzzle.
	30	Slight brown staining on upper dorsal surface.
	31	NAD
	32	NAD
	33	NAD
	34	Brown staining on head, muzzle and upper dorsal thorax.
	35	NAD
	36	NAD
	37	Slight brown staining on upper dorsal thorax.
	38	NAD

NAD No abnormalities detected.

APPENDIX 11 – continued

Necropsy observations for adult females.

Day 21 of lactation

Group	Animal number	Necropsy observations
3	48	NAD
	49	Brown staining on upper dorsal surface.
	50	Slight brown staining on head.
	51	NAD
	52	NAD
	53	Slight brown staining on head.
	54	NAD
	55	NAD
	56	NAD
	57	NAD
4	67	NAD
	68	NAD
	69	Slight brown staining on upper dorsal thorax.
	70	Brown staining on upper dorsal thorax.
	71	Hairloss on forelimbs.
	72	NAD
	73	NAD
	74	NAD
	75	Brown staining on forelimbs. Left eye opaque.
	76	Hairloss on ventral surface, forelimbs and upper hindlimbs.

NAD No abnormalities detected.

APPENDIX 11 – continued

Necropsy observations for adult females.

Day 11 of lactation

Group	Animal number	Necropsy observations
5	77	Hairloss on dorsal and ventral surfaces and forelimbs.
	78	NAD
	79	NAD
	80	NAD
	81	NAD
	82	NAD
	83	NAD
	84	NAD

NAD No abnormalities detected.

APPENDIX 12

Litter data – individual values on Day 20 of gestation

Group 1: Control

Animal number	Corpora lutea	Implants	Resorptions		Total	Live young		Sex ratio (% M)	Implantation loss (%)	
			Early	Late		Male	Female		Pre-	Post-
1	20	11	1	0	1	6	4	60.0	45.0	9.1
2	17	14	3	0	3	6	5	54.5	17.6	21.4
3	16	16	0	0	0	7	9	43.8	0.0	0.0
4	16	16	0	0	0	9	7	56.3	0.0	0.0
5	16	15	1	0	1	7	7	50.0	6.3	6.7
6	18	17	0	0	0	11	6	64.7	5.6	0.0
7	13	13	0	0	0	5	8	38.5	0.0	0.0
8	16	16	0	0	0	8	8	50.0	0.0	0.0
9	15	15	0	0	0	5	10	33.3	0.0	0.0

Group 2: 0.1 mg/kg/day

Animal number	Corpora lutea	Implants	Resorptions		Total	Live young		Sex ratio (% M)	Implantation loss (%)	
			Early	Late		Male	Female		Pre-	Post-
20	17	17	1	0	1	11	5	68.8	0.0	5.9
21	16	16	1	0	1	7	8	46.7	0.0	6.3
22	16	16	1	13	14	2	0	100.0	0.0	87.5
23	17	19	1	0	1	8	10	44.4	0.0	5.3
24	19	18	1	0	1	6	11	35.3	5.3	5.6
25	15	15	1	0	1	8	6	57.1	0.0	6.7
26	15	14	2	0	2	4	8	33.3	6.7	14.3
27	15	14	1	0	1	6	7	46.2	6.7	7.1
28	15	15	1	0	1	6	8	42.9	0.0	6.7

APPENDIX 12 - continued

Litter data – individual values on Day 20 of gestation

Group 3: 0.5 mg/kg/day

Animal number	Corpora lutea	Implants	Resorptions		Total	Live young		Sex ratio (% M)	Implantation loss (%)	
			Early	Late		Male	Female		Pre-	Post-
39	18	15	1	0	1	8	6	57.1	16.7	6.7
40	19	18	1	0	1	8	9	47.1	5.3	5.6
41	17	16	0	0	0	10	6	62.5	5.9	0.0
42	15	15	1	0	1	6	8	42.9	0.0	6.7
43	15	15	2	0	2	7	6	53.8	0.0	13.3
44	17	15	0	0	0	7	8	46.7	11.8	0.0
45	14	15	1	0	1	8	6	57.1	0.0	6.7
46	15	13	1	0	1	6	6	50.0	13.3	7.7
47	17	16	0	0	0	3	13	18.8	5.9	0.0

Group 4: 3 mg/kg/day

Animal number	Corpora lutea	Implants	Resorptions		Total	Live young		Sex ratio (% M)	Implantation loss (%)	
			Early	Late		Male	Female		Pre-	Post-
58	16	16	0	0	0	6	10	37.5	0.0	0.0
59	18	17	1	0	1	9	7	56.3	5.6	5.9
60	18	19	0	0	0	4	15	21.1	0.0	0.0
61	18	16	2	0	2	8	6	57.1	11.1	12.5
62	15	15	0	0	0	7	8	46.7	0.0	0.0
63	18	17	0	0	0	9	8	52.9	5.6	0.0
64	14	15	1	0	1	8	6	57.1	0.0	6.7
65	17	16	2	0	2	7	7	50.0	5.9	12.5
66	15	15	2	0	2	7	6	53.8	0.0	13.3

APPENDIX 13

Fetal and litter weights -- individual values (g) at Day 20 of gestation

Group 1: Control

Animal number	Litter weight	Fetal weights					
		Males		Females		Total	
		Mean	SD	Mean	SD	Mean	SD
1	38.77	3.88	0.23	3.87	0.27	3.88	0.23
2	43.13	4.03	0.10	3.79	0.13	3.92	0.17
3	60.26	3.83	0.21	3.72	0.20	3.77	0.21
4	62.25	3.97	0.23	3.79	0.18	3.89	0.22
5	57.56	4.27	0.18	3.96	0.22	4.11	0.25
6	67.73	4.00	0.21	3.95	0.15	3.98	0.19
7	53.54	4.18	0.08	4.08	0.18	4.12	0.15
8	62.33	4.05	0.25	3.74	0.12	3.90	0.25
9	52.09	3.41	0.36	3.50	0.19	3.47	0.25

SD Standard deviation.

APPENDIX 13 - continued

Fetal and litter weights – individual values (g) at Day 20 of gestation

Group 2: 0.1 mg/kg/day

Animal number	Litter weight	Fetal weights (g)					
		Males		Females		Total	
		Mean	SD	Mean	SD	Mean	SD
20	63.25	3.99	0.10	3.86	0.17	3.95	0.14
21	59.91	4.11	0.16	3.90	0.22	3.99	0.22
22	7.15	3.58	0.11	-	-	3.58	0.11
23	69.94	4.07	0.17	3.74	0.14	3.89	0.22
24	65.06	3.95	0.16	3.76	0.20	3.83	0.20
25	58.96	4.21	0.42	4.22	0.34	4.21	0.37
26	46.84	4.00	0.18	3.85	0.09	3.90	0.14
27	53.63	4.15	0.18	4.11	0.12	4.13	0.14
28	56.27	4.19	0.10	3.89	0.12	4.02	0.19

SD Standard deviation.

- No females in litter.

APPENDIX 13 - continued

Fetal and litter weights – individual values (g) at Day 20 of gestation

Group 3: 0.5 mg/kg/day

Animal number	Litter weight	Fetal weights (g)					
		Males		Females		Total	
		Mean	SD	Mean	SD	Mean	SD
39	59.90	4.43	0.16	4.08	0.06	4.28	0.21
40	61.26	3.63	0.49	3.58	0.20	3.60	0.36
41	63.68	4.14	0.18	3.72	0.75	3.98	0.50
42	59.49	4.46	0.17	4.09	0.19	4.25	0.26
43	46.14	3.64	0.23	3.45	0.23	3.55	0.24
44	54.99	3.77	0.07	3.57	0.13	3.67	0.15
45	60.16	4.38	0.23	4.18	0.25	4.30	0.25
46	48.85	4.18	0.19	3.97	0.15	4.07	0.20
47	59.08	3.90	0.25	3.64	0.19	3.69	0.22

SD Standard deviation.

APPENDIX 13 - continued

Fetal and litter weights -- individual values (g) at Day 20 of gestation

Group 4: 3 mg/kg/day

Animal number	Litter weight	Fetal weights (g)					
		Males		Females		Total	
		Mean	SD	Mean	SD	Mean	SD
58	60.30	3.98	0.25	3.64	0.25	3.77	0.29
59	67.39	4.24	0.21	4.18	0.22	4.21	0.21
60	74.24	4.16	0.16	3.84	0.21	3.91	0.24
61	62.49	4.64	0.15	4.23	0.24	4.46	0.28
62	57.39	3.95	0.11	3.71	0.22	3.83	0.21
63	67.28	4.07	0.24	3.83	0.15	3.96	0.23
64	59.81	4.45	0.22	4.04	0.18	4.27	0.29
65	56.05	4.09	0.11	3.92	0.23	4.00	0.19
66	53.19	4.28	0.18	3.87	0.22	4.09	0.28

SD Standard deviation.

APPENDIX 14

Brain weights – individual values (g) for dams and individual litter mean values (g) for fetuses on Day 20 of gestation

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal Number	Dam brain weight	Fetuses		
			Pooled brain weight	Number of fetuses	Mean brain weight (g)
1	1	1.827	1.672	10	0.167
	2	1.958	1.884	11	0.171
	3	1.705	2.629	16	0.164
	4	1.999	2.618	16	0.164
	6	1.928	2.833	17	0.167
	7	1.997	2.203	13	0.169
	8	1.808	2.442	16	0.153
	9	2.003	2.234	15	0.149
2	20	1.849	2.585	16	0.162
	21	2.086	2.482	15	0.165
	22	1.958	0.284	2	0.142
	23	1.835	2.882	18	0.160
	25	1.920	2.248	14	0.161
	26	1.886	2.025	12	0.169
	27	1.930	2.246	13	0.173
	28	2.008	2.191	14	0.157

APPENDIX 14 - continued

Brain weights – individual values (g) for dams and individual litter mean values (g) for fetuses on Day 20 of gestation

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal Number	Dam brain weight	Fetuses		
			Pooled brain weight	Number of fetuses	Mean brain weight (g)
3	39	1.832	2.318	14	0.166
	40	1.911	2.702	17	0.159
	41	1.823	2.741	16	0.171
	43	1.844	2.099	13	0.161
	44	1.823	2.618	15	0.175
	45	1.769	2.242	14	0.160
	46	1.862	2.019	12	0.168
	47	1.830	2.488	16	0.156
4	58	1.847	2.590	16	0.162
	59	2.032	2.868	16	0.179
	60	1.840	3.079	19	0.162
	62	1.927	2.626	15	0.175
	63	1.926	2.892	17	0.170
	64	1.810	2.202	14	0.157
	65	1.296	2.209	14	0.158
	66	1.846	2.221	13	0.171

APPENDIX 15

Litter size up to Day 11 of age – individual values

Group 1: Control

Animal number	Implants	Total litter size Day 1	Live litter size on Day						
			Before cull		After cull				
			1	4	4	4	7	7	11
10	16	12	12	12	8	8	8	8	8
11	15	15	15	15	8	8	8	8	8
12	15	15	15	15	8	8	8	8	8
13	14	12	12	12	8	8	8	8	8
14	13	12	12	12	8	8	8	8	8
15	15	14	14	14	8	8	8	8	8
16	15	15	15	15	8	8	8	8	8
17	15	14	14	14	8	8	8	8	8
18	14	12	12	12	8	8	8	8	8
19	14	12	12	12	8	8	8	8	8

APPENDIX 15 - continued

Litter size up to Day 11 of age – individual values

Group 2: 0.1 mg/kg/day

Animal number	Implants	Total litter size Day 1	Live litter size on Day						
			Before cull			After cull			
			1	4	4	4	7	11	
29	13	12	12	12	8	8	8	8	8
30	17	14	14	14	8	8	8	8	8
31	17	16	16	16	8	8	8	8	8
32	15	14	14	14	8	8	8	8	8
33	18	17	17	17	8	8	8	8	8
34	14	13	13	13	8	8	8	8	8
35	17	16	16	15	8	8	8	8	8
36	16	14	14	14	8	8	8	8	8
37	17	15	15	15	8	8	8	8	8
38	16	11	11	10	8	8	8	8	8

APPENDIX 15 - continued

Litter size up to Day 11 of age – individual values

Group 3: 0.5 mg/kg/day

Animal number	Implants	Total litter size Day 1	Live litter size on Day						
			Before cull		After cull				
			1	4	4	4	7	11	
48	14	10	10	10	8	8	8	8	
49	8	5	5	5	5	5	5	5	
50	11	11	11	11	8	8	8	8	
51	18	17	17	17	8	8	8	8	
52	15	15	15	15	8	8	8	8	
53	15	14	14	13	8	8	8	8	
54	16	16	16	16	8	8	8	8	
55	16	13	13	12	8	8	8	8	
56	15	14	14	14	8	8	8	8	
57	18	18	18	18	8	8	8	8	

APPENDIX 15 - continued

Litter size up to Day 11 of age -- individual values

Group 4: 3 mg/kg/day

Animal number	Implants	Total litter size Day 1	Live litter size on Day						
			Before cull			After cull			
			1	4	4	4	7	7	11
67	16	16	16	16	16	8	8	8	8
68	16	15	15	15	15	8	8	8	8
69	16	16	16	16	16	8	8	8	8
70	16	16	16	16	16	8	8	8	8
71	14	14	14	14	14	8	8	8	8
72	15	15	15	14	14	8	8	8	8
73	15	14	14	14	14	8	8	8	8
74	8	7	7	7	7	7	7	7	7
75	12	12	12	12	12	8	8	8	8
76	12	12	12	12	12	8	8	8	7

APPENDIX 15 - continued

Litter size up to Day 11 of age – individual values

Group 5: Untreated

Animal number	Implants	Total litter size Day 1	Live litter size on Day						
			Before cull		After cull				
			1	4	4	7	11		
77	18	15	15	15	8	8	8		
78	17	16	16	16	8	8	8		
79	16	14	14	14	8	8	8		
80	15	14	14	14	8	8	8		
81	18	17	17	17	8	8	8		
82	16	16	16	16	8	8	8		
83	17	17	17	17	8	8	8		
84	14	13	13	13	8	8	8		

APPENDIX 16

Offspring survival indices up to Day 11 of age- individual values

Group 1: Control

Animal number	Post-implantation Survival index (%)	Live birth Index (%)	Viability index (%)	Lactation index (%)	
				Day 7	Day 11
10	75	100	100	100	100
11	100	100	100	100	100
12	100	100	100	100	100
13	86	100	100	100	100
14	92	100	100	100	100
15	93	100	100	100	100
16	100	100	100	100	100
17	93	100	100	100	100
18	86	100	100	100	100
19	86	100	100	100	100

APPENDIX 16 - continued

Offspring survival indices up to Day 11 of age- individual values

Group 2: 0.1 mg/kg/day

Animal number	Post-implantation Survival index (%)	Live birth Index (%)	Viability index (%)	Lactation index (%)	
				Day 7	Day 11
29	92	100	100	100	100
30	82	100	100	100	100
31	94	100	100	100	100
32	93	100	100	100	100
33	94	100	100	100	100
34	93	100	100	100	100
35	94	100	94	100	100
36	88	100	100	100	100
37	88	100	100	100	100
38	69	100	91	100	100

APPENDIX 16 - continued

Offspring survival indices up to Day 11 of age- individual values

Group 3: 0.5 mg/kg/day

Animal number	Post-implantation Survival index (%)	Live birth Index (%)	Viability index (%)	Lactation index (%)	
				Day 7	Day 11
48	71	100	100	100	100
49	63	100	100	100	100
50	100	100	100	100	100
51	94	100	100	100	100
52	100	100	100	100	100
53	93	100	93	100	100
54	100	100	100	100	100
55	81	100	92	100	100
56	93	100	100	100	100
57	100	100	100	100	100

APPENDIX 16 - continued

Offspring survival indices up to Day 11 of age- individual values

Group 4: 3 mg/kg/day

Animal number	Post-implantation Survival index (%)	Live birth Index (%)	Viability index (%)	Lactation index (%)	
				Day 7	Day 11
67	100	100	100	100	100
68	94	100	100	100	100
69	100	100	100	100	100
70	100	100	100	100	100
71	100	100	100	100	100
72	100	100	93	100	100
73	93	100	100	100	100
74	88	100	100	100	100
75	100	100	100	100	100
76	100	100	100	100	88

APPENDIX 16 - continued

Offspring survival indices up to Day 11 of age- individual values

Group 5: Untreated

Animal number	Post-implantation Survival index (%)	Live birth Index (%)	Viability index (%)	Lactation index (%)	
				Day 7	Day 11
77	83	100	100	100	100
78	94	100	100	100	100
79	88	100	100	100	100
80	93	100	100	100	100
81	94	100	100	100	100
82	100	100	100	100	100
83	100	100	100	100	100
84	93	100	100	100	100

APPENDIX 17

Sex ratio up to Day 11 of age – individual litter values

Group 1: Control

Animal number	Total on Day 1			Live (before cull)						Live (after cull)					
	M		%M	Day 1		%M	Day 4		%M	Day 4		%M	Day 7		%M
	F	%F		M	F		M	F		M	F		M	F	
10	8	4	66.7	8	4	66.7	8	4	66.7	4	4	50.0	4	4	50.0
11	9	6	60.0	9	6	60.0	9	6	60.0	4	4	50.0	4	4	50.0
12	7	8	46.7	7	8	46.7	7	8	46.7	4	4	50.0	4	4	50.0
13	9	3	75.0	9	3	75.0	9	3	75.0	5	3	62.5	5	3	62.5
14	5	7	41.7	5	7	41.7	5	7	41.7	4	4	50.0	4	4	50.0
15	9	5	64.3	9	5	64.3	9	5	64.3	4	4	50.0	4	4	50.0
16	9	6	60.0	9	6	60.0	9	6	60.0	4	4	50.0	4	4	50.0
17	7	7	50.0	7	7	50.0	7	7	50.0	4	4	50.0	4	4	50.0
18	8	4	66.7	8	4	66.7	8	4	66.7	4	4	50.0	4	4	50.0
19	6	6	50.0	6	6	50.0	6	6	50.0	4	4	50.0	4	4	50.0

M Males.
F Females.

APPENDIX 17 - continued

Sex ratio up to Day 11 of age – individual litter values

Group 2: 0.1 mg/kg/day

Animal number	Total on Day 1			Live (before cull)						Live (after cull)								
	M		%M	Day 1		%M	Day 4		%M	Day 4		%M	Day 7		%M	Day 11		%M
	M	F		M	F		M	F		M	F		M	F		M	F	
29	10	2	83.3	10	2	83.3	10	2	83.3	6	2	75.0	6	2	75.0	6	2	75.0
30	2	12	14.3	2	12	14.3	2	12	14.3	2	6	25.0	2	6	25.0	2	6	25.0
31	7	9	43.8	7	9	43.8	7	9	43.8	4	4	50.0	4	4	50.0	4	4	50.0
32	5	9	35.7	5	9	35.7	5	9	35.7	4	4	50.0	4	4	50.0	4	4	50.0
33	6	11	35.3	6	11	35.3	6	11	35.3	4	4	50.0	4	4	50.0	4	4	50.0
34	4	9	30.8	4	9	30.8	4	9	30.8	4	4	50.0	4	4	50.0	4	4	50.0
35	9	7	56.3	9	7	56.3	8	7	53.3	4	4	50.0	4	4	50.0	4	4	50.0
36	7	7	50.0	7	7	50.0	7	7	50.0	4	4	50.0	4	4	50.0	4	4	50.0
37	6	9	40.0	6	9	40.0	6	9	40.0	4	4	50.0	4	4	50.0	4	4	50.0
38	6	5	54.5	6	5	54.5	6	4	60.0	4	4	50.0	4	4	50.0	4	4	50.0

M Males.
F Females.

APPENDIX 17 - continued

Sex ratio up to Day 11 of age – individual litter values

Group 3: 0.5 mg/kg/day

Animal number	Total on Day 1			Live (before cull)						Live (after cull)								
	I		%M	Day 1			Day 4			Day 4			Day 7			Day 11		
	M	F		M	F	%M	M	F	%M	M	F	%M	M	F	%M	M	F	%M
48	5	5	50.0	5	5	50.0	5	5	50.0	4	4	50.0	4	4	50.0	4	4	50.0
49	3	2	60.0	3	2	60.0	3	2	60.0	3	2	60.0	3	2	60.0	3	2	60.0
50	3	8	27.3	3	8	27.3	3	8	27.3	3	5	37.5	3	5	37.5	3	5	37.5
51	5	12	29.4	5	12	29.4	5	12	29.4	4	4	50.0	4	4	50.0	4	4	50.0
52	8	7	53.3	8	7	53.3	8	7	53.3	4	4	50.0	4	4	50.0	4	4	50.0
53	9	5	64.3	9	5	64.3	8	5	61.5	4	4	50.0	4	4	50.0	4	4	50.0
54	10	6	62.5	10	6	62.5	10	6	62.5	4	4	50.0	4	4	50.0	4	4	50.0
55	9	4	69.2	9	4	69.2	8	4	66.7	4	4	50.0	4	4	50.0	4	4	50.0
56	10	4	71.4	10	4	71.4	10	4	71.4	4	4	50.0	4	4	50.0	4	4	50.0
57	10	8	55.6	10	8	55.6	10	8	55.6	4	4	50.0	4	4	50.0	4	4	50.0

M Males.
F Females.

APPENDIX 17 - continued

Sex ratio up to Day 11 of age – individual litter values

Group 4: 3 mg/kg/day

Animal number	Total on Day 1			Live (before cull)						Live (after cull)					
	M		%M	Day 1		%M	Day 4		%M	Day 4		%M	Day 7		%M
	M	F		M	F		M	F		M	F		M	F	
67	6	10	37.5	6	10	37.5	6	10	37.5	4	4	50.0	4	4	50.0
68	8	7	53.3	8	7	53.3	8	7	53.3	4	4	50.0	4	4	50.0
69	7	9	43.8	7	9	43.8	7	9	43.8	4	4	50.0	4	4	50.0
70	8	8	50.0	8	8	50.0	8	8	50.0	4	4	50.0	4	4	50.0
71	5	9	35.7	5	9	35.7	5	9	35.7	4	4	50.0	4	4	50.0
72	8	7	53.3	8	7	53.3	7	7	50.0	4	4	50.0	4	4	50.0
73	8	6	57.1	8	6	57.1	8	6	57.1	4	4	50.0	4	4	50.0
74	1	6	14.3	1	6	14.3	1	6	14.3	1	6	14.3	1	6	14.3
75	6	6	50.0	6	6	50.0	6	6	50.0	4	4	50.0	4	4	50.0
76	9	3	75.0	9	3	75.0	9	3	75.0	5	3	62.5	4	3	57.1

M Males.
F Females.

APPENDIX 17 - continued

Sex ratio up to Day 11 of age – individual litter values

Group 5: Untreated

Animal number	Total on Day 1			Live (before cull)						Live (after cull)					
	M		%M	Day 1		%M	Day 4		%M	Day 4		%M	Day 7		%M
	M	F		M	F		M	F		M	F		M	F	
77	8	7	53.3	8	7	53.3	8	7	53.3	4	4	50.0	4	4	50.0
78	5	11	31.3	5	11	31.3	5	11	31.3	4	4	50.0	4	4	50.0
79	6	8	42.9	6	8	42.9	6	8	42.9	4	4	50.0	4	4	50.0
80	6	8	42.9	6	8	42.9	6	8	42.9	4	4	50.0	4	4	50.0
81	11	6	64.7	11	6	64.7	11	6	64.7	4	4	50.0	4	4	50.0
82	8	8	50.0	8	8	50.0	8	8	50.0	4	4	50.0	4	4	50.0
83	13	4	76.5	13	4	76.5	13	4	76.5	4	4	50.0	4	4	50.0
84	4	9	30.8	4	9	30.8	4	9	30.8	4	4	50.0	4	4	50.0

M Males.
F Females.

APPENDIX 18

Bodyweight up to Day 11 of age -- individual litter mean values (g) for male offspring

Group 1: Control

Animal Number	Day of age											
	Before cull				After cull							
	1		4		4		7		11			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
10	8.0	0.4	11.0	0.5	11.3	0.6	18.7	1.4	29.6	1.5		
11	6.3	0.3	11.0	0.8	10.7	0.3	16.0	0.5	25.7	1.1		
12	6.3	0.3	10.3	0.6	10.3	0.5	16.0	0.5	26.3	1.1		
13	7.0	0.3	10.5	0.7	10.1	0.5	16.1	0.2	25.5	0.8		
14	5.9	0.8	7.5	0.6	7.7	0.5	11.6	0.7	22.6	1.6		
15	6.5	0.5	9.3	0.5	9.4	0.5	16.5	1.2	26.5	1.4		
16	6.6	0.3	9.2	0.4	9.2	0.2	15.3	0.4	27.1	0.8		
17	6.3	0.3	9.0	0.5	9.3	0.3	14.5	0.8	24.5	1.0		
18	7.0	0.4	10.1	0.5	10.2	0.5	15.7	0.9	25.8	1.4		
19	6.1	0.3	9.2	0.5	9.0	0.4	13.7	0.8	22.1	1.1		

SD Standard deviation.

APPENDIX 18 - continued

Bodyweight up to Day 11 of age – individual litter mean values (g) for male offspring

Group 2: 0.1 mg/kg/day

Animal Number	Day of age											
	Before cull						After cull					
	1		4		4		7		7		11	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
29	6.0	0.9	10.3	1.4	10.0	1.7	14.6	2.9	14.6	2.9	22.7	4.3
30	5.8	1.0	8.2	0.2	8.2	0.2	13.7	1.7	13.7	1.7	22.6	2.2
31	6.6	0.6	10.2	0.8	9.9	1.0	15.8	0.8	15.8	0.8	25.1	1.6
32	6.2	0.4	10.2	0.5	10.3	0.5	16.9	0.6	16.9	0.6	26.7	0.9
33	6.6	0.3	8.8	0.4	9.0	0.4	14.4	1.0	14.4	1.0	26.2	0.8
34	6.9	0.3	9.8	0.1	9.8	0.1	15.1	0.8	15.1	0.8	26.8	0.6
35	5.5	1.5	8.6	2.2	9.6	0.5	16.1	0.7	16.1	0.7	26.5	0.6
36	6.5	0.4	10.2	0.4	10.2	0.6	16.6	0.6	16.6	0.6	27.6	1.4
37	6.8	0.5	9.4	0.4	9.3	0.4	15.3	0.7	15.3	0.7	25.3	0.8
38	7.3	0.5	10.2	0.6	10.1	0.7	16.7	1.6	16.7	1.6	27.2	3.2

SD Standard deviation.

APPENDIX 18 - continued

Bodyweight up to Day 11 of age – individual litter mean values (g) for male offspring

Group 3: 0.5 mg/kg/day

Animal Number	Day of age									
	Before cull				After cull					
	1		4		4		7		11	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
48	6.5	0.8	10.4	0.9	10.1	0.5	14.9	1.2	24.4	1.5
49	8.0	0.2	12.4	0.5	12.4	0.5	18.3	0.6	27.0	0.3
50	8.2	0.4	11.1	0.4	11.1	0.4	17.3	0.3	27.4	0.4
51	5.7	0.7	10.0	1.2	10.2	1.2	14.6	1.3	25.4	1.9
52	6.0	0.4	9.1	0.8	9.3	0.5	15.4	0.7	26.4	0.9
53	5.4	1.1	8.7	1.0	8.9	0.5	14.2	1.3	24.7	1.4
54	6.0	0.4	9.2	0.4	9.2	0.3	14.2	0.8	23.8	1.2
55	5.8	0.5	9.8	0.9	9.9	0.8	16.0	2.2	26.1	2.2
56	6.6	0.4	9.1	0.5	9.6	0.6	14.8	1.1	23.5	1.6
57	7.4	0.5	10.5	1.1	10.3	0.8	17.4	0.7	27.6	1.6

SD Standard deviation.

APPENDIX 18 - continued

Bodyweight up to Day 11 of age – individual litter mean values (g) for male offspring

Group 4: 3 mg/kg/day

Animal Number	Day of age									
	Before cull				After cull					
	1	4			4			7		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
67	6.0	0.4	9.0	0.7	9.1	0.9	14.6	0.5	23.8	0.5
68	6.5	0.4	10.0	0.7	10.5	0.7	15.3	0.8	24.6	0.9
69	5.6	0.5	10.4	0.9	10.4	1.0	15.5	1.1	25.1	1.9
70	6.0	0.2	9.3	0.6	9.4	0.6	16.3	1.2	24.9	1.5
71	7.7	0.1	10.5	0.7	10.3	0.6	15.6	0.6	25.5	0.2
72	5.7	0.4	7.8	0.6	7.9	0.9	11.6	0.5	19.5	0.7
73	6.4	0.2	9.1	0.4	9.0	0.3	13.6	0.9	23.7	0.8
74	8.6	x	12.3	x	12.3	x	19.0	x	30.5	x
75	6.4	0.3	9.6	0.3	9.6	0.3	16.6	1.1	25.5	0.8
76	7.7	0.7	11.2	0.4	11.4	0.3	16.7	1.1	29.3	1.0

SD Standard deviation.
 x Single pup, SD not calculable.

APPENDIX 18 - continued

Bodyweight up to Day 11 of age -- individual litter mean values (g) for male offspring

Group 5: untreated

Animal Number	Day of age									
	Before cull					After cull				
	1		4		4		7		11	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
77	7.8	0.3	11.1	0.6	11.3	0.4	18.4	1.4	29.1	0.9
78	7.6	0.5	9.9	0.7	10.1	0.6	16.2	0.9	25.9	1.2
79	6.1	0.5	9.2	0.6	9.2	0.6	15.9	1.2	25.9	1.8
80	6.8	0.6	8.4	0.4	8.2	0.4	15.1	1.3	25.7	1.0
81	6.7	0.4	8.3	0.6	8.9	0.2	15.3	0.9	24.0	0.6
82	7.1	0.3	9.5	0.7	10.1	0.3	16.6	0.3	24.9	0.8
83	6.7	0.3	8.3	0.5	8.7	0.4	15.2	0.7	25.2	0.8
84	6.7	0.3	9.8	0.6	9.8	0.6	18.4	0.8	27.3	1.2

SD Standard deviation.

APPENDIX 19

Bodyweight up to Day 11 of age – individual litter mean values (g) for female offspring

Group 1: Control

Animal Number	Day of age											
	Before cull						After cull					
	1		4		4		7		7		11	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
10	7.7	0.3	10.5	0.3	10.5	0.3	17.3	0.7	28.9	0.4		
11	5.8	0.3	10.4	0.9	10.6	0.8	15.9	1.0	24.4	1.6		
12	6.2	0.7	9.9	0.5	9.8	0.7	15.3	1.2	25.7	1.0		
13	6.4	0.1	9.9	0.5	9.9	0.5	14.5	0.6	23.6	0.6		
14	5.8	0.7	7.5	0.8	7.2	0.8	11.4	0.7	20.8	2.4		
15	6.2	0.3	8.5	0.4	8.6	0.4	14.7	0.5	24.3	1.4		
16	6.4	0.4	9.0	0.4	9.1	0.5	14.9	1.1	26.5	1.2		
17	6.6	0.7	8.6	0.4	8.8	0.5	14.3	1.0	24.4	1.3		
18	6.6	0.4	9.3	0.8	9.3	0.8	14.6	0.7	24.8	2.0		
19	5.9	0.2	8.2	0.9	7.9	0.7	12.5	0.7	20.8	0.7		

SD Standard deviation.

APPENDIX 19 - continued

Bodyweight up to Day 11 of age – individual litter mean values (g) for female offspring

Group 2: 0.1 mg/kg/day

Animal Number	Day of age									
	Before cull					After cull				
	1	4				4	7			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
29	6.1	0.4	10.5	0.3	10.5	0.3	15.1	1.4	24.4	1.4
30	6.4	0.4	8.6	0.5	8.9	0.5	14.6	0.7	23.7	1.0
31	5.6	0.5	9.5	0.8	9.0	0.6	14.1	0.5	23.8	0.8
32	5.6	0.4	10.0	0.5	10.2	0.4	15.8	0.8	25.5	0.8
33	6.2	0.5	7.9	0.9	8.2	0.8	13.5	1.2	24.0	1.8
34	6.3	0.3	9.2	0.6	9.3	0.7	15.0	0.9	25.8	1.2
35	5.9	1.0	8.6	1.6	9.3	0.6	15.7	1.2	25.9	0.6
36	6.1	0.3	9.5	0.5	9.7	0.4	16.0	0.8	26.5	1.3
37	6.3	0.5	8.9	0.7	8.8	0.7	14.4	1.2	24.8	1.4
38	6.2	1.3	9.6	0.8	9.6	0.8	15.0	1.3	23.0	1.9

SD Standard deviation.

APPENDIX 19 - continued

Bodyweight up to Day 11 of age – individual litter mean values (g) for female offspring

Group 3: 0.5 mg/kg/day

Animal Number	Day of age											
	Before cull				After cull							
	1		4		4		7		7		11	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
48	6.3	0.7	9.5	1.2	9.9	0.9	14.5	1.3	23.9	1.7		
49	7.5	0.8	11.9	0.1	11.9	0.1	18.2	0.3	26.5	1.4		
50	7.6	0.4	10.9	0.5	10.9	0.7	16.5	0.5	27.2	0.7		
51	5.3	0.5	9.3	0.4	9.4	0.7	13.8	0.9	24.2	1.0		
52	5.5 A	0.4 A	8.4	0.3	8.4	0.3	13.8	1.0	24.8	0.7		
53	4.9	1.2	7.7	2.5	8.5	2.1	12.9	2.9	22.1	5.4		
54	6.0	0.5	8.7	0.3	8.8	0.4	13.7	0.6	22.7	0.8		
55	5.3	0.2	9.0	0.4	9.0	0.4	14.7	0.8	24.3	1.1		
56	6.3	0.1	8.9	0.3	8.9	0.3	13.9	0.4	22.7	0.7		
57	7.1	0.2	9.7	0.6	9.6	0.6	16.0	0.3	27.1	1.4		

SD Standard deviation.

A Pup weighed on Day 2 of age.

APPENDIX 19 - continued

Bodyweight up to Day 11 of age – individual litter mean values (g) for female offspring

Group 4: 3 mg/kg/day

Animal Number	Day of age									
	Before cull				After cull					
	1	4			4			7		
	Mean	SD	Mean	SD	Mean	SD	Mean	Mean	SD	SD
67	5.4	0.3	8.6	0.8	8.2	1.1	12.6	1.0	21.2	1.3
68	6.0	0.5	10.0	0.6	9.9	0.7	14.7	1.1	23.4	0.9
69	5.4	0.4	8.8	0.5	9.2	0.4	14.6	1.4	24.1	1.6
70	5.6	0.3	9.1	0.8	9.2	1.1	15.4	0.5	24.3	0.6
71	6.8	0.4	9.9	0.7	10.2	0.8	15.5	1.3	25.2	1.6
72	5.7	0.4	7.4	0.5	7.4	0.6	11.7	1.2	19.4	1.8
73	5.8	0.4	8.6	0.5	8.7	0.3	14.0	0.9	22.9	0.7
74	8.6	0.3	12.7	0.5	12.7	0.5	18.8	0.3	30.0	0.4
75	5.9	0.3	9.4	0.3	9.5	0.2	15.7	0.5	24.8	0.7
76	7.1	0.9	11.2	0.1	11.2	0.1	16.3	0.7	28.3	0.7

SD Standard deviation.

APPENDIX 19 - continued

Bodyweight up to Day 11 of age – individual litter mean values (g) for female offspring

Group 5: untreated

Animal Number	Day of age											
	Before cull				After cull							
	1		4		4		7		7		11	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
77	7.3	0.3	10.4	0.9	10.8	0.3	17.7	1.0	29.0	0.7		
78	7.2	0.4	9.1	0.5	9.3	0.7	15.3	1.4	24.3	1.3		
79	5.7	0.3	9.1	0.7	9.2	0.8	16.5	1.3	26.2	2.0		
80	7.1	0.4	8.4	0.7	8.3	0.9	14.6	1.6	24.4	2.2		
81	5.9	0.5	7.6	0.3	7.7	0.2	13.9	0.4	22.2	0.5		
82	6.8	0.5	8.5	0.7	8.6	1.0	16.0	1.0	24.9	1.4		
83	6.3	0.5	8.4	0.7	8.4	0.7	14.0	0.7	22.5	1.1		
84	6.1	0.2	9.5	0.4	9.5	0.3	16.9	0.1	26.8	1.4		

SD Standard deviation.

APPENDIX 20

Bodyweight - individual values (g) for dosed male offspring

Group 1: Control

Animal number	Bodyweight on Day of age												
	1	4	7	11	14	17	21	28	35	42	49	56	60
1001	7.7	11.3	18.5	30.0	39.5	45.4	60.5						
1002	7.8	10.4	16.8	27.6	36.5	42.1	54.7						
1003	8.2	11.5	19.2	29.8	40.4	46.0	64.5						
1004	7.6	11.9	20.1	31.1	39.8	47.5	67.5	105.8	178.7	236.0	313.2	379.5	414.4
1101	6.5	10.3	15.5	24.6	32.1	41.0	52.4						
1102	6.0	10.9	16.0	25.1	32.1	40.0	49.2						
1103	6.0	11.0	16.0	25.8	31.9	39.3	51.3	85.4	127.3	173.9	220.7	270.9	S
1104	6.7	10.6	16.6	27.1	33.8	41.6	53.6	91.7	143.6	200.3	256.3	312.6	334.8
1201	6.0	10.1	15.2	25.3	32.8	40.3	52.1						
1202	6.3	10.2	16.3	26.2	34.2	40.7	53.5						
1203	6.0	9.8	16.2	25.9	33.3	41.0	53.4						
1204	6.6	10.9	16.3	27.9	36.5	42.3	56.9	101.3	160.5	232.9	304.1	373.5	407.2
1301	6.9	10.1	15.9	25.5	32.3	39.6	52.5						
1302	7.4	10.8	16.3	26.8	33.1	40.5	50.7						
1303	6.9	9.5	16.2	24.8	32.1	39.4	51.7						
1304	6.6	9.7	15.8	24.9	31.3	38.1	52.7						
1305	7.1	10.2	16.1	25.3	31.8	38.3	51.7	92.6	146.0	210.9	268.5	331.1	352.8
1401	6.0	8.0	12.1	23.3	31.1	37.6	46.4						
1402	7.0	8.0	12.3	24.4	34.3	40.5	51.7						
1403	5.3	6.9	10.9	21.0	30.9	37.0	46.8						
1404	6.2	7.9	11.0	21.6	29.5	36.7	45.1	75.3	124.6	187.3	242.0	298.6	331.6
1601	7.3	9.1	14.7	25.9	34.4	40.3	52.9						
1602	6.6	9.0	15.5	27.8	35.6	42.3	56.1						
1603	6.5	9.5	15.4	27.4	35.7	42.9	54.3						
1604	6.5	9.3	15.4	27.1	34.4	42.2	54.8	89.8	142.3	174.8	253.6	306.7	325.1
1701	6.9	9.6	14.9	25.7	33.5	37.5	50.5						
1702	6.2	9.3	15.1	24.4	32.5	36.1	47.2						
1703	6.2	9.0	14.6	24.5	32.3	34.8	46.5						
1704	6.5	9.1	13.4	23.3	31.0	36.2	47.4	83.6	131.2	186.3	246.0	300.2	327.4
1901	6.4	8.6	14.1	22.8	31.0	35.3	46.0						
1902	5.8	9.4	14.6	23.3	31.9	36.5	45.5						
1903	5.8	8.6	13.1	21.0	29.4	32.3	41.4						
1904	6.0	9.2	13.1	21.2	30.0	34.5	45.3	84.1	133.4	188.3	246.8	292.3	316.6

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

S Spare pup allocated to kill on day 61.

Day 60 bodyweight not recorded.

APPENDIX 20 - continued

Bodyweight - individual values (g) for dosed male offspring

Group 2: 0.1 mg/kg/day

Animal number	Bodyweight on Day of age												
	1	4	7	11	14	17	21	28	35	42	49	56	60
2901	6.4	10.8	15.9	24.6	22.8	28.4	38.7						
2902	6.5	11.2	15.7	24.1	31.0	38.3	49.2						
2903	6.4	10.8	15.3	24.4	33.2	38.1	48.4						
2904	6.4	10.6	16.0	24.5	34.4	40.8	52.7						
2905	5.9	9.8	15.7	24.7	31.1	37.7	46.9						
2906	3.6	6.6	8.7	14.0	20.3	24.9	30.0	57.3	101.7	158.2	219.5	282.8	306.8
3001	6.5	8.3	14.9	24.1	31.4	37.5	46.7						
3002	5.1	8.0	12.5	21.0	28.1	35.0	41.2	79.0	125.5	181.3	238.3	295.9	316.8
3101	6.6	11.1	16.4	26.1	34.9	41.5	53.0						
3102	6.2	10.1	16.4	26.3	35.0	41.6	47.7						
3103	5.9	8.9	15.0	22.9	30.9	37.5	44.8	80.7	127.2	188.7	253.9	316.4	S
3104	6.0	9.3	15.2	25.2	32.8	39.3	44.9	83.7	136.0	202.3	270.0	342.8	378.2
3201	6.6	10.8	17.1	27.3	36.2	42.6	57.7						
3202	5.6	10.1	16.1	26.3	34.3	41.8	52.6						
3203	6.6	9.7	16.8	25.5	33.3	40.0	51.4						
3204	6.1	10.4	17.4	27.5	36.1	42.6	56.6	97.9	154.3	215.9	278.0	346.2	370.5
3301	6.5	8.8	14.6	27.0	34.5	43.3	54.0						
3302	6.9	8.5	13.1	25.5	34.4	44.0	55.6						
3303	6.8	9.0	15.6	26.6	35.3	45.7	55.9						
3304	6.5	9.5	14.4	25.5	34.1	43.8	54.3	102.2	162.3	232.7	311.9	391.2	427.9
3401	6.8	9.9	14.5	27.4	34.3	43.5	54.3						
3402	7.2	9.8	16.1	27.1	34.5	42.0	54.1						
3403	7.0	9.9	14.5	26.7	34.9	43.3	56.7						
3404	6.6	9.6	15.2	26.0	33.6	42.8	53.8	90.2	140.7	194.9	255.2	313.9	341.3
3501	7.2	9.5	16.5	26.1	31.6	40.0	51.8						
3502	6.1	9.9	16.3	27.1	30.5	38.4	50.8						
3503	6.5	10.0	16.6	27.0	33.4	40.7	57.8						
3504	6.2	9.0	15.0	25.9	30.3	39.1	51.1	88.8	137.3	193.2	250.7	308.8	333.4
3601	6.8	10.5	17.4	29.0	38.1	46.8	58.8						
3602	6.8	9.9	16.1	27.9	36.4	41.3	51.6						
3603	5.9	9.5	16.1	25.6	33.4	42.6	59.6						
3604	6.9	10.7	16.9	27.7	35.6	43.8	57.7	103.6	162.0	234.3	292.9	352.2	371.9

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

S Spare pup allocated to kill on Day 61.

Day 60 bodyweight not recorded.

APPENDIX 20 - continued

Bodyweight - individual values (g) for dosed male offspring

Group 3: 0.5 mg/kg/day

Animal number	Bodyweight on Day of age												
	1	4	7	11	14	17	21	28	35	42	49	56	60
4801	7.0	10.3	15.1	25.5	32.1	38.8	51.7						
4802	6.5	10.1	15.0	25.3	31.8	39.4	52.8						
4803	5.2	9.3	13.3	22.3	29.4	35.7	47.1						
4804	7.0	10.5	16.1	24.6	31.1	36.5	49.5	92.8	150.6	214.2	283.4	355.4	389.2
4901	8.2	11.8	18.2	26.9	32.6	39.6	51.0						
4902	7.8	12.7	17.8	26.8	33.6	40.8	55.1						
4903	7.9	12.6	18.9	27.3	33.4	41.2	56.4	101.5	157.2	217.1	283.3	349.2	378.1
5001	7.8	11.6	17.6	27.3	35.5	41.5	49.8						
5002	8.5	10.9	17.2	27.9	36.3	42.5	54.1	97.5	156.3	220.8	290.8	354.0	S
5003	8.2	10.9	17.0	27.1	35.0	40.6	46.9	88.8	142.6	209.3	272.6	334.3	368.9
5101	6.5	10.7	15.4	27.1	35.3	44.1	56.3						
5102	4.8	8.4	12.6	22.8	32.1	41.3	52.3						
5103	5.5	10.7	15.4	25.4	33.8	42.4	52.6						
5104	6.4	11.1	14.9	26.2	35.3	44.1	56.7	100.4	156.6	225.7	296.0	357.9	387.1
5201	5.2	8.7	14.9	25.3	32.8	43.1	54.0						
5202	6.0	9.7	15.8	27.1	34.3	44.5	55.2						
5203	6.3	9.0	14.8	26.0	35.8	46.3	54.1						
5204	5.8	9.7	16.2	27.3	34.4	46.8	54.9	93.1	150.5	219.4	291.2	362.1	400.9
5301	5.5	8.6	13.8	24.9	33.3	41.4	48.6						
5302	6.0	9.5	16.0	26.4	34.0	41.3	50.0						
5303	6.2	8.9	13.0	24.7	19.8x	39.7	49.1						
5304	5.5	8.4	13.8	22.9	29.5	37.4	46.6	80.8	123.4	181.7	246.6	310.4	337.9
5401	6.0	9.3	14.6	24.0	33.1	41.9	52.6						
5402	6.8	9.5	14.3	24.5	32.9	42.7	49.5						
5403	6.2	9.2	14.9	24.6	29.6	38.4	49.3						
5404	5.7	8.7	13.0	22.1	30.3	41.6	48.3	85.8	139.4	204.3	272.2	330.9	356.9
5502	5.7	9.5	15.4										
5503	5.5	9.0	13.2										
5504	6.4	10.9	18.2										
5505	6.3	10.1	17.1				50.0	81.8	122.4	171.1	226.9	284.8	316.2

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

S Spare pup allocated to kill on Day 61.

Day 60 bodyweight not recorded.

X Suspected weighing error, value excluded from group mean values.

APPENDIX 20 - continued

Bodyweight - individual values (g) for dosed male offspring

Group 4: 3 mg/kg/day

Animal number	Bodyweight on Day of age												
	1	4	7	11	14	17	21	28	35	42	49	56	60
6701	6.1	8.4	14.6	23.8	31.1	38.7	47.1						
6702	6.5	10.3	15.1	24.4	32.2	39.6	50.9						
6703	5.4	8.6	13.8	23.2	29.3	37.6	46.6	84.1	139.1	207.2	271.6	340.6	S
6704	5.8	9.1	14.7	23.8	30.6	38.7	48.4	89.0	144.1	211.6	277.2	348.6	383.9
6801	5.9	10.4	16.1	25.9	34.2	44.9	55.4						
6802	6.6	10.4	15.0	24.2	31.9	43.0	53.5						
6803	6.5	9.7	14.3	24.4	32.5	41.9	52.0						
6804	6.7	11.4	15.7	24.0	32.4	42.5	55.7	97.0	152.2	222.2	282.1	336.1	366.3
6901	6.3	11.2	16.5	26.6	34.3	42.1	53.1						
6902	4.9	9.0	13.9	22.3	29.7	37.6	46.2						
6903	6.0	10.2	15.8	25.8	34.2	43.0	54.2						
6904	5.7	11.0	15.9	25.5	33.4	42.1	57.0	94.9	151.1	218.1	286.6	348.9	378.6
7001	6.1	8.8	16.0	24.8	32.8	41.2	51.6						
7002	6.2	9.0	17.3	25.6	33.5	41.8	52.0						
7003	5.6	9.6	14.7	22.9	29.9	38.5	47.2						
7004	6.4	10.1	17.2	26.4	34.3	41.9	51.4	92.8	138.2	197.7	261.0	317.0	343.4
7201	6.0	7.3	12.2	19.5	27.2	34.7	44.9						
7202	5.8	7.5	11.7	20.4	27.1	31.2	46.1						
7203	5.9	9.2	11.0	18.6	26.3	33.1	41.6						
7204	5.9	7.6	11.6	19.5	27.2	33.8	42.2	78.2	134.3	205.4	276.6	345.5	372.2
7301	6.4	8.8	14.2	23.2	30.6	37.4	48.5						
7302	6.5	8.9	12.4	23.4	31.2	38.0	49.5						
7303	6.6	9.5	14.3	24.8	33.2	40.5	53.9						
7304	6.4	8.8	13.3	23.3	30.5	38.9	50.9	87.7	137.8	201.5	261.8	321.4	342.5
7401	8.6	12.3	19.0	30.5	38.8	45.1	63.7						
7601	6.6	10.9	15.4	27.8	34.1	40.8	55.1						
7602	7.9	11.3	16.5	29.6	37.5	44.1	57.8						
7603	8.9	11.4	18.0	30.1	36.9	43.9	57.1	106.0	173.5	242.6	318.9	381.6	416.7
7604	7.7	11.8	17.6	29.6	37.3	45.5	60.9	111.7	174.9	243.0	324.8	391.4	429.4

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

S Spare pup allocated to kill on Day 61.

Day 60 bodyweight not recorded.

APPENDIX 21

Bodyweight - individual values (g) for undosed male offspring

Group 1: Control

Animal number	Bodyweight on Day of age									
	1	4	7	11	14	17	21			
1501	6.2	9.1	15.4	26.0	34.8	42.8	56.2			
1502	6.9	9.8	18.1	28.4	38.1	46.0	54.4			
1503	6.1	8.8	15.6	25.1	31.7	39.2	50.0			
1504	6.7	9.7	16.7	26.4	34.7	42.8	52.8			
1801	7.4	10.5	14.9	26.1	35.4	45.1	55.4			
1802	7.1	10.7	16.9	27.3	36.1	44.8	58.5			
1803	7.3	10.1	15.6	25.8	35.8	43.6	53.8			
1804	6.3	9.6	15.3	24.0	33.1	42.5	53.4			

Last two digits of animal number denote pup number. Other digit(s) denote litter number.

APPENDIX 21 - continued

Bodyweight - individual values (g) for undosed male offspring

Group 2: 0.1 mg/kg/day

Animal number	Bodyweight on Day of age									
	1	4	7	11	14	17	21			
3701	6.5	9.0	14.3	24.5	35.1	43.8	55.0			
3702	7.3	9.5	15.9	26.3	35.6	45.8	58.9			
3703	7.1	9.0	15.1	25.5	34.5	44.1	56.7			
3704	6.2	9.8	15.7	25.0	34.0	42.0	52.9			
3801	6.8	9.4	16.4	28.3	36.1	42.1	54.6			
3802	7.2	9.6	14.6	22.5	34.4	40.3	49.1			
3803	8.1	10.8	18.0	28.7	39.8	47.9	62.9			
3804	7.6	10.6	17.9	29.3	38.0	42.6	54.4			

Last two digits of animal number denote pup number. Other digit(s) denote litter number.

APPENDIX 21 - continued

Bodyweight - individual values (g) for undosed male offspring

Group 3: 0.5 mg/kg/day

Animal number	Bodyweight on Day of age									
	1	4	7	11	14	17	21			
5601	7.1	9.8	15.0	23.6	31.1	37.9	53.7			
5602	7.2	9.1	14.5	23.1	30.3	38.0	48.9			
5603	6.9	10.3	16.2	25.6	33.3	41.5	54.6			
5604	6.3	9.1	13.5	21.7	29.4	36.0	46.3			
5701	7.3	10.4	17.9	28.1	38.5	47.1	61.7			
5702	7.6	11.0	17.8	29.6	35.5	46.1	59.0			
5703	7.2	9.1	16.3	26.8	37.8	45.5	57.9			
5704	6.8	10.6	17.6	25.8	38.3	45.5	57.7			

Last two digits of animal number denote pup number. Other digit(s) denote litter number.

APPENDIX 21 - continued

Bodyweight - individual values (g) for undosed male offspring

Group 4: 3 mg/kg/day

Animal number	Bodyweight on Day of age									
	1	4	7	11	14	17	21			
7101	7.7	9.8	15.3	25.5	34.6	39.7	52.1			
7102	7.6	10.4	15.0	25.4	33.9	40.6	53.6			
7103	7.6	10.0	16.3	25.3	34.3	38.7	54.7			
7104	7.7	11.1	15.7	25.8	36.1	40.3	50.4			
7501	6.7	9.4	15.4	25.5	32.4	41.9	53.1			
7502	6.5	9.4	16.0	25.2	32.4	42.2	55.8			
7503	6.3	10.0	18.0	26.6	34.4	45.3	56.2			
7504	6.0	9.6	16.8	24.8	31.9	41.5	51.7			

Last two digits of animal number denote pup number. Other digit(s) denote litter number.

APPENDIX 22

Bodyweight - individual values (g) for dosed female offspring

Group 1: Control

Animal number	Bodyweight on Day of age												
	1	4	7	11	14	17	21	28	35	42	49	56	60
1009	8.1	10.8	17.9	28.9	37.3	43.1	56.5						
1010	7.7	10.1	17.2	28.4	37.5	43.5	56.2						
1011	7.8	10.3	16.3	29.0	37.2	44.0	58.2						
1012	7.3	10.6	17.8	29.3	38.7	44.9	58.7	87.3	131.7	159.9	186.9	212.8	224.0
1110	6.2	11.4	17.2	26.4	32.3	40.0	51.9						
1111	5.8	10.8	15.4	25.0	31.7	39.1	47.9						
1112	5.7	10.5	15.0	22.8	30.9	39.4	49.3	82.5	122.8	154.3	179.5	200.7	S
1113	5.8	9.5	15.8	23.3	30.6	38.8	48.0	83.2	129.4	163.7	190.5	214.1	222.7
1208	5.6	9.7	13.7	24.3	32.6	39.8	51.7						
1209	7.8	9.6	16.7	26.5	34.9	42.4	54.0						
1210	6.2	10.7	15.5	26.3	34.7	42.4	56.4						
1211	6.0	9.0	15.2	25.7	33.9	40.8	52.2	87.0	130.5	165.3	192.5	207.6	213.3
1310	6.5	9.4	14.4	23.2	29.6	35.6	48.4						
1311	6.4	10.0	14.0	23.3	28.9	35.9	47.1						
1312	6.4	10.3	15.2	24.3	31.3	38.1	49.7	77.5	116.5	144.1	177.4	195.1	200.5
1406	4.7	6.8	11.4	19.7	27.8	34.0	42.6						
1407	5.7	7.9	11.8	23.5	33.0	39.0	48.1						
1408 HK	5.7	6.2	10.3	18.1									
1409	7.1	7.7	11.9	21.8	30.2	35.9	44.5	67.6	103.0	133.8	158.1	183.4	191.6
1610	6.6	9.6	16.2	27.0	35.7	41.1	55.6						
1611	6.6	8.8	14.7	26.0	33.4	38.7	51.0						
1612	6.4	9.4	15.2	27.9	35.2	41.1	52.2						
1613	6.0	8.6	13.5	25.1	33.0	39.2	51.8	78.7	121.7	148.8	171.6	193.4	198.7
1708	7.1	8.6	14.3	24.5	31.1	35.6	48.7						
1709	7.4	8.5	13.5	23.7	31.7	36.3	45.4						
1710	7.4	9.5	15.7	26.2	32.6	36.6	48.2						
1711	6.2	8.5	13.8	23.2	30.4	33.1	45.2	77.3	125.7	161.2	189.9	217.2	224.6
1907	5.9	7.6	12.8	21.4	30.0	33.7	44.1						
1908	5.6	7.4	11.6	19.7	26.8	31.6	40.0						
1909	6.0	7.6	12.4	20.9	28.8	32.1	41.3						
1910	5.7	9.0	13.2	21.0	29.2	32.4	42.5	80.6	129.9	164.4	198.6	224.3	237.3

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

HK Killed for reasons of animal welfare.

S Spare pup allocated to kill on Day 61.

Day 60 bodyweight not recorded.

APPENDIX 22 - continued

Bodyweight - individual values (g) for dosed female offspring

Group 2: 0.1 mg/kg/day

Animal number	Bodyweight on Day of age												
	1	4	7	11	14	17	21	28	35	42	49	56	60
2911	6.4	10.7	16.1	25.4	31.6	37.1	49.2						
2912	5.8	10.3	14.1	23.4	29.7	36.6	51.0	92.3	139.7	174.5	213.5	240.2	249.2
3003	7.2	9.1	14.9	24.9	31.8	38.9	49.8						
3004	6.5	9.2	14.7	23.6	31.2	37.7	42.9						
3005	6.8	9.5	14.6	22.9	30.4	36.7	45.6						
3006	5.8	8.2	13.3	22.6	31.7	36.9	43.6						
3007	6.7	8.6	15.3	24.8	29.5	39.7	46.5						
3008	5.8	8.8	14.9	23.1	29.8	36.8	45.3	80.9	121.7	164.7	195.8	223.0	231.3
3108	5.9	9.7	14.2	24.6	32.8	37.9	48.6						
3109	6.1	9.0	14.7	24.3	32.2	38.7	47.2						
3110	5.2	9.1	14.0	23.2	30.8	37.8	44.8	78.5	117.2	148.1	164.8	177.5	S
3111	5.1	8.3	13.6	23.0	30.9	37.5	43.4	75.7	110.9	141.2	168.9	190.7	195.7
3206	5.9	10.1	16.5	26.3	34.4	40.4	53.6						
3207	6.0	10.4	16.3	25.9	34.6	41.0	52.7						
3208	6.3	9.7	15.3	24.5	32.8	38.9	51.6						
3209	5.3	10.7	14.9	25.3	33.3	41.1	52.1	85.7	125.8	161.3	194.2	218.4	231.4
3307	6.4	8.4	14.0	25.1	33.7	42.0	53.6						
3308	6.1	9.0	13.9	25.0	33.0	42.2	53.1						
3309	6.8	8.3	14.4	24.6	33.7	42.4	52.0						
3310	6.1	7.0	11.7	21.3	28.7	37.3	49.4	85.6	133.8	170.1	202.4	237.6	251.3
3405	6.0	9.4	15.2	25.7	32.5	41.7	52.2						
3406	6.8	9.2	14.9	24.9	32.8	41.4	50.7						
3407	6.6	10.2	16.0	27.5	36.4	45.0	56.8						
3408	6.2	8.5	13.9	25.0	32.1	41.7	50.0	85.0	130.6	167.0	195.7	215.2	224.3
3510	6.6	9.9	17.1	26.1	33.5	41.5	55.8						
3511	6.6	8.5	14.4	25.8	31.3	39.5	52.9						
3512	6.0	9.4	15.2	26.4	31.5	38.3	51.4						
3513	6.1	9.3	16.2	25.1	30.7	36.1	50.6	81.2	123.1	153.1	184.0	200.2	206.8
3608	6.0	9.6	15.7	25.5	33.4	41.9	53.8						
3609	5.8	9.4	15.0	25.8	32.5	40.6	50.6						
3610	6.1	9.4	16.1	26.4	33.9	41.4	56.4						
3611	6.5	10.3	17.0	28.4	36.9	44.6	57.2	94.9	140.6	178.5	210.9	232.2	236.7

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

S Spare pup allocated to kill on Day 61.

Day 60 bodyweight not recorded.

APPENDIX 22 - continued

Bodyweight - individual values (g) for dosed female offspring

Group 3: 0.5 mg/kg/day

Animal number	Bodyweight on Day of age												
	1	4	7	11	14	17	21	28	35	42	49	56	60
4806	7.1	10.6	14.8	25.4	31.7	38.1	53.3						
4807	7.0	10.3	15.9	24.4	30.7	37.6	47.9						
4808	5.6	8.6	12.8	21.4	26.7	34.0	43.4						
4809	6.0	10.1	14.5	24.2	30.6	36.3	46.8	78.2	127.4	160.9	193.0	221.1	229.1
4904	6.9	11.8	18.0	25.5	31.2	38.0	51.9						
4905	8.1	12.0	18.4	27.5	32.8	40.6	53.0	81.8	124.6	161.3	187.1	219.8	228.6
5004	7.2	10.8	16.2	27.5	34.9	40.6	51.7						
5005	7.2	10.4	16.0	26.2	33.5	39.6	49.2						
5006	7.4	10.2	16.5	27.8	36.1	41.4	51.3						
5007	7.8	11.8	17.2	26.8	34.9	39.9	51.0	87.3	122.3	142.2	165.0	185.7	S
5008	7.6	11.3	16.7	27.5	35.3	41.0	50.5	85.7	127.9	158.4	192.1	218.0	227.6
5106	5.0	10.0	13.9	23.7	31.1	37.5	50.5						
5107	5.4	9.9	13.5	24.5	34.2	40.9	54.5						
5108	5.3	9.1	15.0	25.5	33.9	42.5	53.5						
5109	5.1	8.6	12.8	23.2	31.8	39.0	50.2	84.9	128.0	165.8	201.4	233.4	244.0
5209	5.3	8.4	14.9	25.1	33.0	43.2	51.1						
5210	5.7	8.8	14.3	25.4	34.2	44.5	53.9						
5211	5.7	8.0	12.6	23.8	32.1	42.3	51.5						
5212	5.1	8.2	13.5	24.8	33.0	43.1	51.3	79.6	132.7	176.0	214.2	248.4	256.5
5310	6.1	9.6	14.9	26.9	34.4	43.2	53.0						
5311	5.7	8.3	14.5	23.6	31.9	38.6	46.5						
5312	5.5	10.3	13.5	23.5	32.5	40.4	30.9						
5313	4.0	5.6	8.6	14.4	31.2	37.1	47.6	84.4	128.4	166.4	202.0	255.6	240.2
5411	5.9	9.0	14.4	23.3	32.0	41.1	50.8						
5412	5.7	8.3	12.9	21.6	28.8	38.2	51.0						
5413	6.6	8.8	13.6	23.0	28.0	41.7	48.4						
5414	5.9	9.1	13.7	22.9	30.2	39.4	50.3	83.5	122.4	153.5	179.8	192.0	197.3
5510	5.6	9.4	15.3	25.5	33.3	42.8	54.1						
5511	5.2	8.9	13.5	23.4	29.6	39.5	47.6						
5512	5.3	9.2	14.9	24.8	32.2	41.5	48.5						
5513	5.0	8.5	15.0	23.4	30.6	40.2	49.2	79.9	116.3	151.6	179.3	201.4	210.1

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

S Spare pup allocated to kill on Day 61.

Day 60 bodyweight not recorded.

APPENDIX 22 - continued

Bodyweight - individual values (g) for dosed female offspring

Group 4: 3 mg/kg/day

Animal number	Bodyweight on Day of age												
	1	4	7	11	14	17	21	28	35	42	49	56	60
6707	4.9	9.3	12.6	20.3	28.1	34.6	40.1						
6708	5.7	9.1	14.0	23.0	29.7	36.5	43.7						
6709	5.2	7.1	11.8	20.3	26.1	34.6	41.4	76.2	120.5	161.4	188.8	212.7	S
6710	5.0	7.4	11.9	21.1	28.4	36.4	43.3	78.2	119.2	160.0	193.2	219.8	230.0
6809	5.7	9.5	15.2	22.8	30.4	40.0	49.0						
6810	6.2	10.9	16.0	24.7	31.0	40.5	50.9						
6811	6.3	9.6	13.6	23.3	30.9	39.8	50.4						
6812	5.5	9.4	13.8	22.6	30.4	39.4	46.0	76.7	116.8	151.0	174.0	196.4	204.8
6908	6.2	9.6	15.6	25.8	32.9	40.8	50.7						
6909	5.0	8.7	12.9	22.0	28.8	36.9	44.9						
6910	5.6	9.3	16.0	24.9	33.6	41.5	52.1						
6911	5.2	9.2	14.0	23.8	31.2	39.1	48.7	78.6	116.5	147.0	172.4	199.1	207.0
7009	5.1	7.7	14.7	23.5	31.4	38.4	46.0						
7010	6.1	9.1	16.0	24.8	33.1	39.5	47.7						
7011	5.8	10.2	15.4	24.5	31.9	39.8	51.1						
7012	5.3	9.7	15.6	24.5	33.0	41.6	51.6	84.8	121.1	151.7	177.3	193.4	200.2
7209	6.4	8.1	12.7	21.2	29.0	36.8	47.6						
7210	5.9	7.7	12.5	20.5	28.3	36.8	46.9						
7211	5.3	7.0	11.4	18.5	25.3	33.3	42.9						
7212	5.4	6.8	10.1	17.3	24.4	32.0	39.2	70.5	116.7	149.9	174.2	196.4	206.2
7309	6.1	9.2	14.6	23.1	30.1	36.2	46.7						
7310	6.0	8.6	14.3	23.7	30.4	36.9	48.5						
7311	6.1	8.4	12.7	22.5	30.3	36.8	48.5						
7312	5.6	8.6	14.3	22.1	30.1	37.4	50.1	85.1	129.8	171.7	204.7	236.0	246.0
7402	8.0	12.6	18.7	30.0	37.5	44.9	61.2						
7403	8.9	13.3	18.6	29.9	37.8	44.5	64.1						
7404	8.5	12.1	18.5	30.1	37.8	44.7	58.8						
7405	8.6	12.4	18.7	30.4	38.8	46.0	59.0						
7406	8.6	12.8	19.4	30.3	38.2	44.8	58.8						
7407	8.8	13.2	18.9	29.4	38.3	44.0	57.9	89.4	132.1	169.8	202.3	226.4	241.2
7610	7.3	11.2	15.6	27.5	34.5	40.0	53.0						
7611	7.9	11.3	16.9	28.9	35.7	42.3	57.4						
7612	6.2	11.2	16.3	28.5	35.8	41.5	55.4	98.0	147.2	173.0	192.0	211.1	226.4

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

S Spare pup allocated to kill on Day 61.

Day 60 bodyweight not recorded.

APPENDIX 23

Bodyweight - individual values (g) for undosed female offspring

Group 1: Control

Animal number	Bodyweight on Day of age									
	1	4	7	11	14	17	21			
1510	6.1	8.2	14.2	23.8	31.5	38.2	49.9			
1511	5.9	8.9	15.3	25.0	32.5	39.3	50.0			
1512	6.7	8.3	14.6	22.7	30.4	36.7	46.8			
1513	6.2	9.0	14.6	25.8	32.7	38.9	51.6			
1809	7.1	10.3	15.0	25.8	36.1	44.6	56.5			
1810	6.6	9.1	13.9	24.8	33.4	40.9	53.0			
1811	6.2	8.3	14.0	22.0	31.9	39.7	49.7			
1812	6.6	9.3	15.4	26.7	33.9	42.1	55.9			

Last two digits of animal number denote pup number.
Other digit(s) denote litter number.

APPENDIX 23 - continued

Bodyweight - individual values (g) for undosed female offspring

Group 2: 0.1 mg/kg/day

Animal number	Bodyweight on Day of age									
	1	4	7	11	14	17	21			
3707	7.2	9.8	16.0	26.5	35.0	45.0	58.6			
3708	6.3	8.3	13.5	23.1	32.3	39.7	51.0			
3709	6.4	8.5	13.5	25.0	33.6	42.1	54.1			
3710	5.8	8.6	14.6	24.4	32.6	41.9	53.7			
3807	7.1	9.7	16.2	24.2	33.3	40.6	50.3			
3808	6.3	8.5	13.4	20.1	27.9	34.4	41.6			
3809	6.9	10.1	15.9	24.2	33.1	40.6	53.2			
3810	6.6	10.1	14.3	23.3	31.0	37.9	45.7			

Last two digits of animal number denote pup number.
Other digit(s) denote litter number.

APPENDIX 23 - continued

Bodyweight - individual values (g) for undosed female offspring

Group 3: 0.5 mg/kg/day

Animal number	Bodyweight on Day of age									
	1	4	7	11	14	17	21			
5611	6.3	9.0	14.2	23.1	29.8	36.7	48.6			
5612	6.1	8.5	13.6	21.8	28.4	35.3	48.2			
5613	6.2	9.1	13.6	22.4	29.6	36.8	47.5			
5614	6.4	8.8	14.3	23.3	30.6	37.3	44.2			
5711	6.8	9.2	16.2	26.0	37.3	43.3	54.4			
5712	7.2	9.8	15.7	25.7	39.0	43.8	55.2			
5713	7.1	10.4	16.3	28.4	38.1	45.2	63.0			
5714	7.4	9.0	15.7	28.2	35.5	43.5	53.6			

Last two digits of animal number denote pup number.
Other digit(s) denote litter number.

APPENDIX 23 - continued

Bodyweight - individual values (g) for undosed female offspring

Group 4: 3 mg/kg/day

Animal number	Bodyweight on Day of age									
	1	4	7	11	14	17	21			
7106	6.6	10.2	15.7	26.0	34.5	38.5	53.7			
7107	6.4	9.1	14.1	23.3	30.3	35.4	49.0			
7108	6.7	10.6	14.9	24.6	33.5	37.5	48.5			
7109	6.9	11.0	17.2	27.0	35.2	40.3	53.1			
7507	6.4	9.6	15.2	25.6	32.6	42.6	55.9			
7508	5.8	9.3	16.1	24.0	32.1	41.3	53.6			
7509	6.1	9.7	15.3	24.5	30.9	40.8	52.3			
7510	6.0	9.3	16.2	25.0	31.5	41.5	51.7			

Last two digits of animal number denote pup number.
 Other digit(s) denote litter number.

APPENDIX 24

Clinical signs – individual observations for offspring

Group	:	1	2	3	4	5
Compound	:	Control	-----	Dimethoate-----		untreated
Dosage (mg/kg/day)	:	0	0.1	0.5	3	0

Signs observed prior to weaning

Group	Litter number	Clinical observations
1	14	One pup with domed shaped head Day 12 of age - killed for reasons of animal welfare
	17	All pups with patchy coats Days 14 to 18 of age
2	29	All pups with patchy coats Days 10 to 19 of age
	32	All pups with patchy coats Days 14 to 19 of age
	33	One pup with bruising on head Days 2 and 3 of age
	35	Two pups small in size Days 1 to 4 of age, culled Day 4
		One pup small in size Day 1 of age, missing Day 2
3	37	All pups with patchy coats Days 12 to 18 of age
	38	One pup small in size Day 1 of age, missing Day 2
	52	One pup with cut on right forelimb, right forepaw swollen and bruised Days 2 and 3 of age; right forepaw swollen and had encrustations Day 4 of age, culled Day 4
	53	One pup small in size Days 1 to 3 of age, missing Day 4 Two pups small in size Days 1 to 4 of age, one pup culled Day 4
4	75	One pup with bruising on head Days 1 to 3 of age
	76	One pup with skin abrasion on left forelimb Day 4 of age
5	81	One pup with cut on back Day 10 of age, found dead Day 11
		One pup with bruise and swelling on left hindlimb Day 1 of age

APPENDIX 24 - continued

Clinical signs – individual observations for offspring

Group	:	1	2	3	4	5
Compound	:	Control	-----	Dimethoate-----		untreated
Dosage (mg/kg/day)	:	0	0.1	0.5	3	0

Signs observed from weaning to termination

Group	Animal number/sex	Clinical observations
1	1004M	Hairloss on head Week 5
	1012F	Vocalisation Weeks 6 and 7
	1711F	Hairloss on ventral body surface Week 6
2	3304M	Brown staining on head Weeks 6 and 7
	2912F	Brown staining on head Week 5
	3110F	Brown staining on upper dorsal thorax Week 7
	3111F	Brown staining on head Weeks 5 and 6
3	5002M	Hairloss and encrustations on head Week 7
	5003M	Brown staining on head Week 6
	4903M	Kinked tail Weeks 5 to 7
	5304M	Brown staining on right orbital region Week 7
	5007F	Kinked tail Weeks 3 to 7; brown staining on head Weeks 5 to 7
	5008F	Vocalisation Week 7
	5513F	Brown staining on head Weeks 5 to 7
4	6704M	Brown staining on head Weeks 6 and 7
	7204M	Kinked tail Weeks 5 to 7
	6911F	Brown staining on head Week 7
	7312F	Brown staining on muzzle Weeks 6 and 7
	7612F	Brown staining on head Week 5

M - Male; F - Female

Week 1 = first physical examination after weaning

APPENDIX 25

Brain weights – individual values (g) for male and female offspring at Day 4 of age

Group 1: Control

Male offspring		Female offspring	
Animal number	Brain weight	Animal number	Brain weight
1005	0.474	1114	0.356
1006	0.484	1115	0.404
1105	0.412	1212	0.404
1106	0.418	1213	0.405
1205	0.404	1410	0.347
1206	0.255	1411	0.363
1306	0.383	1514	0.370
1307	0.392	1614	0.428
1405	0.323	1615	0.427
1505	0.405	1712	0.375
1506	0.402	1713	0.401
1605	0.424	1911	0.381
1606	0.429	1912	0.367
1705	0.413		
1706	0.388		
1805	0.418		
1806	0.428		
1905	0.386		
1906	0.395		

Last two digits of animal numbers denote pup number.
 Other digit(s) denote litter numbers.

APPENDIX 25 - continued

Brain weights – individual values (g) for male and female offspring at Day 4 of age

Group 2: 0.1 mg/kg/day

Male offspring		Female offspring	
Animal number	Brain weight	Animal number	Brain weight
2907	0.407	3009	0.345
2908	0.393	3010	0.344
3105	0.417	3112	0.405
3106	0.420	3113	0.364
3205	0.402	3210	0.387
3305	0.362	3211	0.388
3306	0.374	3311	0.331
3505	0.434	3312	0.283
3506	0.396	3409	0.348
3605	0.396	3410	0.391
3606	0.365	3514	0.375
3705	0.365	3515	0.377
3706	0.391	3612	0.391
3805	0.444	3613	0.394
3806	0.438	3711	0.394
		3712	0.368

Last two digits of animal numbers denote pup number.
 Other digit(s) denote litter numbers.

APPENDIX 25 - continued

Brain weights – individual values (g) for male and female offspring at Day 4 of age

Group 3: 0.5 mg/kg/day

Male offspring		Female offspring	
Animal number	Brain weight	Animal number	Brain weight
4805	0.468	4810	0.367
5105	0.347	5009	0.390
5205	0.414	5010	0.405
5206	0.364	5110	0.391
5305	0.393	5111	0.356
5306	0.382	5213	0.362
5405	0.433	5214	0.364
5406	0.425	5314	0.259
5506	0.386	5415	0.397
5507	0.384	5416	0.431
5605	0.388	5715	0.423
5606	0.392	5716	0.423
5705	0.388		
5706	0.488		

Last two digits of animal numbers denote pup number.
Other digit(s) denote litter numbers.

APPENDIX 25 - continued

Brain weights – individual values (g) for male and female offspring at Day 4 of age

Group 4: 3 mg/kg/day

Male offspring		Female offspring	
Animal number	Brain weight	Animal number	Brain weight
6705	0.383	6711	0.369
6706	0.374	6712	0.369
6805	0.392	6813	0.418
6806	0.432	6814	0.400
6905	0.397	6912	0.398
6906	0.386	6913	0.392
7005	0.396	7013	0.391
7006	0.405	7014	0.381
7105	0.455	7110	0.417
7205	0.351	7111	0.423
7206	0.345	7213	0.334
7305	0.401	7214	0.349
7306	0.401	7313	0.395
7505	0.403	7314	0.392
7506	0.379	7511	0.367
7606	0.459	7512	0.381
7607	0.430		

Last two digits of animal numbers denote pup number.
Other digit(s) denote litter numbers.

APPENDIX 26

Brain weights – individual values (g) for dosed male and female offspring at Day 11 of age

Group 5: Control

Male offspring		Female offspring	
Animal number	Brain weight	Animal number	Brain weight
7701	1.162	7709	1.124
7801	1.143	7806	1.095
7901	1.019	7907	1.054
8001	1.022	8007	1.034
8101	0.978	8112	0.928
8201	1.041	8209	1.053
8301	1.060	8314	0.991
8401	1.014	8405	1.113

Group 5: 0.1 mg/kg/day

Male offspring		Female offspring	
Animal number	Brain weight	Animal number	Brain weight
7702	1.164	7710	1.115
7802	1.077	7807	1.091
7902	1.061	7908	0.998
8002	1.023	8008	0.984
8102	0.981	8113	0.949
8202	1.032	8210	1.056
8302	1.037	8315	0.966
8402	1.083	8406	1.119

Last two digits of animal number denote pup number.
Other digit(s) denote litter number.

APPENDIX 26 - continued

Brain weights – individual values (g) for male and female offspring at Day 11 of age

Group 5: 0.5 mg/kg/day

Male offspring		Female offspring	
Animal number	Brain weight	Animal number	Brain weight
7703	1.196	7711	1.133
7803	1.124	7808	1.039
7903	0.956	7909	0.962
8003	1.088	8009	0.996
8103	0.966	8114	0.942
8203	1.061	8211	1.008
8303	1.022	8316	0.983
8403	1.108	8407	1.054

Group 5 : 3 mg/kg/day

Male offspring		Female offspring	
Animal number	Brain weight	Animal number	Brain weight
7704	1.198	7712	1.132
7804	1.071	7809	1.054
7904	0.970	7910	1.045
8004	1.029	8010	0.991
8104	0.972	8115	0.995
8204	1.069	8212	1.021
8304	0.992	8317	1.026
8404	1.132	8408	1.062

Last two digits of animal number denote pup number.
 Other digit(s) denote litter number.

APPENDIX 27

Brain weights – individual values (g) for dosed male and female offspring at Day 21 of age

Group 1: Control

Male offspring		Female offspring	
Animal number	Brain weight	Animal number	Brain weight
1001	1.561	1009	1.537
1101	1.519	1110	1.525
1201	1.483	1208	1.396
1301	1.496	1310	1.431
1401	1.424	1406	1.349
1601	1.574	1610	1.538
1701	1.505	1708	1.460
1901	1.453	1907	1.435

Group 2 : 0.1 mg/kg/day

Male offspring		Female offspring	
Animal number	Brain weight	Animal number	Brain weight
2901	1.333	2911	1.409
3001	1.453	3003	1.447
3101	1.576	3108	1.449
3201	1.475	3206	1.602
3301	1.475	3307	1.435
3401	1.475	3405	1.456
3501	1.507	3510	1.444
3601	1.579	3608	1.495

Last two digits of animal number denote pup number.
Other digit(s) denote litter number.

APPENDIX 27 - continued

Brain weights – individual values (g) for male and female offspring at Day 21 of age

Group 3 : 0.5 mg/kg/day

Male offspring		Female offspring	
Animal number	Brain weight	Animal number	Brain weight
4801	1.451	4806	1.473
4901	1.520	4904	1.431
5001	1.434	5004	1.411
5101	1.548	5106	1.410
5201	1.493	5209	1.472
5301	1.391	5310	1.527
5401	1.540	5411	1.450
5502	1.457	5510	1.446

Group 4 : 3 mg/kg/day

Male offspring		Female offspring	
Animal number	Brain weight	Animal number	Brain weight
6701	1.451	6707	1.315
6801	1.461	6809	1.440
6901	1.385	6908	1.458
7001	1.454	7009	1.472
7201	1.433	7209	1.383
7301	1.474	7309	1.448
7401	1.523	7402	1.526
7601	1.483	7610	1.446

Last two digits of animal number denote pup number.
Other digit(s) denote litter number.

APPENDIX 28

Brain weights – individual values (g) for male and female offspring at Day 60 of age

Group 1: Control

Male offspring		Female offspring	
Animal number	Brain weight	Animal number	Brain weight
1004	2.121	1012	1.944
1104	2.026	1113	1.798
1204	2.072	1211	1.824
1305	1.937	1312	1.868
1404	1.900	1409	1.736
1604	2.080	1613	1.883
1704	2.040	1711	1.813
1904	1.929	1910	1.800

Group 2 : 0.1 mg/kg/day

Male offspring		Female offspring	
Animal number	Brain weight	Animal number	Brain weight
2906	1.826	2912	1.872
3002	1.816	3008	1.834
3104	2.028	3111	1.832
3204	2.059	3209	1.890
3304	2.070	3310	1.928
3404	1.955	3408	1.931
3504	1.973	3513	1.855
3604	2.045	3611	1.943

Last two digits of animal number denote pup number.
Other digit(s) denote litter number.

APPENDIX 28 - continued

Brain weights – individual values (g) for male and female offspring at Day 60 of age

Group 3: 0.5 mg/kg/day

Male offspring		Female offspring	
Animal number	Brain weight	Animal number	Brain weight
4804	1.830	4809	1.839
4903	2.078	4905	1.888
5003	1.922	5008	1.854
5104	1.952	5109	1.824
5204	1.993	5212	1.818
5304	1.882	5313	1.985
5404	1.911	5414	1.841
5505	1.982	5513	1.820

Group 4 : 3 mg/kg/day

Male offspring		Female offspring	
Animal number	Brain weight	Animal number	Brain weight
6704	1.964	6710	1.796
6804	1.958	6812	1.798
6904	2.016	6911	1.724
7004	2.029	7012	1.779
7204	1.886	7212	1.742
7304	2.023	7312	1.888
7603	2.046	7407	1.949
7604	2.015	7612	1.850

Last two digits of animal number denote pup number.
Other digit(s) denote litter number.

APPENDIX 29

Necropsy findings – individual observations for offspring on Day 4 of age

Group 1: Control

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
1005	NAD	1114	NAD
1006	NAD	1115	NAD
1105	NAD	1212	NAD
1106	NAD	1213	NAD
1205	NAD	1410	NAD
1206	NAD	1411	NAD
1306	NAD	1514	NAD
1307	NAD	1614	NAD
1405	NAD	1615	NAD
1505	NAD	1712	NAD
1506	NAD	1713	NAD
1605	NAD	1911	NAD
1606	NAD	1912	NAD
1705	NAD		
1706	NAD		
1805	NAD		
1806	NAD		
1905	NAD		
1906	NAD		

NAD No abnormalities detected.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

APPENDIX 29 - continued

Necropsy findings – individual observations for offspring on Day 4 of age

Group 2: 0.1 mg/kg/day

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
2907	NAD	3009	NAD
2908	NAD	3010	NAD
3105	NAD	3112	NAD
3106	NAD	3113	NAD
3205	NAD	3210	NAD
3305	NAD	3211	NAD
3306	NAD	3311	NAD
3505	NAD	3312	NAD
3506	NAD	3409	NAD
3605	NAD	3410	NAD
3606	NAD	3514	NAD
3705	NAD	3515	NAD
3706	NAD	3612	NAD
3805	NAD	3613	NAD
3806	NAD	3711	NAD
		3712	NAD

NAD No abnormalities detected.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

APPENDIX 29 - continued

Necropsy findings – individual observations for offspring on Day 4 of age

Group 3: 0.5 mg/kg/day

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
4805	NAD	4810	NAD
5105	NAD	5009	NAD
5205	NAD	5010	NAD
5206	NAD	5110	NAD
5305	NAD	5111	NAD
5306	NAD	5213	NAD
5405	NAD	5214	NAD
5406	NAD	5314	NAD
5506	NAD	5415	NAD
5507	NAD	5416	NAD
5605	NAD	5715	NAD
5606	NAD	5716	NAD
5705	NAD		
5706	NAD		

NAD No abnormalities detected.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

APPENDIX 29 - continued

Necropsy findings – individual observations for offspring on Day 4 of age

Group 4: 3 mg/kg/day

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
6705	NAD	6711	NAD
6706	NAD	6712	NAD
6805	NAD	6813	NAD
6806	NAD	6814	NAD
6905	NAD	6912	NAD
6906	NAD	6913	NAD
7005	NAD	7013	NAD
7006	NAD	7014	NAD
7105	NAD	7110	NAD
7205	NAD	7111	NAD
7206	NAD	7213	NAD
7305	NAD	7214	NAD
7306	NAD	7313	NAD
7505	NAD	7314	NAD
7506	NAD	7511	NAD
7606	NAD	7512	NAD
7607	NAD		

NAD No abnormalities detected.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

APPENDIX 29 - continued

Necropsy findings – individual observations for offspring on Day 11 of age

Group 5: Untreated

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
7701	NAD	7709	NAD
7702	NAD	7710	NAD
7703	NAD	7711	NAD
7704	NAD	7712	NAD
7801	NAD	7806	NAD
7802	NAD	7807	NAD
7803	NAD	7808	NAD
7804	NAD	7809	NAD
7901	NAD	7907	NAD
7902	NAD	7908	NAD
7903	NAD	7909	NAD
7904	NAD	7910	NAD
8001	NAD	8007	NAD
8002	NAD	8008	NAD
8003	NAD	8009	NAD
8004	NAD	8010	NAD
8101	NAD	8112	NAD
8102	NAD	8113	NAD
8103	NAD	8114	NAD
8104	NAD	8115	NAD
8201	NAD	8209	NAD
8202	NAD	8210	NAD
8203	NAD	8211	NAD
8204	NAD	8212	NAD
8301	NAD	8314	NAD
8302	NAD	8315	NAD
8303	NAD	8316	NAD
8304	NAD	8317	NAD
8401	NAD	8405	NAD
8402	NAD	8406	NAD
8403	NAD	8407	NAD
8404	NAD	8408	NAD

NAD No abnormalities detected.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

APPENDIX 29 - continued

Necropsy findings – individual observations for offspring on Day 21 of age

Group 1: Control

Pups selected for blood and brain sampling

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
1001	NAD	1009	NAD
1101	NAD	1110	NAD
1201	NAD	1208	NAD
1301	NAD	1310	NAD
1401	NAD	1406	NAD
1601	NAD	1610	NAD
1701	NAD	1708	NAD
1901	NAD	1907	NAD

Group 2: 0.1 mg/kg/day

Pups selected for blood and brain sampling

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
2901	NAD	2911	NAD
3001	NAD	3003	NAD
3101	NAD	3108	NAD
3201	NAD	3206	NAD
3301	NAD	3307	NAD
3401	NAD	3405	NAD
3501	NAD	3510	NAD
3601	Raised area on median liver lobe adhered to diaphragm.	3608	NAD

NAD No abnormalities detected.

APPENDIX 29 – continued

Necropsy findings – individual observations for offspring on Day 21 of age

Group 3: 0.5 mg/kg/day

Pups selected for blood and brain sampling

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
4801	NAD	4806	NAD
4901	NAD	4904	NAD
5001	NAD	5004	NAD
5101	NAD	5106	NAD
5201	NAD	5209	NAD
5301	NAD	5310	NAD
5401	NAD	5411	NAD
5502	NAD	5510	NAD

Group 4: 3 mg/kg/day

Pups selected for blood and brain sampling

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
6701	NAD	6707	NAD
6801	NAD	6809	NAD
6901	NAD	6908	NAD
7001	NAD	7009	NAD
7201	NAD	7209	NAD
7301	NAD	7309	NAD
7401	NAD	7402	NAD
7601	NAD	7610	NAD

NAD No abnormalities detected.

APPENDIX 29 – continued

Necropsy findings – individual observations for offspring on Day 21 of age

Group 1: Control

Pups not selected for blood and brain sampling

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
1002	NAD	1010	NAD
1003	NAD	1011	NAD
1102	NAD	1111	NAD
1202	NAD	1209	NAD
1203	NAD	1210	NAD
1302	NAD	1311	NAD
1303	NAD	1407	NAD
1304	NAD	1408HK	Domed head Skin of head detached from cranium and resulting pocket filled with air Thoracic cavity: large amount of white gelatinous material with small amount of clotted blood adjacent to diaphragm
1402	NAD	1510#	NAD
1403	NAD	1511#	NAD
1501#	NAD	1512#	NAD
1502#	NAD	1513#	NAD
1503#	NAD	1611	NAD
1504#	NAD	1612	NAD
1602	NAD	1709	NAD
1603	NAD	1710	NAD
1702	NAD	1809#	NAD
1703	NAD	1810#	NAD
1801#	NAD	1811#	NAD
1802#	NAD	1812#	NAD
1803#	NAD	1908	NAD
1804#	NAD	1909	NAD
1902	NAD		
1903	NAD		

NAD No abnormalities detected.

HK Humane kill.

Pups not directly dosed.

APPENDIX 29 – continued

Necropsy findings – individual observations for offspring on Day 21 of age

Group 2: 0.1 mg/kg/day

Pups not selected for blood and brain sampling

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
2902	NAD	3004	NAD
2903	NAD	3005	NAD
2904	NAD	3006	NAD
2905	NAD	3007	NAD
3102	NAD	3109	NAD
3202	NAD	3207	NAD
3203	NAD	3208	NAD
3302	NAD	3308	NAD
3303	NAD	3309	NAD
3402	NAD	3406	NAD
3403	NAD	3407	NAD
3502	NAD	3511	NAD
3503	NAD	3512	NAD
3602	NAD	3609	NAD
3603	NAD	3610	NAD
3701#	NAD	3707#	NAD
3702#	NAD	3708#	NAD
3703#	NAD	3709#	NAD
3704#	NAD	3710#	NAD
3801#	NAD	3807#	NAD
3802#	NAD	3808#	NAD
3803#	NAD	3809#	NAD
3804#	NAD	3810#	NAD

NAD No abnormalities detected.

Pups not directly dosed.

APPENDIX 29 – continued

Necropsy findings – individual observations for offspring on Day 21 of age

Group 3: 0.5 mg/kg/day

Pups not selected for blood and brain sampling

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
4802	NAD	4807	NAD
4803	NAD	4808	NAD
4902	NAD	5005	NAD
5102	NAD	5006	NAD
5103	NAD	5107	NAD
5202	NAD	5108	NAD
5203	NAD	5210	NAD
5302	NAD	5211	NAD
5303	NAD	5311	NAD
5402	NAD	5312	NAD
5403	NAD	5412	NAD
5503	NAD	5413	NAD
5504	NAD	5511	NAD
5601#	NAD	5512	NAD
5602#	NAD	5611#	NAD
5603#	NAD	5612#	NAD
5604#	NAD	5613#	NAD
5701#	NAD	5614#	NAD
5702#	NAD	5711#	NAD
5703#	NAD	5712#	NAD
5704#	NAD	5713#	NAD
		5714#	NAD

NAD No abnormalities detected.

Pups not directly dosed.

APPENDIX 29 – continued

Necropsy findings – individual observations for offspring on Day 21 of age

Group 4: 3 mg/kg/day

Pups not selected for blood and brain sampling

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
6702	NAD	6708	NAD
6802	NAD	6810	NAD
6803	NAD	6811	NAD
6902	NAD	6909	NAD
6903	NAD	6910	NAD
7002	NAD	7010	NAD
7003	NAD	7011	NAD
7101#	NAD	7106#	NAD
7102#	NAD	7107#	NAD
7103#	NAD	7108#	NAD
7104#	NAD	7109#	NAD
7202	NAD	7210	NAD
7203	NAD	7211	NAD
7302	NAD	7310	NAD
7303	NAD	7311	NAD
7501#	NAD	7403	NAD
7502#	NAD	7404	NAD
7503#	NAD	7405	NAD
7504#	NAD	7406	NAD
7602	NAD	7507#	NAD
		7508#	Kidneys: bilateral renal cavitation
		7509#	Kidneys: bilateral renal cavitation Right hydroureter
		7510#	Kidneys: right renal cavitation Right hydroureter
		7611	NAD

NAD No abnormalities detected.

Pups not directly dosed.

APPENDIX 29 – continued

Necropsy findings – individual observations for offspring on Day 60 of age

Group 1: Control

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
1004	NAD	1012	NAD
1104	NAD	1113	NAD
1204	NAD	1211	NAD
1305	NAD	1312	NAD
1404	NAD	1409	NAD
1604	NAD	1613	NAD
1704	NAD	1711	NAD
1904	NAD	1910	NAD

NAD No abnormalities detected.

APPENDIX 29 – continued

Necropsy findings – individual observations for offspring on Day 60 of age

Group 2: 0.1 mg/kg/day

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
2906	NAD	2912	NAD
3002	NAD	3008	NAD
3104	NAD	3111	NAD
3204	NAD	3209	NAD
3304	NAD	3310	NAD
3404	NAD	3408	NAD
3504	NAD	3513	NAD
3604	NAD	3611	NAD

NAD No abnormalities detected.

APPENDIX 29 – continued

Necropsy findings – individual observations for offspring on Day 60 of age

Group 3: 0.5 mg/kg/day

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
4804	NAD	4809	NAD
4903	NAD	4905	NAD
5003	NAD	5008	NAD
5104	NAD	5109	NAD
5204	NAD	5212	NAD
5304	NAD	5313	NAD
5404	NAD	5414	NAD
5505	NAD	5513	NAD

NAD No abnormalities detected.

APPENDIX 29 – continued

Necropsy findings – individual observations for offspring on Day 60 of age

Group 4: 3 mg/kg/day

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
6704	NAD	6710	NAD
6804	NAD	6812	NAD
6904	NAD	6911	NAD
7004	NAD	7012	NAD
7204	NAD	7212	NAD
7304	NAD	7312	NAD
7603	NAD	7407	NAD
7604	NAD	7612	NAD

NAD No abnormalities detected.

APPENDIX 30

Plasma, erythrocyte and brain cholinesterase activity - individual values for adult males on Day 1 of treatment

Group : 6 7 8 9
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
6	201	424	1050	13700
	202	432	1350	13900
	203	425	675	13550
	204	337	1375	13850
	205	367	1225	13750
	206	382	1225	13500
	207	321	1050	13800
	208	313	1025	14300
7	217	345	1350	14250
	218	339	1550	14350
	219	373	1500	11900
	220	369	1175	12850
	221	417	1050	13700
	222	547	1250	13800
	223	407	1025	13750
	224	299	1075	13750

CHE Cholinesterase.

APPENDIX 30 - continued

Plasma, erythrocyte and brain cholinesterase activity - individual values for adult males on Day 1 of treatment

Group : 6 7 8 9
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
8	233	356	1175	13500
	234	332	1125	13500
	235	407	1200	12850
	236	296	1150	13450
	237	362	1075	13000
	238	500	1125	13300
	239	341	1200	13400
9	240	320	1000	13350
	249	292	825	11400
	250	306	850	12150
	251	343	1025	14000
	252	237	800	11500
	253	270	825	10800
	254	363	1050	12400
	255	319	1000	11400
	256	310	1050	13400

CHE Cholinesterase.

APPENDIX 30 - continued

Plasma, erythrocyte and brain cholinesterase activity - individual values for adult females on Day 1 of treatment

Group : 6 7 8 9
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
6	85	621	1375	13850
	86	940	1100	14200
	87	624	1325	13650
	88	559	1125	14200
	89	580	1475	13300
	90	638	1200	15100
	91	720	1100	14500
	92	822	975	14400
7	101	737	1225	13750
	102	661	1100	13150
	103	533	1200	13100
	104	544	1100	13300
	105	921	950	13750
	106	650	1075	13500
	107	500	1075	14150
	108	711	1300	14300

CHE Cholinesterase.

APPENDIX 30 - continued

Plasma, erythrocyte and brain cholinesterase activity - individual values for adult females on Day 1 of treatment

Group : 6 7 8 9
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
8	117	807	1150	14850
	118	649	1050	13450
	119	703	1200	14500
	120	753	950	13450
	121	700	1125	14150
	122	839	1225	13750
	123	784	1050	14000
	124	598	1100	12650
9	133	314	975	12100
	134	708	925	11200
	135	716	950	12600
	136	588	900	12200
	137	638	725	11500
	138	626	925	12600
	139	544	875	13550
	140	683	775	11100

CHE Cholinesterase.

APPENDIX 31

Plasma, erythrocyte and brain cholinesterase activity - individual values for adult males on Day 11 of treatment

Group : 6 7 8 9
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
6	209	357	875	14300
	210	329	1275	13900
	211	299	875	14350
	212	403	1175	14650
	213	321	975	14350
	214	353	1200	12950
	215	363	1200	13900
7	216	321	1175	14400
	225	368	900	15100
	226	311	1000	14000
	227	351	950	13650
	228	370	2225	14700
	229	370	1025	13100
	230	270	1175	13700
	231	265	1100	14200
	232	308	975	13450

CHE Cholinesterase.

APPENDIX 31 - continued

Plasma, erythrocyte and brain cholinesterase activity - individual values for adult males on Day 11 of treatment

Group : 6 7 8 9
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
8	241	325	1150	13300
	242	315	950	13100
	243	269	750	12850
	244	319	725	12950
	245	329	850	11900
	246	244	1050	11850
	247	271	1025	13050
	248	343	725	12600
9	257	293	550	9050
	258	287	875	9850
	259	141	300	5350
	260	176	275	6300
	261	218	300	6200
	262	214	275	4800
	263	238	750	11850
	264	155	325	6350

CHE Cholinesterase.

APPENDIX 31 - continued

Plasma, erythrocyte and brain cholinesterase activity - individual values for adult females on Day 11 of treatment

Group : 6 7 8 9
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
6	93	817	950	13900
	94	745	950	14400
	95	893	1100	17100
	96	744	900	16700
	97	885	1050	13100
	98	898	925	13900
	99	802	950	15200
	100	539	1325	14650
	109	1412	1000	14100
	110	1514	1025	13650
7	111	810	1125	14150
	112	732	900	14250
	113	906	1125	14250
	114	728	1000	13300
	115	708	875	13250
	116	783	875	14350

CHE Cholinesterase.

APPENDIX 31 - continued

Plasma, erythrocyte and brain cholinesterase activity - individual values for adult females on Day 11 of treatment

Group : 6 7 8 9
 Compound : Control ----- Dimethoate -----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
8	125	788	825	12350
	126	707	850	11500
	127	559	1050	13650
	128	919	950	14200
	129	847	1025	12550
	130	790	1025	13250
	131	892	950	13100
	132	655	925	12450
9	141	742	275	5350
	142	817	650	7850
	143	479	350	5850
	144	557	250	5250
	145	624	375	6000
	146	860	400	7900
	147	456	375	5900
	148	458	325	5400

CHE Cholinesterase.

APPENDIX 32

Plasma, erythrocyte and brain cholinesterase activity - individual values for dams on Day 20 of gestation

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1	1	1481	1525	13550
	2	1497	1625	13300
	3	1635	1625	13400
	4	1323	1925	12150
	6	1206	1600	13650
	7	1493	1575	13900
	8	1160	1500	9700
	9	1254	1975	13050
2	20	1376	1450	13150
	21	1205	1525	12500
	22	1506	1475	13700
	23	1019	1350	12650
	25	1250	2075	12750
	26	1501	1525	13900
	27	845	1450	13150
	28	1025	1650	12550

CHE Cholinesterase.

APPENDIX 32 - continued

Plasma, erythrocyte and brain cholinesterase activity - individual values for dams on Day 20 of gestation

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal Number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
3	39	1368	1250	11450
	40	1392	1500	12000
	41	1425	1325	11800
	43	1355	1350	11500
	44	912	1375	11350
	45	977	1375	11800
	46	827	2125	11550
4	47	1214	1375	11050
	58	1009	675	4750
	59	806	725	4750
	60	252	700	6200
	62	931	650	4000
	63	738	775	5200
	64	1052	925	7100
	65	596	625	4850
	66	823	600	3900

CHE Cholinesterase.

APPENDIX 33

Plasma, erythrocyte and brain cholinesterase activity - individual litter values for male and female fetuses on Day 20 of gestation

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal Number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1	101	243	1200	1750
	201	268	1250	1800
	301	229	1225	1700
	401	259	1250	1650
	601	259	1225	1700
	701	299	1325	1750
	801	266	1175	2200
	901	240	1050	1700
2	2001	219	1175	1800
	2101	268	1225	1550
	2201	INS	1175	1650
	2301	273	1300	1500
	2501	246	1050	1450
	2601	299	1350	1800
	2701	235	1325	1300
	2801	259	1200	1500

CHE Cholinesterase.

INS Insufficient sample for testing.

Last two digits of animal number denote pooled fetal sample; litter number given by remaining digit(s).

APPENDIX 33 - continued

Plasma, erythrocyte and brain cholinesterase activity - individual litter values for male and female fetuses on Day 20 of gestation

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal Number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
3	3901	204	1550	1550
	4001	299	1225	1650
	4101	225	1125	1900
	4301	224	1050	1550
	4401	224	1175	1500
	4501	240	1250	1600
	4601	250	1075	1450
4	4701	244	1000	1600
	5801	155	700	1200
	5901	150	925	1250
	6001	184	1250	1400
	6201	123	775	1000
	6301	126	725	1150
	6401	176	825	1400
	6501	127	750	1150
	6601	134	725	950

CHE Cholinesterase.

Last two digits of animal number denote pooled fetal sample; litter number given by remaining digit(s).

APPENDIX 34

Plasma, erythrocyte and brain cholinesterase activity – individual values for male offspring on Day 4 of age

Group Compound : 1 Control : 2 ----- Dimethoate ----- 3 4
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal Number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1	1005	623	1050	3450
	1006	613	1300	3400
	1105	710	1525	3200
	1106	651	1325	2850
	1205	667	1500	3250
	1206	681	1550	2050
	1306	646	1325	3350
	1307	584	1050	3100
	1405	658	1050	2800
	1505	605	1000	3100
	1506	498	925	3150
	1605	532	1725	3200
	1606	583	1275	3300
	1705	623	1375	3250
	1706	561	1575	3350
	1805	505	1425	3450
	1806	543	1125	3200
	1905	714	1100	2900
	1906	635	1325	3250

CHE Cholinesterase.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

APPENDIX 34 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for male offspring on Day 4 of age

Group : 1 2 3 4
 Compound : Control ----- Dimethoate -----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
2	2907	574	1275	3350
	2908	513	1050	1900
	3105	719	1525	3350
	3106	634	1350	2350
	3205	637	1675	2950
	3305	584	1450	2650
	3306	606	1325	3050
	3505	582	1775	3400
	3506	570	1150	3100
	3605	611	1475	3100
	3606	639	1450	2350
	3705	592	1425	2750
	3706	539	1600	2900
	3805	742	1425	2600
	3806	558	1100	2450

CHE Cholinesterase.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

APPENDIX 34 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for male offspring on Day 4 of age

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
3	4805	609	1075	3000
	5105	580	1000	2400
	5205	532	1100	2950
	5206	602	1225	3050
	5305	514	1325	2600
	5306	644	1375	2950
	5405	606	1475	3200
	5406	677	1650	3000
	5506	593	1050	2800
	5507	477	1375	3100
	5605	605	1100	2950
	5606	604	1050	3000
	5705	573	1500	2700
	5706	612	1250	2750

CHE Cholinesterase.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

APPENDIX 34 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for male offspring on Day 4 of age

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
4	6705	618	1000	2700
	6706	645	1125	2350
	6805	INS	1025	2000
	6806	608	875	2250
	6905	586	1000	2800
	6906	616	1250	2950
	7005	440	900	3200
	7006	475	975	3300
	7105	608	1275	3200
	7205	558	850	2750
	7206	532	1025	2650
	7305	566	1200	2700
	7306	588	1325	2900
	7505	551	1225	2700
	7506	593	1225	2600
	7606	511	1075	2850
	7607	555	850	2750

CHE Cholinesterase.

INS Insufficient sample for testing.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

APPENDIX 34 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for female offspring on Day 4 of age

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1	1114	645	1325	2700
	1115	637	1200	2500
	1212	662	1250	3300
	1213	712	1575	3200
	1410	587	1150	2650
	1411	663	875	2800
	1514	636	1025	2300
	1614	635	975	3150
	1615	577	975	2850
	1712	546	1775	2750
	1713	623	2050	2800
	1911	693	950	2500
	1912	702	1250	3200

CHE Cholinesterase.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

APPENDIX 34 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for female offspring on Day 4 of age

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
2	3009	652	1175	3150
	3010	616	1250	3200
	3112	INS	675	2600
	3113	672	1050	2500
	3210	665	1300	3050
	3211	622	1375	2850
	3311	602	1325	2700
	3312	552	1275	2550
	3409	618	1475	3150
	3410	645	1675	3300
	3514	661	975	3100
	3515	591	1325	2850
	3612	567	1400	2850
	3613	520	1450	2900
	3711	529	1325	3200
	3712	569	1125	3100

CHE Cholinesterase.

INS Insufficient sample for testing.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

APPENDIX 34 - continued

Plasma, erythrocyte and brain cholinesterase activity -- individual values for female offspring on Day 4 of age

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
3	4810	656	1025	2850
	5009	563	1400	2800
	5010	517	1275	2400
	5110	617	1450	3200
	5111	532	1100	2300
	5213	613	1250	2550
	5214	595	1225	2950
	5314	631	1000	2200
	5415	563	1325	2750
	5416	615	1925	2750
	5715	609	1625	2550
	5716	575	1625	2500

CHE Cholinesterase.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

APPENDIX 34 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for female offspring on Day 4 of age

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
4	6711	INS	950	2450
	6712	567	900	2300
	6813	566	1250	2500
	6814	620	1000	2450
	6912	555	1200	2850
	6913	569	1025	2450
	7013	496	1025	2700
	7014	491	850	2650
	7110	562	950	3050
	7111	552	1125	2900
	7213	563	700	2350
	7214	576	925	2200
	7313	676	1775	3050
	7314	645	1700	2600
	7511	621	925	2950
	7512	575	1100	2750

CHE Cholinesterase.

INS Insufficient sample for testing.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

APPENDIX 35

Plasma, erythrocyte and brain cholinesterase activity – individual values for dosed male offspring on Day 11 of age

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal Number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1	7701	681	1325	6450
	7801	970	1475	6900
	7901	879	1450	6200
	8001	676	1900	6300
	8101	747	1725	6300
	8201	733	1800	6550
	8301	638	2150	6350
2	8401	720	1475	6750
	7702	752	1175	6450
	7802	847	1375	6600
	7902	793	1650	6500
	8002	752	1650	6300
	8102	758	2275	6150
	8202	738	1825	6450
	8302	624	1725	5900
	8402	723	1400	6550

CHE Cholinesterase.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

APPENDIX 35 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for dosed male offspring on Day 11 of age

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
3	7703	705	1600	6150
	7803	772	1350	6750
	7903	707	1650	6300
	8003	634	1500	5450
	8103	720	1525	6250
	8203	648	1975	6100
	8303	626	1725	6150
4	8403	693	1450	6000
	7704	484	1200	5150
	7804	703	1050	5850
	7904	646	1475	5600
	8004	539	1375	4950
	8104	642	1950	5350
	8204	696	1475	5400
	8304	620	2650	5550
	8404	579	1175	5150

CHE Cholinesterase.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

APPENDIX 35 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for dosed female offspring on Day 11 of age

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1	7709	685	2525	6250
	7806	963	3225	6650
	7907	787	1850	6150
	8007	690	2175	6050
	8112	695	1650	6100
	8209	809	1600	6400
	8314	601	1600	6300
	8405	704	1350	6150
2	7710	657	1475	6650
	7807	864	2275	7000
	7908	715	1400	6400
	8008	723	1625	6350
	8113	791	1500	6000
	8210	714	1725	6250
	8315	449	1775	6100
	8406	687	1400	6050

CHE Cholinesterase.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

APPENDIX 35 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for dosed female offspring on Day 11 of age

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
3	7711	697	1450	6050
	7808	888	1725	6750
	7909	695	1725	6000
	8009	737	2750	6150
	8114	682	1850	5900
	8211	747	1650	6250
	8316	615	1900	5750
4	8407	698	2100	6150
	7712	593	1175	5600
	7809	802	1175	5750
	7910	629	1600	5450
	8010	645	1850	5100
	8115	513	1525	4600
	8212	619	1400	5200
	8317	517	1725	5300
	8408	554	1350	4150

CHE Cholinesterase.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

APPENDIX 36

Plasma, erythrocyte and brain cholinesterase activity – individual values for dosed male offspring on Day 21 of age

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1	1001	433	1450	10300
	1101	483	1275	10200
	1201	673	1000	10100
	1301	437	1350	10450
	1401	500	2425	10700
	1601	529	1950	10200
	1701	450	1850	10550
	1901	546	1800	10500
2	2901	471	1100	10000
	3001	604	1350	10650
	3101	640	2425	9850
	3201	433	1625	9650
	3301	548	1800	10000
	3401	538	1550	10000
	3501	551	1875	9850
	3601	497	1550	9550

CHE Cholinesterase.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

APPENDIX 36 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for dosed male offspring on Day 21 of age

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
3	4801	482	1325	9350
	4901	493	1475	8950
	5001	443	1025	8500
	5101	498	1150	8800
	5201	493	1725	9400
	5301	523	2000	9250
4	5401	444	1750	8750
	5502	451	1500	9350
	6701	382	650	6250
	6801	248	625	5450
	6901	370	850	6600
	7001	245	325	5350
	7201	370	625	5400
	7301	288	725	4950
	7401	221	775	5400
	7601	329	775	6000

CHE Cholinesterase.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

APPENDIX 36 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for dosed female offspring on Day 21 of age

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1	1009	500	1500	10900
	1110	532	1700	10000
	1208	616	1550	10750
	1310	412	1375	10100
	1406	499	2425	10200
	1610	428	3125	10100
	1708	407	1800	9800
2	1907	503	1725	10350
	2911	445	1275	10150
	3003	540	1525	10150
	3108	626	1475	9650
	3206	424	1425	9800
	3307	498	1950	10050
	3405	506	2175	9450
	3510	443	1525	9650
	3608	573	1600	10350

CHE Cholinesterase.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

APPENDIX 36 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for dosed female offspring on Day 21 of age

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
3	4806	429	1500	8650
	4904	445	1500	8950
	5004	394	1075	9150
	5106	406	1200	9100
	5209	515	1750	9300
	5310	468	1525	9200
	5411	548	1825	9150
4	5510	495	1350	8650
	6707	363	825	7000
	6809	353	825	7050
	6908	235	375	5150
	7009	227	350	4950
	7209	290	700	5050
	7309	323	575	5200
	7402	289	825	6250
	7610	349	825	7000

CHE Cholinesterase.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

APPENDIX 37

Plasma, erythrocyte and brain cholinesterase activity – individual values for male offspring on Day 60 of age

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1	1004	360	1150	13000
	1104	307	950	13050
	1204	395	1200	12000
	1305	310	1000	13600
	1404	411	1100	13200
	1604	326	1125	13050
	1704	328	1000	13100
2	1904	548	1075	13000
	2906	316	1000	13000
	3002	411	1075	13100
	3104	434	1025	12950
	3204	341	1100	13750
	3304	375	1175	13700
	3404	378	1125	12900
	3504	312	1200	12650
	3604	383	1100	12750

CHE Cholinesterase.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

APPENDIX 37 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for male offspring on Day 60 of age

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
3	4804	352	1025	13150
	4903	378	1075	12500
	5003	346	1200	13350
	5104	261	1150	12550
	5204	353	1125	12600
4	5304	313	1100	13700
	5404	352	975	13000
	5505	364	1150	13050
	6704	352	1150	14050
	6804	312	1125	13300
	6904	361	1075	13400
	7004	400	950	13200
	7204	317	1025	13800
	7304	299	1200	12350
	7603	336	825	12250
	7604	317	950	12000

CHE Cholinesterase.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

APPENDIX 37 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for female offspring on Day 60 of age

Group : 1 2 3 4
 Compound : Control ----- Dimethoate-----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1	1012	999	1250	13500
	1113	732	1100	13150
	1211	1219	1125	13250
	1312	1082	1000	13200
	1409	745	1175	13800
	1613	902	1350	13250
	1711	616	975	12850
	1910	957	900	13200
2	2912	763	1200	12750
	3008	1183	1250	12750
	3111	1178	1025	12850
	3209	874	1150	13150
	3310	838	1050	13600
	3408	838	1000	12750
	3513	1024	1200	12650
	3611	624	1075	13100

CHE Cholinesterase.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

APPENDIX 37 - continued

Plasma, erythrocyte and brain cholinesterase activity -- individual values for female offspring on Day 60 of age

Group : 1 2 3 4
 Compound : Control ----- Dimethoate -----
 Dosage (mg/kg/day) : 0 0.1 0.5 3

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
3	4809	1029	800	12700
	4905	1070	850	12650
	5008	758	975	12500
	5109	660	1050	12800
	5212	941	1050	13200
	5313	965	1025	12400
	5414	1389	1075	12800
4	5513	745	1100	12850
	6710	1236	1175	13500
	6812	710	1175	12700
	6911	987	1150	13550
	7012	714	950	12950
	7212	870	1000	12650
	7312	684	1000	12000
	7407	720	900	12000
	7612	843	1000	12600

CHE Cholinesterase.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

ADDENDUM 1

Certificate of Analysis for diet



CONTROL DATA

VRF1C lot 00314

Date of Manufacture 2000/03/14 Sell by date 2000/07/14
Use by date 2001/03/14

Bag numbers	1 to 30
Quantity manufactured	(tonnes) 32
Variation from theoretical weight	Conform

SIEVE ANALYSIS (mm)

Diameter 0.00 - 0.10	0.1
Diameter 0.10 - 0.25	3.7
Diameter 0.25 - 0.50	41.8
Diameter 0.50 - 1.00	43.7
Diameter 1.00 - 2.00	4.7
Diameter 2.00 - 3.15	0.0
Diameter > 3.15	0.0

NUTRITIVE QUALITY

Incorporation of macro-mineral mix	(%)	Positive	
Incorporation of micro-mineral premix	(Zn and Cu)	Positive	
Incorporation of vitamin premix	(Vit. A and B)	Positive	
Moisture	(%)	12.8	(9 to 13.5)
Crude protein	(%)	18.5	(16.1 to 19.0)
Crude oil	(%)	4.0	(1.7 to 6.1)
Nitrogen free extract	(%)	54.0	
of which starch	(%)	41.0	
of which total sugars	(%)	1.9	
Crude fibre	(%)	4.0	(2.8 to 5.2)
Hemicellulose	(%)		
True cellulose	(%)		
Lignine	(%)		
Total minerals	(%)	6.7	(4.5 to 7.0)
Calcium	(mg / Kg)	10 900	(8 000 to 12 000)
Phosphorus	(mg / Kg)	5 900	(4 000 to 8 300)
Sodium	(mg / Kg)	3 400	(2 500 to 3 700)
Potassium	(mg / Kg)	6 600	(5 600 to 6 800)
Manganese	(mg / Kg)	70	(20 to 100)
Copper	(mg / Kg)	22	(13 to 25)
Vitamin A	(UI / Kg)	37 000	(10 000 to 40 000)
Vitamin C	(mg / Kg)		
Vitamin D3	(UI / Kg)	1 580	(ca 3 000)
Vitamin E	(mg / Kg)	90	

CONTAMINANTS

BACTERIOLOGY			MYCOTOXINS (µg / Kg)	
Viable organisms	(/g)	≤ 200 (< 100 000)	Aflatoxin	< 1 (< 5)
Moulds and yeasts	(/g)	< 10 (< 1 000)	Mycotoxin global risk	Negative
Total coliforms	(/g)	0 (< 5)	Optional Notes	
Faecal coliforms	(/g)	0 (0)		
Anaerobes S.R.	(/g)	40 (< 100)		
Salmonella	(/25 g)	0 (0)		

VRF1C lot 00314

2000/03/14

HEAVY METALS

Lead - Pb	(µg / Kg)	20	(< 1 500)
Mercury - Hg	(µg / Kg)	51	(< 100)
Arsenic - Ar	(µg / Kg)	20	(< 1 000)
Cadmium - Cd	(µg / Kg)	10	(< 250)
Selenium	(µg / Kg)	80	(< 600)

PESTICIDES ORGANOS-CHLORINE (µg / Kg)

Lindane	< 1	(< 100)
a HCH	< 1	(< 20)
b HCH	< 5	(< 10)
d HCH	< 5	(< 100)
HCB	< 1	(< 10)
PCB	< 50	(< 50)
Aldrin	< 1	(< 10)
Dieldrin	< 1	(< 20)
Endosulfan	< 1	(< 100)

PESTICIDES ORGANOS-PHOSPHORUS (µg / Kg)

Acéphate	< 500	(< 5 000)
Azinphos ethyl	< 30	(< 5 000)
Azinphos methyl	< 30	(< 5 000)
Bromophos ethyl	< 10	(< 5 000)
Bromophos methyl	< 20	(< 5 000)
Carbophenothion ethyl	< 50	(< 5 000)
Carbophenothion methyl	< 20	(< 5 000)
Chlorfenvinphos	< 10	(< 5 000)
Chloromphos	< 10	(< 5 000)
Chlorpyrifos ethyl	< 15	(< 5 000)
Chlorpyrifos methyl	< 15	(< 5 000)
Chlorthalckos	< 15	(< 5 000)
Diazinon	< 15	(< 5 000)
Dichlofenthion	< 10	(< 5 000)
Dichlorvos	< 20	(< 5 000)
Diethion	< 10	(< 5 000)
Dimefox	< 20	(< 5 000)
Disethoate	< 30	(< 5 000)
Dioxathion	< 15	(< 5 000)
Disulfoton	< 30	(< 5 000)
Ethoprophos	< 20	(< 5 000)
Fenchlorphos	< 20	(< 5 000)
Fenitrothion	< 15	(< 5 000)
Fenthion	< 30	(< 5 000)
Esofos	< 20	(< 5 000)
Formothion	< 20	(< 5 000)
Heptenophos	< 30	(< 5 000)

SYNTHETIC ESTERESTERMOIDS (µg / Kg)

ND

NITROGEN DERIVATIVES

NO2	(µg / Kg)	0.5	
NO3	(µg / Kg)	14.2	(< 500)
NDMA	(µg / Kg)	1.3	(< 10)
NDEA	(µg / Kg)	< 0.2	(< 10)
NDPA	(µg / Kg)	< 0.3	(< 10)
NDMA	(µg / Kg)	< 0.3	(< 10)
NFIP	(µg / Kg)	< 0.3	(< 10)
NFIR	(µg / Kg)	< 0.5	(< 10)
NDSH	(µg / Kg)	< 0.6	(< 10)

(Total < 200)

Heptachlor	< 1	
Heptachlor Epoxide	< 1	(< 10)
Endrin	< 1	(< 10)
o,p'-DDD	< 5	
p,p'-DDD	< 5	
o,p'-DDE	< 1	
p,p'-DDE	< 1	(< 50)
o,p'-DDT	< 5	
p,p'-DDT	< 5	

(Total < 7 000)

Iodoxyphos	< 25	(< 5 000)
Malathion	65	(< 5 000)
Methamidophos	< 15	(< 5 000)
Methidathion	< 25	(< 5 000)
Mevinphos	< 10	(< 5 000)
Monocrotophos	< 90	(< 5 000)
Naled	< 15	(< 5 000)
Oxydemeton methyl	< 400	(< 5 000)
Parathion ethyl	< 20	(< 5 000)
Parathion methyl	< 20	(< 5 000)
Phosalone	< 50	(< 5 000)
Phosmet	< 50	(< 5 000)
Phosphamidon	< 25	(< 5 000)
Profenofos	< 50	(< 5 000)
Prothoate	< 20	(< 5 000)
Pyridaphenthion	< 15	(< 5 000)
Pyrimiphos ethyl	< 20	(< 5 000)
Pyrimiphos methyl	< 15	(< 5 000)
Sulfotep	< 20	(< 5 000)
Temphos	< 15	(< 5 000)
Tetrachlorvinphos	< 30	(< 5 000)
Thiomethon	< 40	(< 5 000)
Triazophos	< 30	(< 5 000)
Trichlorfon	< 10	(< 5 000)
Trichloronate	< 25	(< 5 000)

ND

ND

NOTESLaboratoire Contrôle AQ
Le Responsable

2000/04/05

Le Responsable AQ

ADDENDUM 2

Certificate of Analysis for drinking water

WATER SUPPLY ZONE SYLEHAM
Essex and Suffolk Water : Period 1-JAN-2000 to 31-DEC-2000 incl.

Parameter	U/A & Freq.	No. of samples planned per annum	No. of samples taken in year	PCV	No. of samples contraven- ing PCV		Concentration or value (all samples)		
					Relaxed	ing PCV	Min.	Mean	Max
CONDUCTIVITY	US/cm @20	12	7	>1500	0	0.000	787.000	821.286	855.000
ODOUR (QUAL)	S	12	7		0	0.000	0.000	0.000	0.000
TASTE (QUAL)	S	12	7		0	0.000	0.000	0.000	0.000
ALUMINIUM	S	4	2	>200	0	0.000	< 14.600	< 14.600	< 14.600
COLOUR	U	4	2	>20	0	0.000	1.100	1.415	1.730
IRON	S	4	2	>200	0	0.000	11.000	15.000	19.000
MANGANESE	S	4	2	>50	0	0.000	< 1.200	< 1.250	1.300
AMMONIUM	S	4	2	>0.5	0	0.000	< 0.025	< 0.025	< 0.025
NO2 mg/l	S	4	2	>0.1	0	0.000	< 0.009	< 0.009	< 0.009
NITRATE	S	4	2	>50	0	0.000	1.600	1.650	1.700
ODOUR (QUANT)	S	4	2	>3	0	0.000	0.000	0.000	0.000
HYDROGEN ION (pH)	S	4	2	9.5	0	0.000	7.370	7.375	7.380
DN	S	4	2	>3	0	0.000	0.000	0.000	0.000
TASTE (QUANT)	S	4	2	>25	0	0.000	7.500	9.350	11.200
TEMP	S	4	2	>4	0	0.000	0.110	0.205	0.300
TURBIDITY	S	4	2	>0.1	0	0.000	< 0.009	< 0.009	< 0.009
2.4-DB	S	4	2	>0.1	0	0.000	< 0.012	< 0.012	< 0.012
236-TBA	S	4	2	>0.1	0	0.000	< 0.007	< 0.007	< 0.007
2.4.5-T	S	4	2	>0.1	0	0.000	< 0.010	< 0.010	< 0.010
2.4-D	S	4	2	>0.1	0	0.000	< 0.011	< 0.011	< 0.011
BENTAZONE	S	4	2	>0.1	0	0.000	< 0.010	< 0.010	< 0.010
BROMOXNYL	S	4	2	>0.1	0	0.000	< 0.009	< 0.009	< 0.009
CLOPYRALID	S	4	2	>0.1	0	0.000	< 0.008	< 0.008	< 0.008
DICAMBA	S	4	2	>0.1	0	0.000	< 0.012	< 0.012	< 0.012
DICHLORPROP	S	4	2	>0.1	0	0.000	< 0.008	< 0.008	< 0.008
IOXNYL	S	4	2	>0.1	0	0.000	< 0.009	< 0.009	< 0.009
MCPA	S	4	2	>0.1	0	0.000	< 0.008	< 0.008	< 0.008
MCPB	S	4	2	>0.1	0	0.000	< 0.013	< 0.013	< 0.013
MECOPROP(MCPP)	S	4	2	>0.1	0	0.000	< 0.009	< 0.009	< 0.009
COPPER	S	4	2	>3000	0	0.000	33.000	39.500	46.000
Cu ug/l	S	4	2	>0.1	0	0.000	< 0.010	< 0.010	< 0.010
CHLOROTHALONIL	S	4	2	...	0	0.000	< 0.008	< 0.013	< 0.018
HEXACHLOROBENZENE	S	4	2	>0.1	0	0.000	< 0.010	< 0.010	< 0.010
HEPTACHLOR	S	4	2	>0.1	0	0.000	< 0.017	< 0.017	< 0.017
TECHNAZENE	S	4	2	>0.1	0	0.000	< 0.012	< 0.012	< 0.012
TRIFLURALIN	S	4	2	>0.1	0	0.000	< 0.018	< 0.018	< 0.018
INDENO	S	4	2	>0.2	0	0.000	< 0.003	< 0.003	< 0.003
BENZO-1.1.12-FLUORANTHENE	S	4	2	>0.2	0	0.000	0.001	< 0.001	< 0.002
BENZO-3.4-FLUORANTHENE	S	4	2	>0.2	0	0.000	< 0.003	< 0.003	< 0.003
BENZO-3.4-PYRENE	S	4	2	>10	0	0.000	< 0.600	< 0.600	< 0.600
BENZO-GHI-PERYLENE	S	4	2	>0.2	0	0.000	< 0.003	< 0.003	< 0.003

FLUORANTHENE	ug/l	4	2	>0.2	0	0.000	0.003	0.004	0.004
PAH (TOTAL)	ug/l	4	2	>0.2	0	0.000	0.003	0.004	0.004
LEAD	Pb ug/l	12	7	>50	0	0.000	< 3.100	< 3.100	< 3.100
TETRACHLOROETHENE	ug/l	4	2	>10	0	0.000	< 0.180	< 0.270	< 0.360
TETRACHLOROMETHANE	ug/l	4	2	>3	0	0.000	< 0.050	< 0.090	< 0.130
TRICHLOROETHENE	ug/l	4	2	>30	0	0.000	< 0.250	< 0.400	< 0.550
TRICHLOROMETHANE	ug/l	4	2	>30	0	0.000	< 0.520	< 0.815	< 1.110
BROMODICHLOROMETHANE	ug/l	4	2	>100	0	0.000	4.520	4.530	4.540
BROMOFORM	ug/l	4	2	>100	0	0.000	14.500	14.905	15.310
CHLOROFORM	ug/l	4	2	>100	0	0.000	< 1.180	< 1.440	< 1.700
DIBROMOCHLOROMETHANE	ug/l	4	2	>100	0	0.000	12.400	12.730	13.060
TOTAL HALOFORMS	ug/l	4	2	>100	0	0.000	31.420	33.015	34.610
TOTAL PESTICIDE	ug/l	4	2	>0.5	0	0.000	0.000	0.000	0.000
ATRAZINE	ug/l	4	2	>0.1	0	0.000	< 0.016	< 0.016	< 0.016
FLURICARB	ug/l	4	2	>0.1	0	0.000	< 0.012	< 0.012	< 0.012
PHOMETRYNE	ug/l	4	2	>0.1	0	0.000	< 0.017	< 0.017	< 0.017
PROPACINE	ug/l	4	2	>0.1	0	0.000	< 0.010	< 0.010	< 0.010
SIMAZINE	ug/l	4	2	>0.1	0	0.000	< 0.012	< 0.012	< 0.012
TERBUTHYRIN	ug/l	4	2	>0.1	0	0.000	< 0.030	< 0.030	< 0.030
TERBUTHYLAZINE	ug/l	4	2	>0.1	0	0.000	< 0.015	< 0.015	< 0.015
TRIFLAZINE	ug/l	4	2	>0.1	0	0.000	< 0.019	< 0.019	< 0.019
CHLOROTOLURON	ug/l	4	2	>0.1	0	0.000	< 0.005	< 0.008	< 0.011
DIURON	ug/l	4	2	>0.1	0	0.000	< 0.005	< 0.010	< 0.014
ISOPROTURON	ug/l	4	2	>0.1	0	0.000	< 0.006	< 0.011	< 0.015
LINURON	ug/l	4	2	>0.1	0	0.000	< 0.010	< 0.011	< 0.012
METHYLBENZURON (MBT)	ug/l	4	2	>0.1	0	0.000	< 0.003	< 0.008	< 0.013
MONURON	ug/l	4	2	>0.1	0	0.000	< 0.005	< 0.008	< 0.011
TERBUTHIURON	ug/l	4	2	>0.1	0	0.000	< 0.009	< 0.011	< 0.013
ZINC	Zn ug/l	4	2	>5000	0	0.000	10.000	167.000	324.000
SILVER	Ag ug/l	1	1	>10	0	0.000	< 0.860	< 0.860	< 0.860
ALKALINITY	HCO3 mg/l	1	1	<30	0	0.000	381.000	381.000	381.000
ARSENIC	As ug/l	1	1	>50	0	0.000	< 0.400	< 0.400	< 0.400
BORON	B ug/l	1	1	>2000	0	0.000	50.000	50.000	50.000
BARIUM	Ba ug/l	1	1	>1000	0	0.000	43.000	43.000	43.000
CALCIUM	Ca mg/l	1	1	>250	0	0.000	138.000	138.000	138.000
CADMIUM	Cd ug/l	1	1	>5	0	0.000	< 0.340	< 0.340	< 0.340
CHLORIDE	Cl mg/l	1	1	>400	0	0.000	57.000	57.000	57.000
CYANIDE	CN ug/l	1	1	>50	0	0.000	< 5.000	< 5.000	< 5.000
CHROMIUM	Cr ug/l	1	1	>50	0	0.000	< 1.600	< 1.600	< 1.600
FLUORIDE	F ug/l	1	1	>1500	0	0.000	292.000	292.000	292.000
MERCURY	Hg ug/l	1	1	>1	0	0.000	< 0.004	< 0.004	< 0.004
POTASSIUM	K mg/l	1	1	>12	0	0.000	4.440	4.440	4.440
MAGNESIUM	Mg mg/l	1	1	>50	0	0.000	10.300	10.300	10.300
SODIUM	Na mg/l	1	1	>150	0	0.000	39.200	39.200	39.200
NICKEL	Ni ug/l	1	1	>50	0	0.000	< 2.800	< 2.800	< 2.800
PHOSPHOROUS	P ug/l	1	1	>2200	0	0.000	17.000	17.000	17.000
OXIDISABILITY	O2 mg/l	1	1	>5	0	0.000	0.710	0.710	0.710
ANTIMONY	Sb ug/l	1	1	>10	0	0.000	< 0.400	< 0.400	< 0.400
SELENIUM	Se ug/l	1	1	>10	0	0.000	< 1.000	< 1.000	< 1.000
SULPHATE	SO4 mg/l	1	1	>250	0	0.000	101.200	101.200	101.200

SURFACTANT	ug/l	S	1	1	>200	0	0.000	< 8.000	< 8.000	< 8.000
TOTAL ORGANIC CARBON	C mg/l	S	1	1		0	0.000	2.510	2.510	< 8.000
TOTAL HARDNESS	Ca mg/l	S	1	1	<60	0	0.000	164.000	164.000	164.000
COLONY COUNT AT 22	/ml	S	24	13		0	0.000	0.000	4.231	20.000
COLONY COUNT AT 37	/ml	S	24	13		0	0.000	0.000	0.769	7.000
TOTAL CHLORINE	mg/l	S	24	13		0	0.000	0.150	0.206	0.330
FAECAL COLIFORMS	/100ml	S	24	13	>0.1	0	0.000	0.000	0.000	0.000
TOTAL COLIFORMS	/100ml	S	24	13	>0.1	0	0.000	0.000	0.000	0.000

ADDENDUM 3

Certificate of Analysis for Dimethoate



Cheminova Agro A/S
P.O. Box 9
DK-7620 Lemvig
Denmark

Phone (+45) 97834100
Fax (+45) 97834585
Telex 86514 CHEMV DK
A/S reg.no. 177.122

BATCH ANALYTICAL CERTIFICATE

CONFIDENTIAL
PROPERTY OF
CHEMINOVA
DK-7620 LEMVIG
DENMARK

ARTICLE IDENTIFICATION					
Article Name: Dimethoate		Reg. Denmark Code: <input type="checkbox"/>			
Manufacturer: Cheminova Agro A/S		Batch No.: 20522-00			
Origin of Production: Commercial <input checked="" type="checkbox"/> ; Pilot plant <input type="checkbox"/> ; Laboratory <input type="checkbox"/> ;					
PHYSICAL PROPERTIES					
Technical <input checked="" type="checkbox"/> ; Preparation of technical Product <input type="checkbox"/> ; Analytical <input type="checkbox"/> ; Liquid <input type="checkbox"/> ; Solid <input checked="" type="checkbox"/> ; Colour: White					
Recommended Storage Conditions					
Ambient temperature in the dark _____		Expiry Date:			
In refrigerator <input type="checkbox"/>		The article is stable at least <u>5</u> years from date			
In deep freezer <input checked="" type="checkbox"/>		of analysis/last date of reanalysis when stored at			
Additional Comments: recommended conditions.					
ACTIVE INGREDIENT IDENTIFICATION					
Common Name/ISO-Name: Dimethoate		CAS-Name: Phosphorodithioic acid, 0,0-dimethyl S-[2-(methyl-amino)-2-oxoethyl] ester			
CAS No.: 60-51-5					
Empirical Formula: C ₅ H ₁₂ NO ₃ PS ₂		Structural Formula:			
Molecular Weight: 229.3		$\begin{array}{c} \text{CH}_3\text{-O} \\ \text{CH}_3\text{-O} \end{array} \text{P} \begin{array}{c} \text{S} \\ \text{S} \end{array} \text{S-C} \begin{array}{c} \text{O} \\ \parallel \end{array} \text{C-N-CH}_3 \\ \text{H}_2 \quad \text{H} \end{array}$			
Identified by means of:					
NMR <input checked="" type="checkbox"/> ; IR <input checked="" type="checkbox"/> ; UV <input checked="" type="checkbox"/> ; MS <input checked="" type="checkbox"/> ; Other Methods:					
ANALYTICAL DATA					
Certified Purity/Content of a.i.: 99.1% w/w					
Analytical Method: VAM 009-01					
Analytical Report (incl. amendments): PR 020					
Date of analysis/ reanalysis (yy-mm-dd)	920603	950518	980513		
-for article stored at -	Cheminova Agro A/S	Cheminova Agro A/S	Cheminova Agro A/S		
GLP-COMPLIANCE					
The identification and determination of purity/content of active ingredient were performed at Cheminova Agro A/S and conducted in accordance with FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 and the OECD Principles of Good Laboratory Practices. All raw data, documentation, records, protocols, test articles, reference samples, and report are retained in the GLP archives of Cheminova Agro A/S, Denmark.					
Date: May 26, 1998		Signature: <u>Elsa V. Sørensen</u> Elsa V. Sørensen			



Cheminova Agro A/S
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A/S reg.no. 177.122

BATCH ANALYTICAL CERTIFICATE
TEST/REFERENCE ARTICLE

CONFIDENTIAL
PROPERTY OF
CHEMINOVA
DK-7620 LEMVIG
DENMARK

Addendum				
Article Name: Dimethoate.				
Batch No.: 20522-00				
ANALYTICAL DATA				
Reference, Analytical Report: PR 020				
Impurities:				
CAS No.	Compound CAS name/Cheminova name	% by weight	Analytical method	Day of analysis
2953-29-9	Phosphorodithioic acid O,O,S-trimethyl ester/ MeOOSPS triester	0.26	VAM 011-01	June 2, 1992
757-86-6	Acetic acid, [(dimethoxyphos- phinothioyl)thio]-methyl ester/ MPEM	0.32	VAM 011-01	June 2, 1992
1113-02-6	Phosphorothioic acid, O,O-di- methyl S-(2-(methylamino)-2- oxoethyl) ester/ Omethoate	<0.02	VAM 011-01	June 2, 1992 and May 13, 1998
3344-11-4	Phosphorodithioic acid, O,S-di- methyl S-(2-(methylamino)-2- oxoethyl) ester/ Isodimethoate	0.04	VAM 011-01	June 2, 1992 and May 13, 1998
152-18-1	Phosphorothioic acid O,O,O-trimethyl ester/ MeOOOPS triester	<0.02	VAM 011-01	June 2, 1992
44988-12-7 (ion)	Phosphorodithioic acid S-methyl S-(2-(methylamino)-2-oxoethyl ester/ Des-O-methyl-isodimethoate	0.02	VAM 012-01	June 4, 1992
60823-19-0	Phosphorodithioic acid, S-(2-(dimethylamino)-2-oxoethyl) O,O-dimethyl ester/ N-Me-Dimethoate	0.02	VAM 012-01	June 4, 1992
1113-01-5	Acetic acid [(dimethoxy-phos- phinothioyl)thio]-/ MP-1-acetic acid	0.02	VAM 012-01	June 4, 1992
2700-77-8	Phosphorodithioic acid, O-methyl S-(2-(methylamino)- 2-oxoethyl) ester/ Des-methyl-Dimethoate	0.05	VAM 012-01	June 4, 1992
7732-18-5	Water	0.11	VAM 013-01	June 1, 1992
GLP - COMPLIANCE				
The identification and determination of the impurities were performed at Cheminova Agro A/S and conducted in accordance with FIFRA Good Laboratory Practice Standards, 40 CFR Part 160 and the OECD Principles of Good Laboratory Practices. All raw data, documentation, records, protocols, test articles, reference samples, and report are retained in the GLP archives of Cheminova Agro A/S, Denmark.				
Date: May 26, 1998		Signature: <u>Elsa V. Sørensen</u> Elsa V. Sørensen		

ADDENDUM 4

Solution Chemistry Report

DIMETHOATE
SOLUTION CHEMISTRY

Authors:

I. Suzanne Dawe,
Emma Mann.

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INTRODUCTION

This report describes the analytical and sampling procedures used and details the results obtained for the concentrations of dimethoate in test solutions analysed during the study.

The solutions for this study were prepared as solutions of dimethoate in water (Reverse osmosis) by Pharmacy personnel at Huntingdon Life Sciences Ltd.

The samples were analysed with reference to a method established at Huntingdon Life Sciences (HRC/FCH/M13/00 issue 01/100500). The method of analysis for dimethoate in aqueous solution involved dilution using water and injection of the diluted test samples onto a high performance liquid chromatograph (HPLC) with ultra-violet detection. The amount of dimethoate in the test samples was quantified by external calibration with reference to five linearly related standards of known concentrations.

The stability was confirmed following storage at ambient temperature for 2 days and under refrigeration for 15 days during an earlier study. The experimental conditions and analytical results obtained were presented in Huntingdon Life Sciences report number CHV 069/003881.

EXPERIMENTAL PROCEDURE

ANALYTICAL PROCEDURE

Apparatus and instrumentation

High performance liquid chromatograph (HPLC):

As detailed in the chromatographic section.

Balances:

Mettler AT261, fitted with an LC-P45 printer.
Sartorius L2200P, fitted with a YDP series printer.

Autodiluter:

Hamilton Ltd, Microlab 1000.

Ultrasonic bath:

Decon FS300.

General laboratory equipment and glassware.

Reagents

Test substance:

dimethoate.

Supplier:

Cheminova Agro A/S.

Technical standard:

Batch 20522-00, stated purity 99.1%

Analytical standard:

Batch 291-BSe-75B, stated purity 99.5%

Control vehicle:

Water, deionised reverse osmosis.

Methanol:

Fisher Scientific UK Ltd, HPLC grade.

Sodium dihydrogen orthophosphate, di-hydrate:

Fisher Scientific UK Ltd, Analytical Reagent.

Phosphate buffer:

Sodium dihydrogen orthophosphate, di-hydrate (5 g)
dissolved in sufficient water to provide 1 litre.

Water:

Elgastat UHP-4, deionised reverse osmosis.

Mobile phase:

Methanol / phosphate buffer (350/650 v/v).

Sample process

A representative sample of test solution was diluted using water to provide a solution containing dimethoate at an expected concentration in the range 20 µg/ml - 40 µg/ml.

The concentration of dimethoate in the final solution was quantified by high performance liquid chromatography using ultraviolet detection as detailed in the following section.

Typical chromatographic conditions**High performance liquid chromatograph (HPLC):**

Pump:	Perkin Elmer LC200.
Autosampler:	Waters Associates WISP 712.
Detector:	Applied Biosystems 785A.
Data handling:	Perkin Elmer Nelson Turbochrom.

Analytical column:	Luna C8(2), 3 μ m, 50 \times 3 mm id, Phenomenex LUNA TM .
--------------------	--------------------------------------------------------------------------------

Guard column:	Inline filter, Upchurch Ltd.
---------------	---------------------------------

Column temperature:	Ambient, nominally 21°C.
---------------------	--------------------------

Mobile phase:	Methanol / phosphate buffer (35/65 v/v).
---------------	------------------------------------------

Flow rate:	0.4 ml/minute.
------------	----------------

Detector wavelength:	UV, 210 nm.
----------------------	-------------

Injection volume:	5 μ l.
-------------------	------------

Integration sensitivity range:	64 mV, 4 mV baseline – 68 mV full scale.
--------------------------------	------------------------------------------

Approximate retention time / volume:	3.1 minutes / 1.2 ml.
--------------------------------------	-----------------------

Calibration

A primary standard solution was prepared for each analytical occasion by dissolving an accurately weighed quantity (*ca* 20 mg) of dimethoate analytical standard in water (100 ml). Solutions for instrument calibration, containing dimethoate at concentrations of 10 μ g/ml, 20 μ g/ml, 30 μ g/ml, 40 μ g/ml and 50 μ g/ml, were prepared by appropriate dilution of the primary standard using water.

Calibration solutions were injected onto the HPLC in order of increasing concentration, at the beginning and end of each sample analysis sequence, using the conditions detailed in the previous section.

Calculation

The peak area response for dimethoate in each calibration chromatogram was measured and a calibration curve was constructed by linear regression of standard response versus standard concentration. The area response of the peak observed at the characteristic retention volume for dimethoate in sample chromatograms was measured and the concentration of dimethoate was determined using the following equation:

$$\text{Analysed concentration, mg/ml} = \frac{Y-I}{S} \times V \times 10^{-3}$$

Where Y = Peak area response for dimethoate in test chromatogram
 I = Intercept derived from linear regression of calibration data
 S = Slope derived from linear regression of calibration data
 V = Dilution factor of sample

CONCENTRATIONS IN TEST SOLUTIONS

At specified intervals (Week 1 and 4) during the study, freshly prepared test solutions were sampled (20 ml) by Pharmacy personnel at the Eye Research Centre and submitted for analysis. Duplicate samples of each solution were analysed in accordance with the analytical procedure.

RESULTS

The concentrations of dimethoate in test solutions analysed during the study and the deviation of mean results from nominal values are detailed in Table 1.

A typical calibration standard graph is presented in Figure 1. Typical analytical chromatograms are presented in Figure 2.

DISCUSSION

The mean concentrations of dimethoate in test solutions analysed during the study were between 1.7% below and 0.7% above nominal values which were within normal limits ($\pm 10\%$), confirming accurate preparation. The precision of duplicate analyses was $\leq 2.0\%$.

The specificity of the HPLC assay was demonstrated by the absence of a peak at the characteristic retention volume for dimethoate in the control sample chromatogram.

CONCLUSION

The mean concentrations for dimethoate in test solutions analysed during the study were within $\pm 2\%$ of nominal values, confirming accurate formulation.

TABLE 1

Concentrations of dimethoate in test solutions

Week of treatment	Group	Nominal inclusion (mg/ml)	Analysed concentration (mg/ml)			RME (%)
			Analysis 1	Analysis 2	Mean	
1	Control, 1, 6	0	ND	ND	ND	-
	2, 7	0.02	0.0196	0.0199	0.0198	-1.0
	3, 8	0.1	0.0993	0.0999	0.0996	-0.4
	4, 9	0.6	0.598	0.610	0.604	+0.7
4	Control, 1	0	ND	ND	ND	-
	2	0.02	0.0197	0.0205	0.0201	+0.5
	3	0.1	0.0985	0.0981	0.0983	-1.7
	4	0.6	0.596	0.595	0.595	-0.8

ND None detected (<0.0015 mg/ml, the value for the limit of detection).

Analysed concentrations were calculated using unrounded figures.

RME Relative Mean error

FIGURE 1

Typical calibration standard graph (Week 1)

Fit Analysis Output For Method File: \\HUNNT3\DATATC\FormulationChemistry\PostVal\Chv070\001\Dimeth_Liq_A.mth
Component Name : Dimethoate
Date : 12/02/01 09:37:08

Page 1 of 1

Curve Parameters:

Curve #1 : 1st Order
Weighting Factor = 1 (No Weighting) R-Squared = 0.999874
Calibration Curve : $Y = (-563.025000) + (12593.299949) X$

Curve #1 : Level Name	Observed X-Value	Calculated X-Value	Delta	%Diff.	Observed Y-Value	Calculated Y-Value	Delta	%Diff.
STD 1	9.735000	9.818199	-0.083199	-0.847	123080.500000	122032.750	1047.750	0.859
STD 1A	9.735000	9.820303	-0.085303	-0.869	123107.000000	122032.750	1074.250	0.880
STD 2	19.470000	19.230109	0.239891	1.247	241607.500000	244628.525	-3021.025	-1.235
STD 2A	19.470000	19.558339	-0.088339	-0.452	245741.000000	244628.525	1112.475	0.455
STD 3	29.205000	29.253732	-0.048732	-0.167	367838.000000	367224.300	613.700	0.167
STD 3A	29.205000	28.899893	0.305107	1.056	363382.000000	367224.300	-3842.300	-1.046
STD 4	38.940000	39.177501	-0.237501	-0.606	492811.000000	489820.075	2990.925	0.611
STD 4A	38.940000	38.995897	-0.055897	-0.143	490524.000000	489820.075	703.925	0.144
STD 5	48.675000	48.689305	-0.014305	-0.029	612596.000000	612415.850	180.150	0.029
STD 5A	48.675000	48.606722	0.068278	0.140	611556.000000	612415.850	-859.850	-0.140

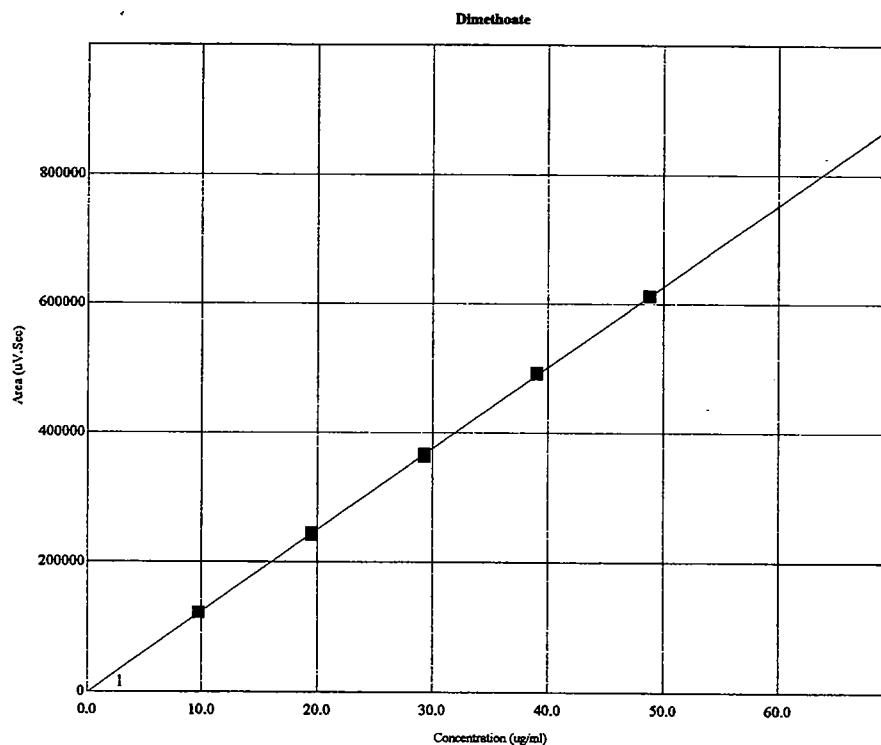
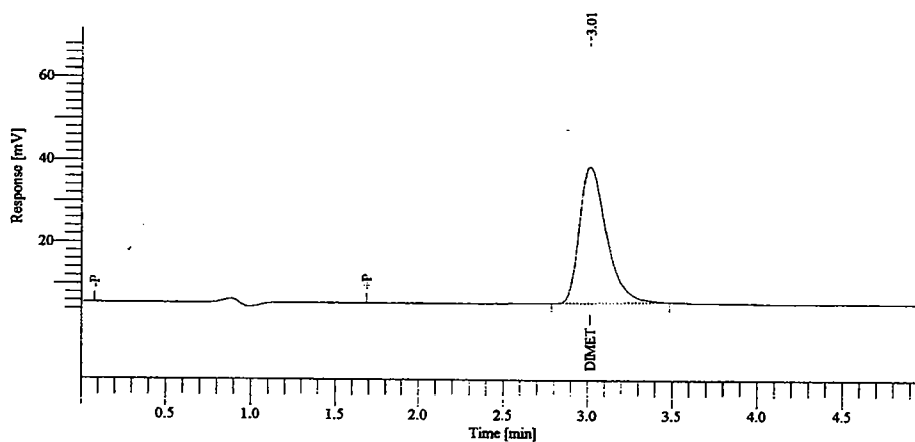


FIGURE 2

Typical analytical chromatograms - Week 1

Calibration standard, 30 µg/ml

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 Sequence File : \\HUNNT3\DATATC\FormulationChemistry\PostVal\Chv070\001\CHV070_WK1_Aseq



Groups 1 and 6, Control (Dilution factor 1)

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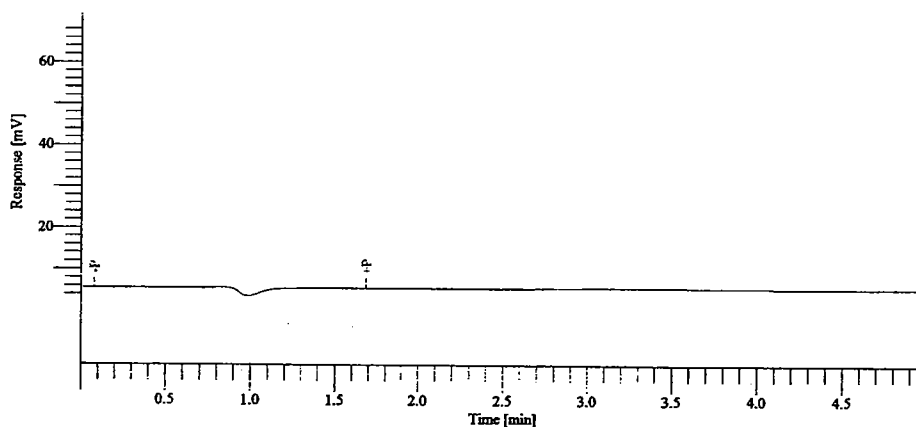
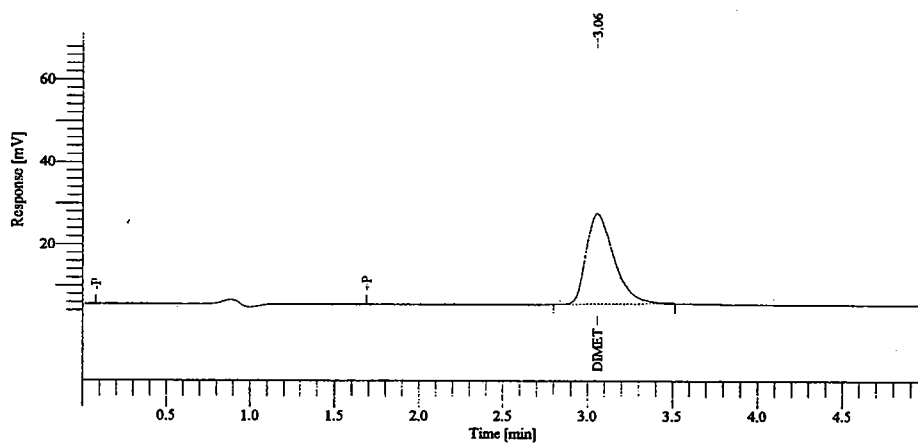


FIGURE 2

Typical analytical chromatograms – Week 1 (continued)

Groups 2 and 7, 0.02 mg/ml (Dilution factor 1)

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 Sequence File : \\HUNNT3\\DATATC\\FormulationChemistry\\PostVal\\Chv070\\001\\CHV070_WK1_A.seq



Groups 3 and 8, 0.1 mg/ml (Dilution factor 1:2.5)

Raw Data File : \\HUNNT3\\DATATC\\FormulationChemistry\\PostVal\\Chv070\\001\\Raw\\S15A18072000_011.raw
 Result File : \\HUNNT3\\DATATC\\FormulationChemistry\\PostVal\\Chv070\\001\\S15A18072000_011.rst
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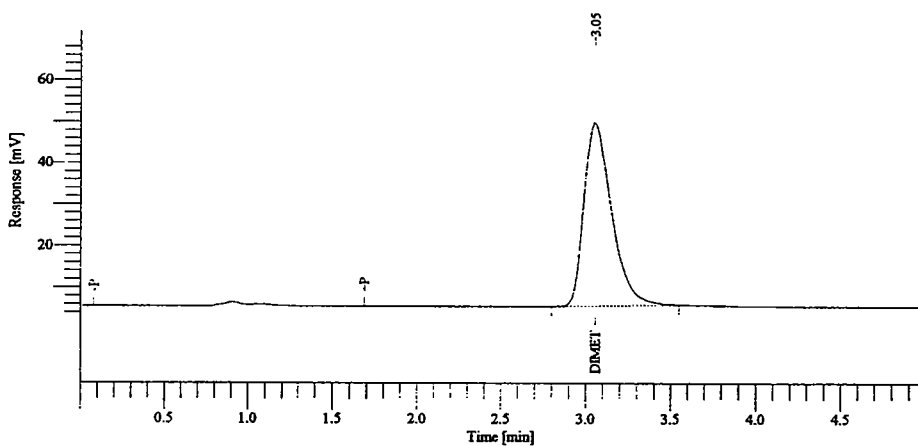
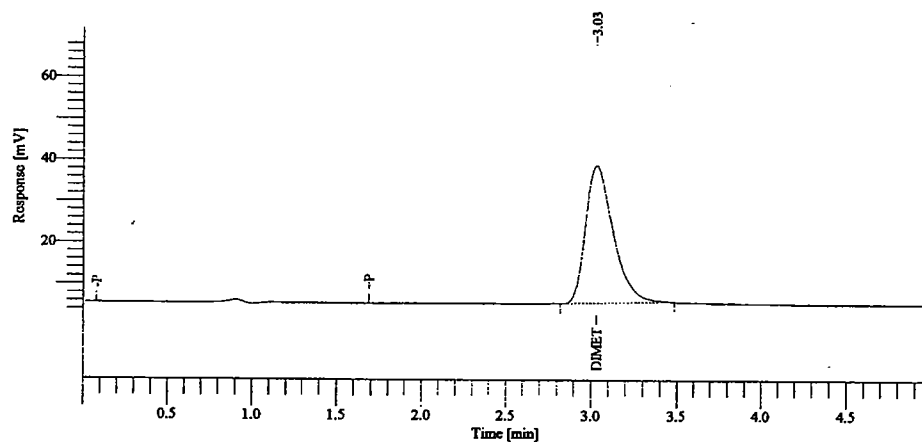


FIGURE 2

Typical analytical chromatograms – Week 1
(continued)

Groups 4 and 9, 0.6 mg/ml (Dilution factor 1:20)

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Sequence File : \HUNNT3\DATATC\FormulationChemistry\PostVal\Chv070\001\CHV070_WK1_A.seq



ADDENDUM 5

Pathology Report

CHV/070
DIMETHOATE
EFFECTS ON CHOLINESTERASE IN THE CD RAT (ADULT AND JUVENILE) BY
ORAL GAVAGE ADMINISTRATION

CHV/070/Dimethoate/CD Rat

16 February 2001

Histology and Histopathology of day 61 offspring

Five offspring were killed on day 61 of age and the tissues fixed by *in situ* gravity perfusion.

Sectioning of the brains of these animals was performed using suitable landmarks on the ventral surface of the brain. A standard set of landmarks was identified which will allow consistent and comparable sectioning of brains from rats at approximately day 61 of age. The sections produced allow for examination of all major brain regions, and produce consistent sections through those areas of the brain used for morphometric analysis.

Epoxy resin sections of the peripheral nerves of these animals were also prepared for evaluation. A comparison was made of the presentation of sciatic nerves which had been stretched on card at necropsy with those which had not been stretched.

Examination of the sections produced showed there to be excellent fixation of the tissues with a low level of fixation artefact.

Histopathological examination revealed no significant lesions in the brains or peripheral nerves of the five animals.

Ian Taylor
Study Pathologist

Pathologist's Comments

Generally the perfusion of the animals was excellent. In one animal however, blood was present in the capillaries and vessels of the brain sections, indicating inadequate flushing of the animal prior to perfusion of fixative. Another of the animals showed occasional vessels with blood present. There was no noticeable difference in the quality of the fixation of the material for these animals however, and it was not considered to have adversely affected the evaluation of the tissues.

Comparison of the sciatic nerves which had been stretched on card with those which had not been stretched did not show any consistent differences in the presentation of the nerves.

Study Number : CHV/070

**Huntingdon
Life Sciences**

**PROTOCOL
DIMETHOATE
EFFECTS ON CHOLINESTERASE
IN THE CD RAT (ADULT AND JUVENILE) BY ORAL
GAVAGE ADMINISTRATION**

Sponsor

Cheminova A/S
P.O. Box 9
DK-7620 Lemvig
DENMARK

Research Laboratory

Huntingdon Life Sciences Ltd
Woolley Road
Alconbury
Huntingdon
Cambridgeshire
PE28 4HS
ENGLAND

Total number of pages: 23

Final Protocol

Page *i*

Huntingdon Life Sciences Ltd, registered in England: 1815730

Study Number : CHV/070

**Huntingdon
Life Sciences**

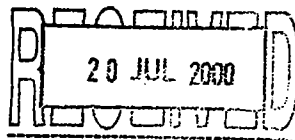
CONTACT DETAILS

Study Monitoring : D. Søndergaard
Cheminova A/S
Lemvig
DENMARK

Final Protocol

Page ii

Study Number : CHV/070

**Huntingdon
Life Sciences****PROTOCOL APPROVAL****DIMETHOATE****EFFECTS ON CHOLINESTERASE****IN THE CD RAT (ADULT AND JUVENILE) BY ORAL****GAVAGE ADMINISTRATION**

D.P. Myers, B.Sc., Ph.D., IDT.
Study Director,
Huntingdon Life Sciences Ltd.

10 July 2000
Date

The signature of the Study Director confirms this protocol as the working document for the study. Any changes made subsequent to the date of the Study Director's signature will be documented in formal amendments.

P. Aughton, B.Sc., D.A.B.T., Dip.R.C.Path., C.Biol., M.I.Biol.
Management,
Huntingdon Life Sciences Ltd.

10 July 2000
Date

D. Søndergaard
Sponsor,
Cheminova Agro A/S

17 July 2000
Date

Please sign both copies of this page, retain one for your records and return one to the Study Director at Huntingdon Life Sciences.

Final Protocol

Page iii

Study Number : CHV/070

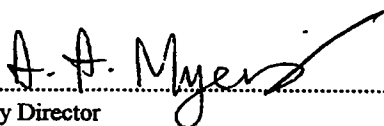
**Huntingdon
Life Sciences**

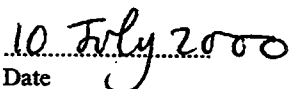
**DIMETHOATE
EFFECTS ON CHOLINESTERASE
IN THE CD RAT (ADULT AND JUVENILE) BY ORAL
GAVAGE ADMINISTRATION**

Enquiry Number: 19579D

Number of pages for internal distribution: 20

This working document is approved for circulation and use:


Study Director


Date

Primary location of study

Eye Research Centre
Eye
Suffolk
IP23 7PX

Building Number: 30

All procedures to be performed at the above site unless otherwise detailed below:

Location of specific tasks

Measurement of cholinesterase activity and Quality control of dosage form. : Huntingdon Research Centre, Huntingdon, Cambridgeshire, PE28 4HS.

Final Protocol

Page 1

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Huntingdon Life Sciences

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Study Number : CHV/070

Huntingdon Life Sciences

1. INTRODUCTION

Management of study

Study Director : D.P. Myers
Monitoring Toxicologist : K.P. Hazelden

In the temporary absence of the Study Director, the scientific responsibilities will be taken over by the Monitoring Toxicologist; other items of routine study management should be referred to the following person in the first instance. : S.M. Fulcher

Objective

The objective of this study is two-fold: first, to assess the effect of acute or prolonged dosing of adult or young pre-weanling rats with Dimethoate on erythrocyte, blood and brain acetyl cholinesterase activity. Second, to evaluate recovery of acetyl cholinesterase activity 39 days after the end of dosing young rats.

Good Laboratory Practice

The study will be conducted in compliance with principles of Good Laboratory Practice Standards as set forth in:

The UK Good Laboratory Practice Regulations 1999 (Statutory Instrument No 3106).

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17.

EC Commission Directive 1999/11/EC of 8 March 1999 (Official Journal No L 77/8).

Animals (Scientific Procedures) Act 1986 compliance

The in-life experimental procedures to be undertaken during the course of this study are subject to the provisions of the United Kingdom Animals (Scientific Procedures) Act 1986 (the Act). The Act, administered by the UK Home Office, regulates all scientific procedures in living animals which may cause pain, suffering, distress or lasting harm and provides for the designation of establishments where procedures may be undertaken, the licensing of trained individuals who perform the practical techniques and the issue of project licences for specified programmes of work.

This study will comply with all applicable sections of the Act and the associated Codes of Practice for the Housing and Care of Animals used in Scientific Procedures and the Humane Killing of Animals under Schedule 1 to the Act, issued under section 21 of the Act.

Animal model : CD rat, requirement for a rodent species by regulatory agencies, used extensively in these laboratories.

Route : Oral by gavage.

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Huntingdon Life Sciences

2. STUDY SCHEDULE AND STRUCTURE

2.1. Identity of treatment groups and duration of treatment

Group	Treatment (mg/kg/day)	Level (mg/kg/day)	Number of animal/sex	Animal numbers
1	Control		19F	1-9 A 10-19 B
2			19F	20-28 A 29-38 B
3			19F	39-47 A 48-57 B
4			19F	58-66 A 67-76 B
5	Untreated		8F	77-84
6	Control		16F 16M	85-92 C, 93-100 D 201-208 C, 209-216 D
7			16F 16M	101-108 C, 109-116 D 217-224 C, 225-232 D
8			16F 16M	117-124 C, 125-132 D 233-240 C, 241-248 D
9			16F 16M	133-140 C, 141-148 D 249-256 C, 257-264 D

F females A treated from Day 6 to Day 20 after mating inclusive.
M males B treated from Day 6 after mating to Day 10 of lactation.
 C treated for one day only
 D treated for up to 11 consecutive days

For groups 1-4 inclusive: Nine adult females will be dosed from Day 6 to Day 20 after mating inclusive. Ten adult females will be treated from Day 6 after mating to Day 10 of lactation. Offspring from 8 of these ten litters per group will be treated from Day 11 to Day 21 of age inclusive.

Group 5: These adult females will be undosed throughout the study. On Day 11 of age, one male and one female offspring per litter will be assigned to each of the following dosing groups for treatment on Day 11 of age: Control group

X mg/kg/day
Y mg/kg/day
Z mg/kg/day

Groups 6-9 inclusive: Eight adult male and female rats will be dosed for one day. The remaining animals in each group will be dosed for 11 consecutive days.

Study Number : CHV/070

**Huntingdon
Life Sciences****2.2. Scheduled time plan**

(to be up-dated as required in an amendment to protocol)

Sample of Dimethoate arrived	:	8 May 2000
Pairing to commence	:	10 July 2000
Draft report to be issued	:	February 2001 (estimated)

3. TEST SUBSTANCE AND FORMULATION

In order for Huntingdon Life Sciences to comply with the Health and Safety at Work etc. Act 1974, and the Control of Substances Hazardous to Health Regulations 1994, it is a condition of undertaking the study that the Sponsor shall provide Huntingdon Life Sciences with all information available to it regarding known or potential hazards associated with the handling and use of any substance supplied by the Sponsor to Huntingdon Life Sciences. The Sponsor shall also comply with all current legislation and regulations concerning shipment of substances by road, rail, sea or air.

Such information in the form of a completed Huntingdon Life Sciences test substance data sheet must be received by Safety Management Services at Huntingdon Life Sciences before the test substance can be handled in the laboratory. At the discretion of Safety Management Services at Huntingdon Life Sciences, other documentation containing the equivalent information may be acceptable.

Information received will be used to set the Huntingdon Life Sciences Hazard Class, which determines safety precautions taken in the workplace.

Huntingdon Life Sciences Hazard Class:

2

Study Number : CHV/070

Huntingdon Life Sciences

3.1. Test substance

- Sponsor's identification : Dimethoate
- Storage conditions : Frozen at approximately -20°C, (Analytical standard also to be stored frozen , at approximately -20°C).
- Sponsor's responsibilities : Documentation of methods of synthesis, fabrication or derivation.
Stability data.
Certificate of analysis.
- Certificate of analysis details : Test substance identity.
Batch number.
Purity.
Composition.
Other appropriate characteristics.
Current expiry date.

3.2. Formulation

- Treatment
- Control : Vehicle.
- Test group : Dimethoate; low mg/ml.
- Test group : Dimethoate; intermediate mg/ml.
- Test group : Dimethoate; high mg/ml.
- Conversion factor : The test substance will be used as supplied, unless otherwise advised by the Sponsor. Any such requirement will be documented in an amendment to protocol.
- Vehicle : Water for formulation.
- Method of preparation : Will be documented in the study data and included in the final report.
- Frequency of preparation : Will depend upon the availability of supporting stability data. Where sufficient stability data is available, batches will cover one week of dosing and may be prepared up to three days in advance of the first day of dosing. Where stability data does not support a period of use of this length, a more frequent mixing regime will be initiated.

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3.3. Quality control of dosage form

Liquid formulation : Before commencement of treatment, the suitability of the proposed mixing procedures will be determined and specimen formulations will be analysed to assess the stability of the test substance in the liquid matrix.

At specified intervals during treatment, the test formulations will be analysed for achieved concentration of the test substance.

Analysis : The formulated samples will be analysed using a method validated with respect to the determination of the specificity of analysis, limits of quantitation and/or detection, linearity of detector response, reproducibility, method accuracy and precision.

Determined by Huntingdon Life Sciences as part of the main developmental neurotoxicity study.

Achieved concentration

Sampling and determination : On two occasions. Formulations prepared for use during the first week of treatment and again during the first week of lactation. Other sampling regimens may be specified by the Sponsor.

Single samples (nominally 20 ml) from all groups; 2 assays for each sample.

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4. ANIMAL MANAGEMENT

4.1. Animals - supply, acclimatisation and allocation

4.1.1. Animals

Species : Rat.

Strain : CrI: CD[®]BR.

Age ordered : Groups 1 to 5:
Females 9-10 weeks of age.
Groups 6 to 9:
Males and females 5-6 weeks of age.

Weight range ordered : Groups 1 to 5:
Females 200-220 g.
Groups 6 to 9:
Males 120-175 g, females 100-150 g.

Supplier : Males and females: Charles River (UK).
Males for mating of Groups 1-5: Stock animals from same source,
retained at Huntingdon Life Sciences.

4.1.2. Acclimatisation

Duration : At least 5 days.

Husbandry conditions : Refer to Section 4.2.

4.1.3. Mating (F₀ generation)

Male/female ratio : 1:1 with stock males.

Daily checks for evidence of mating : Ejaculated copulation plugs in cage tray and vaginal smear.

Day 0 of gestation : When positive evidence of mating detected.

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Huntingdon Life Sciences

4.1.4. Allocation to treatment groups

Females (Groups 1 to 5):

- Allocation : After positive evidence of mating.
- Method : Allocated to group and cage position in sequence of mating.
- Cage distribution : Arrangement designed to minimise environmental variables.
- : Allocation of mated females will be adjusted to avoid any stock male having mated with more than 1 female in each group.
- Allocation of mated females may be adjusted if Day 0 group mean gestation bodyweights differ beyond acceptable limits.

Males and Females (Groups 6-9):

- Allocation : Before the start of study. Grossly atypical animals discarded.
- Method : Divided into bodyweight ranges (5 g blocks), animals taken from each block assigned in rotation to treatment groups.
- Cage distribution : Arrangement designed to minimise environmental variables.

4.1.5. Identification

- Numbering : Unique for each animal within study - from Day 28 of age.
- Method : Tail tattoo (F₀ generation and, selected F₁ generation at 4 weeks of age).
Toe marking (F₁ generation) on Day 1 of age within each litter.
Tail marking with pen (F₁) between Days 21 and 28 of age.
- Cage labels : Uniquely identifying the occupants.

4.1.6. Precommencement animal replacement

Spare animals will be ordered to replace any individuals rejected before the start of treatment.

- Replacement before treatment : Ill-health.
Bodyweight range extremes.
Inadequate mating performance.
- Replacement during treatment : None scheduled.

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4.2. Animals - housing, diet and water supply

4.2.1. Environmental control

- Rodent facility : Full barrier - to minimise entry of external biological and chemical agents.
- Air supply : Filtered, not recirculated.
- Temperature : Maintained within the range of 19-25°C.
- Relative humidity : Maintained within the range of 40-70%.
- Monitored continuously or daily. Excursions outside these ranges documented in the study data.
- Lighting : 12 hours light : 12 hours dark.
- Alarm systems : Activated on ventilation failure and when temperature/humidity limits exceeded.
- Electricity supply : Public supply with automatic stand-by generators.

4.2.2. Animal accommodation

Study period	Number of animals/cage		Cage material	Cage flooring
	Male	Female		
Generation F ₀				
Acclimatisation	Up to 4	Up to 4	Stainless steel	Stainless steel grid
Mating	1 : 1		Polypropylene	Stainless steel grid
Gestation (up to Day 17 after mating)	-	1	Polypropylene	Stainless steel grid
Littering (from Day 17 after mating to Day 14-18 of lactation)	-	1 + litter	Polypropylene	Solid polypropylene
Lactation (Day 14-18 of lactation until weaning)	-	1 + litter	Stainless steel	Stainless steel grid
Generation F ₁				
From weaning	Up to 4	Up to 4	Stainless steel	Stainless steel grid

Grid cages will be suspended above absorbent paper which will be changed at least twice each week or daily during mating: solid bottomed cages with wood flakes bedding changed at least twice each week. Cages, cage-trays, food hoppers and water bottles will be changed at appropriate intervals. Precise details of caging will be included in the final report.

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4.2.3. Bedding, diet and water supply

Copies of all certificates of analysis are stored in the archives. Typical, relevant certificates will be reproduced in the study report.

Bedding supply (for solid bottomed cages)

Bedding name : Lignocel 3/4 wood flakes.
 Certification : Certificates of analysis are routinely received from the supplier.

Diet supply

Diet name : UAR VRF1 Certified.
 Diet type : Pelleted diet.
 Availability : Non-restricted.
 Certification : Before delivery each batch of diet is analysed by the supplier for various nutritional components and chemical and microbiological contaminants.
 Supplier's analytical certificates are scrutinised and approved before any batch of diet is released for use.

This diet contains no added antibiotic or other chemotherapeutic or prophylactic agent.

The certificate of analysis for the batch of diet used on the study will be presented in the final report.

Water supply

Supply : Public drinking water.
 Regulatory agency : U.K. Department of the Environment.
 Availability : Non-restricted via polyethylene or polycarbonate bottles with sipper tubes.
 Certification : Certificates of analysis are routinely received from the supplier and the relevant certificate will be presented in the final report.

4.2.4. Contaminants assay

It is the Sponsor's responsibility to advise Huntingdon Life Sciences of any specific contaminants likely to prejudice the outcome of the study. Analyses for such contaminants may be performed at Huntingdon Life Sciences if requested by the Sponsor.

Study Number : CHV/070

Huntingdon Life Sciences

4.3. Animals - procedures

4.3.1. Administration

Route	:	Oral gavage.
Treated at	:	Constant dosages in mg/kg/day.
Volume dosage	:	5 ml/kg/day.
Individual dose volume - adult males and females	:	Calculated from the most recently recorded scheduled bodyweight up to and including Day 17 after mating (if appropriate); thereafter the dosage volume will remain constant to Day 1 of lactation. From Day 1 of lactation dose volumes will be calculated, once again, from the most recently recorded bodyweight. Animals that are in parturition at the time of dosing will not be dosed. Animals that have completed parturition and can be dosed (prior to Day 1) will be weighed and the weight recorded and dose volumes adjusted and recorded. Where Day 1 weighing is scheduled for later on the same day this earlier weight will be accepted as the Day 1 weight.
Individual dose volume - offspring	:	Calculated from the most recently recorded scheduled bodyweight.
Controls	:	Vehicle at the same volume dosage as treated groups.
Frequency - adult males and females (Groups 6-9)	:	Once daily at approximately the same time each day, for up to eleven consecutive days.
Frequency - adult females (Group 1-5)	:	Once daily at approximately the same time each day, from Day 6 after mating until Day 20 after mating or, Day 10 of lactation (inclusive)
- offspring	:	Once daily at approximately the same time each day, from Day 11 until Day 21 of age (inclusive).
Sequence	:	By group.
Formulation	:	A daily record of the usage of formulation will be maintained based on weights. This balance is compared with the expected usage as a check of correct administration.

In view of the small dose volumes for the offspring, no records of formulation usage will be maintained but records will be kept of volumes dosed to individual offspring.

Formulations are stirred using a magnetic stirrer before and throughout the dosing procedure.

Final Protocol

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Study Number : CHV/070

Huntingdon Life Sciences

4.3.2. Clinical observations (F₀ generation)

- Animals and their cages : Inspected at least twice daily from time of arrival, for evidence of reaction to treatment or ill-health.
- Deviations from normal recorded at the time in respect of : Nature and severity.
Date and time of onset.
Duration and progress of the observed condition.
- Schedule : Daily on each day of study.

Additional observations will be made in association with dosing according to the following schedule and frequency:

- Minimum schedule : Daily, on each day of treatment.
- Frequency : 1. Pre-dose observation.
2. As each animal is returned to its home cage.
3. At the end of dosing each group.
4. Between 1 and 2 hours after completion of dosing all groups.
5. As late as possible in the working day.

The above schedule may be amended, as necessary, in the light of signs observed.

During the acclimatisation period, observations of the animals and their cages will be recorded at least once per day.

4.3.3. Mortality

- Debilitated animals : Observed carefully.
- Premature sacrifice : Animals may be killed on humane grounds or if considered *in extremis*.
- Animals found dead, killed *in extremis* or on humane grounds : A necropsy is performed as soon as possible. Animals found outside the normal workday will be preserved in a refrigerator (approximately 4°C) provided for this purpose.

Study Number : CHV/070

Huntingdon Life Sciences

4.3.4. Bodyweight

- F₀ females : After mating: Days 0, 3, 6, 10, 14, 17 and 20, then daily until parturition.
(Groups 1-5) During lactation: Days 1, 4, 7, 11, 14, 17 and 21.
- F₁ offspring : Days 1, 4, 7, 11, 14, 17, 21 and 28 of age, then weekly until termination
(Groups 1-5) and on Day 60 of age (where appropriate).
- Adult males and : On the day before treatment commences and then daily to termination.
females (Groups 6-9)

4.3.5. Parturition observations and gestation length

Detailed records will be maintained of:

- Duration of gestation : Time elapsing between detection of mating and commencement of parturition.
- Duration of parturition : From Day 20 of gestation, animals checked 3 times daily for evidence of parturition. If difficulties progress of parturition process monitored. Approximate numbers of live and dead offspring recorded (approximate at this stage, because parturition may not be complete).

4.3.6. Offspring observations - littering phase

The following observations will be recorded for all surviving offspring.

Offspring will be examined on each day from Day 1 to Day 21 of age for general clinical signs.

Dose observations on each day of dosing: observations will be made in association with dosing according to the following schedule:

- Frequency : Pre-dose observation
As each animal is returned to its cage
At the end of dosing each group
As late as possible in the working day.
- Litter size (definitive) : Daily on Days 1-21.
Litters culled to 8 (4M + 4F where possible) on Day 4 of age.
- Sex ratio : Recorded Days 1, 4 and 21 of age.

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4.3.7. Blood Chemistry and brain acetyl cholinesterase activity

Group(s)	Day	Animals/Measurements
Groups 1-4	Day 20 after mating	8 dams per group to be killed 3 hours after dosing. Measure plasma, erythrocyte and brain cholinesterase in dams and fetuses.
Groups 1-4	Day 4 of age	Up to 2 male and 2 female culled offspring will be killed per litter 4 hours after dosing for measurement of plasma, erythrocyte and brain cholinesterase.
Group 5	Day 11 of age	All offspring in each litter will be killed 2 hours after dosing for measurement of plasma, erythrocyte and brain cholinesterase.
Groups 6-9	Day 1 of treatment	8 males and 8 females per group will be killed 2 hours after dosing for measurement of plasma, erythrocyte and brain cholinesterase.
Groups 1-4	Day 21 of age	One male and one female offspring in each litter (up to 8 litters per group) will be killed 2 hours after dosing for measurement of plasma, erythrocyte and brain cholinesterase.
Groups 6-9	Day 11 of treatment	8 males and 8 females per group will be killed 2 hours after dosing for measurement of plasma, erythrocyte and brain cholinesterase.
Groups 1-4	Day 60 of age	8 male and 8 female offspring per group will be killed for measurement of plasma, erythrocyte and brain cholinesterase.

Conditions : Samples collected under light general anaesthesia (adult animals and Day 21 pups only).
Anaesthetic : Isoflurane.
Sample site : Retro-orbital sinus – all animals \geq Day 21 of age.
Day 20 fetuses – umbilical cord
Day 4 and 11 pups – following decapitation
Sample volume : 0.7 ml (samples will be pooled from fetuses within each litter (not pooled by sex)/Day 4 and Day 11 pups as necessary to provide the necessary sample volume).

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All samples will be examined for the following characteristics:

Using lithium heparin as anticoagulant

Plasma pseudo cholinesterase
Erythrocyte acetyl cholinesterase.

For assessment of brain acetyl cholinesterase activity: brains will be taken (after decapitation for Day 4 and Day 11 pups) and weighed at necropsy and brain cholinesterase activity will be measured. For the Day 20 fetuses, the brains for all fetuses in the litter will be pooled for analysis.

4.4. Animals – euthanasia

All animals will be subject to terminal investigations (Section 5).

5. PATHOLOGY

5.1. Time of necropsy

Adult males and females (Groups 6-9)	:	On Day 1 or Day 11 of treatment, after dosing.
9 adult females per group (Groups 1-4).	:	Day 20 after mating.
F ₀ females failing to produce viable litter	:	Day 25 after mating.
F ₀ females with litters	:	After recording of bodyweight on Day 21 of lactation or on day of litter loss.
Unallocated F ₁ offspring	:	After Day 21 or Day 60 of age (Groups 1-4).
Selected offspring (Group 5)	:	Day 11 of age.

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5.2. Euthanasia

Adult males and females and Day 21 offspring : Inhaled carbon dioxide.
 Offspring culled on Day 4 of age : Intraperitoneal injection of barbiturate.
 F₁ animals at Day 11 of age : Decapitation.
 Unallocated F₁ offspring : Inhaled Carbon dioxide.

5.3. Macroscopic pathology

All animals scheduled for necropsy will be subjected to a macroscopic examination for evidence of disease or adverse reaction to treatment and abnormal tissues retained.

In addition, the following will be recorded:

Females killed on Day 20 after mating : The reproductive tract, complete with ovaries, will be dissected out. The following will be recorded:

Each ovary/uterine horn : Number of:
 Corpora lutea.
 Implantation sites.
 Resorption sites (early or late).
 Fetuses (live and dead).
 Fetuses dissected from the uterus and weighed individually.
 Fetuses sexed. Fetal blood samples taken and brains weighed (see section 4.3.7).

Females failing to produce viable litter and those killed after weaning (Groups 1-5) : Number of implantation sites.

Females where litter dies before weaning (Groups 1-5) : Number of implantation sites.
 Mammary tissue examined and specimen retained.

For all animals killed for assay of brain acetyl cholinesterase activity (section 4.3.7), the brain will be weighed.

Photographs may be prepared showing representative treatment-related macroscopic findings, at the discretion of the necropsy supervisor or Study Director.

Sporadic deaths in early neonates, any excess offspring culled on Day 4 of age and excess weanlings will be discarded without further examination.

Study Number : CHV/070

Huntingdon Life Sciences

6. DATA TREATMENT

6.1. Data processing

Summary data are presented as mean with standard deviation (SD), as appropriate:

Bodyweight

- Adults : Group mean values and SD calculated from individual data.
- Offspring : Mean and SD calculated for each litter. Group mean and SD calculated from individual litter values.

Gestation index : $\frac{\text{Number of live litters born}}{\text{Number pregnant}} \times 100$

Post-implantation survival index : $\frac{\text{Total number offspring born}}{\text{Total number uterine implantation sites}} \times 100$

Live birth index : $\frac{\text{Number live offspring on Day 1 after littering}}{\text{Total number of offspring born}} \times 100$

Viability index : $\frac{\text{Number live offspring on Day 4 before culling}}{\text{Number live offspring on Day 1 after littering}} \times 100$

Lactation index on Day 7 or 11 : $\frac{\text{Number live offspring on Day 7 or 11 of examination}}{\text{Number live offspring on Day 4 after culling}} \times 100$

Lactation index on Day 21 : $\frac{\text{Number live offspring on Day 21}}{\text{Number live offspring on Day 11}} \times 100$

Sex ratio : Calculated for Day 1, live offspring Days 1, 4 (before and after culling) and at Day 21 of age.

6.2. Statistical analysis

Appropriate tests will be performed as required.

Details of the tests actually used will be included in the final report.

Study Number : CHV/070

Huntingdon Life Sciences

7. REPORTING

Study progress : Periodic verbal and written updates on study progress will be provided by the Study Director. Status reports will be sent after parturition and weaning phases, then following F₁ behavioural assessment and termination of in-life phase.

Draft final report : For review by Sponsor

Authorised final report : After approval from the Sponsor

Routinely reports are supplied on A4 paper. The following numbers of reports are supplied:

Type of report	Printing	Number of copies	
		Bound	Unbound
Draft report	Single-sided	0	2
Authorised final	Double-sided	1	0
	Single-sided	0	1
Photographic report (if any)	Single-sided	1	0

Any additions or corrections to an authorised final report will be documented as a formal addendum/amendment to the final report.

8. QUALITY ASSURANCE AND ARCHIVING PROCEDURES

8.1. Quality Assurance

The following will be inspected or audited in relation to this study.

Protocol Audit : Authorised protocol and any amendments.

Study based inspections : Critical phases of this study will be inspected.

Process based inspections : Routine and repetitive procedures will be inspected on representative studies, not necessarily on this study.

Report Audit : The draft report and study data will be audited after issue of the draft report to the Sponsor.

QA findings will be reported to the Study Director and Company Management promptly on completion of each action, except for process based inspections, which will be reported to appropriate Company Management only.

Study Number : CHV/070

Huntingdon Life Sciences

8.2. Archiving

All raw data, samples and specimens arising from the performance of this study will remain the property of the Sponsor.

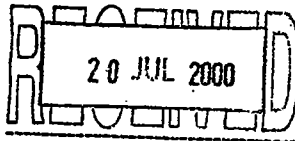
Types of sample and specimen which are unsuitable, by reason of instability, for long term retention and archiving may be disposed of after the periods stated in Huntingdon Life Sciences Standard Operating Procedures.

All other samples and specimens and all raw data will be retained by Huntingdon Life Sciences in its archive for a period of five years from the date on which the Study Director signs the final report. After such time, the Sponsor will be contacted and his advice sought on the return, disposal or further retention of the materials. If requested, Huntingdon Life Sciences will continue to retain the materials subject to a reasonable fee being agreed with the Sponsor.

Huntingdon Life Sciences will retain the Quality Assurance records relevant to this study and a copy of the final report in its archive indefinitely.

Study Number : CHV/070
 Protocol Amendment Number : 1

**Huntingdon
 Life Sciences**



DIMETHOATE
EFFECTS ON CHOLINESTERASE
IN THE CD RAT (ADULT AND JUVENILE) BY ORAL
GAVAGE ADMINISTRATION

Total number of pages: 6

Number of pages for internal distribution: 6

Study Director : D.P. Myers, B.Sc., Ph.D., IDT.

The signature of the Study Director authorises the implementation of this amendment to protocol. In this amendment, deleted statements are struck through and new statements are underlined. Any changes to the study design after the date of this authorising signature will be documented in a further formal amendment.

FIRST AMENDMENT APPROVAL

For Huntingdon Life Sciences Ltd

Authorised by:
 (Study Director)

Date:

A. P. Myers 13 July 2000

For the Sponsor

Approved by:

Date:

D. Spalding July 17, 2000

Study Number : CHV/070
Protocol Amendment Number : 1

**Huntingdon
Life Sciences**

DIMETHOATE
EFFECTS ON CHOLINESTERASE
IN THE CD RAT (ADULT AND JUVENILE) BY ORAL
GAVAGE ADMINISTRATION

Reason for amendment : Section 2.1: Addition of dosages of Dimethoate. Correction of typographical error.

: Section 3.2: Addition of concentrations of formulations in test Groups.

: Section 4.3.1: Correction of typographical error – mated females only dosed in Groups 1-4.

: Section 4.3.2: Clarification of frequency of recording of clinical signs during lactation and for F1 offspring from weaning to termination.

: Section 5.2: Addition of method of euthanasia for offspring culled on Day 4 of age and selected for assessment of blood and brain cholinesterase activity.

Study Number : CHV/070
Protocol Amendment Number : 1

**Huntingdon
Life Sciences**

Amendments

2. STUDY SCHEDULE AND STRUCTURE

2.1 Identity of treatment groups and duration of treatment

Group	Treatment (mg/kg/day)	Level (mg/kg/day)	Number of animal/sex	Animal numbers
1	Control	<u>0</u>	19F	1-9 A 10-19 B
2	<u>Dimethoate</u>	<u>0.1</u>	19F	20-28 A 29-38 B
3	<u>Dimethoate</u>	<u>0.5</u>	19F	39-47 A 48-57 B
4	<u>Dimethoate</u>	<u>3</u>	19F	58-66 A 67-76 B
5	Untreated	<u>0</u>	8F	77-84
6	Control	<u>0</u>	16F 16M	85-92 C, 93-100 D 201-208 C, 209-216 D
7	<u>Dimethoate</u>	<u>0.1</u>	16F 16M	101-108 C, 109-116 D 217-224 C, 225-232 D
8	<u>Dimethoate</u>	<u>0.5</u>	16F 16M	117-124 C, 125-132 D 233-240 C, 241-248 D
9	<u>Dimethoate</u>	<u>3</u>	16F 16M	133-140 C, 141-148 D 249-256 C, 257-264 D

F females A treated from Day 6 to Day 20 after mating inclusive.
M males B treated from Day 6 after mating to Day 10 of lactation.
 C treated for one day only
 D treated for up to 11 consecutive days

For groups 1-4 inclusive: Nine adult females will be dosed from Day 6 to Day 20 after mating inclusive. Ten adult females will be treated from Day 6 after mating to Day 10 of lactation. Offspring from 8 of these ten litters per group will be treated from Day 11 to Day 21 of age inclusive.

Group 5: These adult females will be undosed throughout the study. On Day 11 of age, one male and one female offspring per litter will be assigned to each of the following dosing groups for treatment on Day 11 of age: Control group

0.1 X mg/kg/day

0.5 Y mg/kg/day

3 Z mg/kg/day

Groups 6-9 inclusive: Eight adult male and female rats will be dosed for one day. The remaining animals in each group will be dosed for 11 consecutive days.

Study Number : CHV/070
Protocol Amendment Number : 1

**Huntingdon
Life Sciences**

3. TEST SUBSTANCE AND FORMULATION

3.2 Formulation

Treatment

- Control : Vehicle.
- Test group : Dimethoate; 0.02 ~~Low~~ mg/ml.
- Test group : Dimethoate; 0.1 ~~intermediate~~ mg/ml.
- Test group : Dimethoate; 0.6 ~~high~~ mg/ml.
- Conversion factor : The test substance will be used as supplied, unless otherwise advised by the Sponsor. Any such requirement will be documented in an amendment to protocol.
- Vehicle : Water for formulation.
- Method of preparation : Will be documented in the study data and included in the final report.
- Frequency of preparation : Will depend upon the availability of supporting stability data. Where sufficient stability data is available, batches will cover one week of dosing and may be prepared up to three days in advance of the first day of dosing. Where stability data does not support a period of use of this length, a more frequent mixing regime will be initiated.

Study Number : CHV/070
Protocol Amendment Number : 1

Huntingdon Life Sciences

4. ANIMAL MANAGEMENT

4.3.1 Administration

- | | | |
|-----------------------------------------------------|---|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Route | : | Oral gavage. |
| Treated at | : | Constant dosages in mg/kg/day. |
| Volume dosage | : | 5 ml/kg/day. |
| Individual dose volume -
adult males and females | : | Calculated from the most recently recorded scheduled bodyweight up to and including Day 17 after mating (if appropriate); thereafter the dosage volume will remain constant to Day 1 of lactation. From Day 1 of lactation dose volumes will be calculated, once again, from the most recently recorded bodyweight. Animals that are in parturition at the time of dosing will not be dosed. Animals that have completed parturition and can be dosed (prior to Day 1) will be weighed and the weight recorded and dose volumes adjusted and recorded. Where Day 1 weighing is scheduled for later on the same day this earlier weight will be accepted as the Day 1 weight. |
| Individual dose volume -
offspring | : | Calculated from the most recently recorded scheduled bodyweight. |
| Controls | : | Vehicle at the same volume dosage as treated groups. |
| Frequency - adult males and
females (Groups 6-9) | : | Once daily at approximately the same time each day, for up to eleven consecutive days. |
| Frequency - adult females
(Groups 1-4 & 5) | : | Once daily at approximately the same time each day, from Day 6 after mating until Day 20 after mating or, Day 10 of lactation (inclusive) |
| - offspring | : | Once daily at approximately the same time each day, from Day 11 until Day 21 of age (inclusive). |
| Sequence | : | By group. |
| Formulation | : | A daily record of the usage of formulation will be maintained based on weights. This balance is compared with the expected usage as a check of correct administration. |

In view of the small dose volumes for the offspring, no records of formulation usage will be maintained but records will be kept of volumes dosed to individual offspring.

Formulations are stirred using a magnetic stirrer before and throughout the dosing procedure.

Study Number : CHV/070
Protocol Amendment Number : 1

Huntingdon Life Sciences

4.3.2 Clinical observations (F0 generation)

Animals and their cages : Inspected at least twice daily from time of arrival, for evidence of reaction to treatment or ill-health.

Deviations from normal recorded at the time in respect of : Nature and severity.
Date and time of onset.
Duration and progress of the observed condition.

Schedule : Daily on each day of study until parturition (if applicable), and then weekly during lactation. Weekly for selected F1 offspring from weaning to termination.

Additional observations will be made in association with dosing according to the following schedule and frequency:

Minimum schedule : Daily, on each day of treatment.

Frequency : 1. Pre-dose observation.
2. As each animal is returned to its home cage.
3. At the end of dosing each group.
4. Between 1 and 2 hours after completion of dosing all groups.
5. As late as possible in the working day.

The above schedule may be amended, as necessary, in the light of signs observed.

During the acclimatisation period, observations of the animals and their cages will be recorded at least once per day.

5.2 Euthanasia

Adult males and females and Day 21 offspring : Inhaled carbon dioxide.

Offspring culled on Day 4 of age : Intraperitoneal injection of barbiturate. Decapitation for offspring selected for assessment of blood and brain cholinesterase activity.

F₁ animals at Day 11 of age : Decapitation.

Unallocated F₁ offspring : Inhaled Carbon dioxide.

Study Number : CHV/070
Protocol Amendment Number : 2

**Huntingdon
Life Sciences**

**DIMETHOATE
EFFECTS ON CHOLINESTERASE
IN THE CD RAT (ADULT AND JUVENILE) BY ORAL
GAVAGE ADMINISTRATION**

Total number of pages: 4

Number of pages for internal distribution: 4

Study Director : D.P. Myers, B.Sc., Ph.D., IDT.

The signature of the Study Director authorises the implementation of this amendment to protocol. In this amendment, deleted statements are struck through and new statements are underlined. Any changes to the study design after the date of this authorising signature will be documented in a further formal amendment.

SECOND AMENDMENT APPROVAL

For Huntingdon Life Sciences Ltd

Authorised by:
(Study Director)

A. P. Myers

Date: 15 September 2000

For the Sponsor

Approved by:

Doreen Spence

Date: Sept. 20, 2000

Study Number : CHV/070
Protocol Amendment Number : 2

**Huntingdon
Life Sciences**

DIMETHOATE
EFFECTS ON CHOLINESTERASE
IN THE CD RAT (ADULT AND JUVENILE) BY ORAL
GAVAGE ADMINISTRATION

Reason for amendment : Sections 5.2 and 5.3: As part of the Main Developmental Neurotoxicity Study (Study CHV/069) it is necessary to process and examine brains from Day 65 \pm 2 offspring following gravity perfusion. In order to gain experience in gravity perfusion and sectioning Day 60 brains to allow examination of specific regions of the brain, four offspring will be killed on Day 60 of age, perfused and the brain embedded, sectioned and subjected to light microscopy examination.

Study Number : CHV/070
Protocol Amendment Number : 2

Huntingdon Life Sciences

Amendments

5. PATHOLOGY

5.2 Euthanasia

- Adult males and females and Day 21 offspring : Inhaled carbon dioxide.
Offspring culled on Day 4 of age : Intraperitoneal injection of barbiturate. Decapitation for offspring selected for assessment of blood and brain cholinesterase activity.
F₁ animals at Day 11 of age : Decapitation.
Unallocated F₁ offspring : Inhaled Carbon dioxide. Four offspring will receive an intraperitoneal injection of a lethal dose of barbiturate. Heart exposed to permit perfusion of fixative via left ventricle.

5.3 Macroscopic pathology/histology and light microscopy

All animals scheduled for necropsy will be subjected to a macroscopic examination for evidence of disease or adverse reaction to treatment and abnormal tissues retained.

In addition, the following will be recorded:

- Females killed on Day 20 after mating : The reproductive tract, complete with ovaries, will be dissected out. The following will be recorded:
Each ovary/uterine horn : Number of:
Corpora lutea.
Implantation sites.
Resorption sites (early or late).
Fetuses (live and dead).
Fetuses dissected from the uterus and weighed individually.
Fetuses sexed. Fetal blood samples taken and brains weighed (see section 4.3.7).
Females failing to produce viable litter and those killed after weaning (Groups 1-5) : Number of implantation sites.
Females where litter dies before weaning (Groups 1-5) : Number of implantation sites.
Mammary tissue examined and specimen retained.

For all animals killed for assay of brain acetyl cholinesterase activity (section 4.3.7), the brain will be weighed.

Study Number : CHV/070
Protocol Amendment Number : 2

Huntingdon Life Sciences

Photographs may be prepared showing representative treatment-related macroscopic findings, at the discretion of the necropsy supervisor or Study Director.

Sporadic deaths in early neonates, any excess offspring culled on Day 4 of age and excess weanlings will be discarded without further examination.

For the four offspring which are perfused (see Section 5.2) procedures will be as follows:

Tissue Preparation

Fixation will be by *in situ* gravity perfusion with glutaraldehyde and paraformaldehyde from a reservoir approximately 90 cm above the work bench, followed by immersion.

Embedding

The brain will be embedded in paraffin wax.

Sectioning and Staining

For the brain, sections will be prepared and stained as follows:

<u>Paraffin wax:</u>		
<u>Type of section</u>	<u>Tissue</u>	<u>Area sectioned</u>
<u>4-5 μm sections stained with haematoxylin and eosin</u>	<u>Brain</u>	<u>Coronal sections:</u> <u>Olfactory lobes.</u> <u>Forebrain.</u> <u>Cerebrum, hippocampus, thalamus, hypothalamus.</u> <u>Cerebrum, tectum, tegmentum</u> <u>Medulla oblongata.</u> <u>Mid-sagittal sections:</u> <u>Cerebellum, pons.</u>

Light Microscopy

Tissue

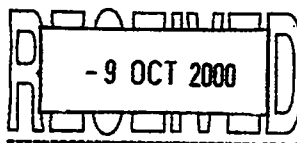
Brain

Regions to be examined

Coronal sections:
Olfactory lobes.
Forebrain.
Cerebrum, hippocampus, thalamus, hypothalamus.
Cerebrum, tectum, tegmentum
Medulla oblongata.
Mid-sagittal sections:
Cerebellum, pons.

Study Number : CHV/070
Protocol Amendment Number : 3

**Huntingdon
Life Sciences**



DIMETHOATE
EFFECTS ON CHOLINESTERASE
IN THE CD RAT (ADULT AND JUVENILE) BY ORAL
GAVAGE ADMINISTRATION

Total number of pages: 3

Number of pages for internal distribution: 3

Study Director : D.P. Myers, B.Sc., Ph.D., IDT.

The signature of the Study Director authorises the implementation of this amendment to protocol. In this amendment, deleted statements are struck through and new statements are underlined. Any changes to the study design after the date of this authorising signature will be documented in a further formal amendment.

THIRD AMENDMENT APPROVAL

For Huntingdon Life Sciences Ltd

Authorised by:
(Study Director)

A. P. Myers

Date:

29 September 2000

For the Sponsor

Approved by:

Dorrie Springgaard

Date:

October 5, 2000

Study Number : CHV/070
Protocol Amendment Number : 3

**Huntingdon
Life Sciences**

DIMETHOATE
EFFECTS ON CHOLINESTERASE
IN THE CD RAT (ADULT AND JUVENILE) BY ORAL
GAVAGE ADMINISTRATION

Reason for amendment : Section 5.1: As part of the Main Developmental Neurotoxicity Study (Study CHV/069) it is necessary to process and examine brains from Day 65 \pm 2 offspring following gravity perfusion. It had been intended that four offspring would be killed on Day 60 of age, perfused and the brain embedded, sectioned and subjected to light microscopy examination in order to gain experience in gravity perfusion and sectioning Day 60 brains to allow examination of specific brain regions. For logistical reasons the four offspring allocated for perfusion will now be killed on Day 61 of age and the same procedures followed.

Study Number : CHV/070
Protocol Amendment Number : 3

Huntingdon Life Sciences

Amendments

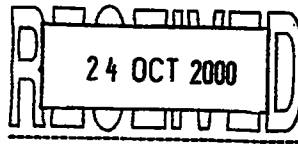
5. PATHOLOGY

5.1 Time of necropsy

- | | | |
|------------------------------------------------------------|---|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Adult males and females
(Groups 6-9) | : | On Day 1 or Day 11 of treatment, after dosing. |
| 9 adult females per group
(Groups 1-4). | : | Day 20 after mating. |
| F ₀ females failing to
produce viable litter | : | Day 25 after mating. |
| F ₀ females with litters | : | After recording of bodyweight on Day 21 of lactation or on day of
litter loss. |
| Unallocated F ₁ offspring | : | After Day 21 or Day 60 of age (Groups 1-4). <u>(Eight "spare"
unallocated offspring will be killed on Day 61 of age. Four of these
offspring will be perfused – see section 5.2).</u> |
| Selected offspring
(Group 5) | : | Day 11 of age. |

Study Number : CHV/070
Protocol Amendment Number : 4

**Huntingdon
Life Sciences**



DIMETHOATE
EFFECTS ON CHOLINESTERASE
IN THE CD RAT (ADULT AND JUVENILE) BY ORAL
GAVAGE ADMINISTRATION

Total number of pages: 5

Number of pages for internal distribution: 5

Study Director : D.P. Myers, B.Sc., Ph.D., IDT.

The signature of the Study Director authorises the implementation of this amendment to protocol. In this amendment, deleted statements are struck through and new statements are underlined. Any changes to the study design after the date of this authorising signature will be documented in a further formal amendment.

FOURTH AMENDMENT APPROVAL

For Huntingdon Life Sciences Ltd

Authorised by:
(Study Director)

D.P. Myers

Date: 16 October 2000

For the Sponsor

Approved by:

David S. Lundgaard

Date: Oct. 23, 2000

Study Number : CHV/070
Protocol Amendment Number : 4

**Huntingdon
Life Sciences**

DIMETHOATE
EFFECTS ON CHOLINESTERASE
IN THE CD RAT (ADULT AND JUVENILE) BY ORAL
GAVAGE ADMINISTRATION

Reason for amendment : Sections 5.2 and 5.3: The study protocol required that four offspring would be perfused on Day 61 of age. However, the perfusion appeared to be unsatisfactory for the first two animals and so an additional fifth animal was subjected to perfusion fixation.

In addition to the brain, specimens were collected from the tibial and sciatic nerves. Procedures for embedding, sectioning, staining and microscopic examination of the nerve tissue are detailed.

Some samples of sciatic nerve were stretched on card in order to see if this improves the presentation of the cross-sections of nerve at microscopic examination.

Study Number : CHV/070
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Huntingdon Life Sciences

Amendments

5. PATHOLOGY

5.2 Euthanasia

- Adult males and females and Day 21 offspring : Inhaled carbon dioxide.
Offspring culled on Day 4 of age : Intraperitoneal injection of barbiturate. Decapitation for offspring selected for assessment of blood and brain cholinesterase activity.
F₁ animals at Day 11 of age : Decapitation.
Unallocated F₁ offspring : Inhaled Carbon dioxide. ~~Four~~ Five offspring will receive an intraperitoneal injection of a lethal dose of barbiturate. Heart exposed to permit perfusion of fixative via left ventricle.

5.3 Macroscopic pathology/histology and light microscopy

All animals scheduled for necropsy will be subjected to a macroscopic examination for evidence of disease or adverse reaction to treatment and abnormal tissues retained.

In addition, the following will be recorded:

- Females killed on Day 20 after mating : The reproductive tract, complete with ovaries, will be dissected out. The following will be recorded:
Each ovary/uterine horn : Number of:
Corpora lutea.
Implantation sites.
Resorption sites (early or late).
Fetuses (live and dead).
Fetuses dissected from the uterus and weighed individually.
Fetuses sexed. Fetal blood samples taken and brains weighed (see section 4.3.7).
Females failing to produce viable litter and those killed after weaning (Groups 1-5) : Number of implantation sites.
Females where litter dies before weaning (Groups 1-5) : Number of implantation sites.
Mammary tissue examined and specimen retained.

For all animals killed for assay of brain acetyl cholinesterase activity (section 4.3.7), the brain will be weighed.

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Photographs may be prepared showing representative treatment-related macroscopic findings, at the discretion of the necropsy supervisor or Study Director.

Sporadic deaths in early neonates, any excess offspring culled on Day 4 of age and excess weanlings will be discarded without further examination.

For the ~~four~~ five offspring which are perfused (see Section 5.2) procedures will be as follows:

Tissue Preparation

Fixation will be by *in situ* gravity perfusion with glutaraldehyde and paraformaldehyde from a reservoir approximately 90 cm above the work bench, followed by immersion. Samples of sciatic and tibial nerves were taken. After primary fixation in glutaraldehyde and paraformaldehyde, the tissues will be washed in phosphate buffer followed by secondary fixation in 1% osmium tetroxide.

Embedding

The brain will be embedded in paraffin wax.

Sciatic and tibial nerves will be embedded in epoxy resin.

Sectioning and Staining

For the brain and nerves, sections will be prepared and stained as follows:

Type of section	Tissue	Area sectioned
4-5 µm sections stained with haematoxylin and eosin	Brain	Coronal sections: Olfactory lobes. Forebrain. Cerebrum, hippocampus, thalamus, hypothalamus. Cerebrum, tectum, tegmentum Medulla oblongata. Mid-sagittal sections: Cerebellum, pons.
<u>Epoxy Resin</u> 2 µm sections stained with toluidine blue	<u>Sciatic nerve</u> <u>Tibial nerve</u>	<u>Longitudinal and transverse sections at the sciatic notch and the mid-thigh.</u> <u>Longitudinal and transverse sections of calf muscle branch(es).</u> <u>Longitudinal and transverse sections at the knee</u>

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**Light Microscopy
Tissue**

Regions to be examined

Brain

- : Coronal sections:
Olfactory lobes.
Forebrain.
Cerebrum, hippocampus, thalamus,
hypothalamus.
Cerebrum, tectum, tegmentum
Medulla oblongata.
Mid-sagittal sections:
Cerebellum, pons.

Sciatic Nerve

- : Longitudinal and transverse sections at the Sciatic notch and the mid-thigh.

Tibial nerve

- : Longitudinal and transverse sections at the knee and of calf muscle branch(es).



**THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM**

GOOD LABORATORY PRACTICE

**STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC**

LABORATORY

**Huntingdon Life Sciences
Eye Research Centre
Eye
Suffolk
IP23 7PX**

TEST TYPE

**Analytical Chemistry
Clinical Chemistry
Ecosystems
Environmental Fate
Environmental Toxicity
Mutagenicity
Phys/Chem Testing
Toxicology**

DATE OF INSPECTION

29th January 2001

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

Roger G. Alexander
3/4/01

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority



**THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM**

GOOD LABORATORY PRACTICE

**STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC**

LABORATORY

**Huntingdon Life Sciences
Huntingdon Research Centre
Wooley Road
Alconbury
Huntingdon
Cambs.
PE28 4HS**

TEST TYPE

**Analytical Chemistry
Clinical Chemistry
Ecosystems
Environmental Fate
Environmental Toxicity
Phys/Chem Testing
Toxicology**

DATE OF INSPECTION

15th January 2001

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

Roger G. Alexander
3/4/01

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority