

**MALATHION**  
**EFFECTS ON CHOLINESTERASE**  
**IN THE CD RAT (ADULT AND JUVENILE) BY ORAL**  
**GAVAGE ADMINISTRATION**

**Huntingdon Life Sciences Project identity** : CHV/067

**Study completed on** : 30 November 2001

**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

Final: 30 November 2001

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

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No claim of confidentiality is made for any information contained in this study.

Submitter/Agent Cheminova A/S

Name Mette K. Jensen, M.Sc.

Signature

*Mette K. Jensen*

Title Monitoring Scientist

Date

*April 15, 2002*

**GOOD LABORATORY PRACTICE STATEMENT**  
**MALATHION**  
**EFFECTS ON CHOLINESTERASE**  
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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.

*S. M. Fulcher*

S. M. Fulcher, B.A., F.I.A.T.  
Study Director  
Huntingdon Life Sciences Ltd.

*30 November 2001*

Date

## FLAGGING STATEMENT

## MALATHION

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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 or exceeds any of the applicable criteria.

Submitter/Agent .....Cheminova A/S.....

Name ...Mette K. Jensen, M.Sc....

Signature

*Mette K. Jensen*

Title ...Monitoring Scientist.....

Date

*April 15, 2002*



**QUALITY ASSURANCE STATEMENT**

**MALATHION**

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

Study Phase	Date of Inspection	Date of Reporting
Protocol Audit	31 October 2000	31 October 2000
<b>Study Based Inspections</b>		
Dose Preparation	08 November 2000	08 November 2000
Dosing & Post dose Observations	09 November 2000	09 November 2000
Umbilical cord bleed	23 November 2000	23 November 2000
Measurement of acetyl cholinesterase	28 November 2000	30 November 2000
Dosing of offspring	05 December 2000	08 December 2000
Report Audit	29 November 2001	29 November 2001

**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.



Helen Comb BSc; M.R.Q.A  
Group Manager  
Department of Quality Assurance,  
Huntingdon Life Sciences Ltd.



Date

**CONTRIBUTING SCIENTISTS**

**MALATHION**

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**STUDY MANAGEMENT**

S. M. Fulcher, B.A., F.I.A.T.  
Study Director

K.P. Hazelden, B.Sc., C Biol, MI Biol  
Monitoring Toxicologist

S. Renaut, B.Sc (Hons)  
Study Supervisor

**MATINGS**

M.J. Collier, B.Sc.  
Behavioural Scientist

**FORMULATION CHEMISTRY**

A D Clemson, PhD, MSc, BSc, C.Chem., FRSC.  
Scientific Manager, Formulation Chemistry

**CHOLINESTERASE ANALYSIS**

M.J. Smith, B.A. (Hons.), FIBMS DMS.  
Section Head, Clinical Chemistry  
Central Laboratory Services

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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 malathion on plasma, erythrocyte and brain acetyl cholinesterase activity in gestating females and their pre-term fetuses at GD 20 (gestation day 20), in pre-weaning offspring at PND (post natal day) 4, 11 and 21 and in young adult rats (7-8 weeks of age). Recovery of cholinesterase activity was also assessed in offspring at PND 60, 39 days after cessation of repeated-dose treatment.

The study was conducted in support of a Developmental Neurotoxicity study in rats with malathion (Huntingdon Life Sciences Report No. CHV066/013331).

Groups of animals were dosed by oral gavage at 0 (control) 5, 50 or 150 mg/kg/day at a dosage volume of 5ml/kg as indicated below. Additional acute investigations were also conducted at 450 mg/kg/day and are indicated below in square parentheses. Control animals received the vehicle, corn oil, at the same dosage volume and over the same period as treated animals. The dosing regimes used on the study were as follows:

Nine mated females per group were treated from GD 6 to GD 20. Eight dams per group were killed 3 hours after dosing on GD20, litter data was assessed and maternal and fetal plasma, erythrocyte and brain cholinesterase activities determined.

Additionally ten mated females per group were treated from GD 6 to PND 10. Offspring in eight litters per group were then dosed from PND 11 to PND 21 inclusive to assess effects on survival, weight gain and plasma, erythrocyte and brain cholinesterase activity. Selected offspring from these litters were killed at PND 4, 21 and 60 and plasma, erythrocyte and brain cholinesterase activities determined.

A group of eight undosed pregnant females were allowed to litter and rear their young (litters culled to 5 males and 5 females on PND 4) to PND 11. On PND 11, one male and one female offspring per litter were assigned to each experimental group [including 450 mg/kg/day], giving eight male and eight female offspring per group, and directly dosed. Offspring were then killed 2 hours after dosing and plasma, erythrocyte and brain cholinesterase activities determined.

Eight male and 8 female young adult rats [including 450 mg/kg/day] per group were dosed on one occasion, killed 2 hours after dosing and plasma, erythrocyte and brain cholinesterase activity determined.

Additionally 8 young adult males and 8 young adult females per group [excluding 450 mg/kg/day] were dosed for 11 consecutive days and were killed 2 hours after dosing on day 11 and plasma, erythrocyte and brain cholinesterase activities determined.

### Results

#### 450 mg/kg/day

A single administration to PND 11 offspring was associated with body tremors 1-2 hours after dosing in 5/16 animals and a further offspring was found in a moribund state and killed 1 hour after dosing. Necropsy findings were unremarkable. A marked inhibition in plasma, erythrocyte and brain cholinesterase activities (approximately 52-54%, 61-72% and 81-84 % respectively) compared to the control was evident.

Plasma and erythrocyte cholinesterase activities for young adult animals, following one single dose administration, were inhibited (24% and 17-25% respectively) compared with the control however, brain cholinesterase activity was unaffected.

#### **150 mg/kg/day**

There was no adverse effect of treatment on dams and young adult animals as assessed by clinical signs, bodyweights and bodyweight gain, including those during pregnancy and lactation, and necropsy observations. Litter data on GD 20 or PND 11, fetal weight at GD 20, offspring bodyweight on PND1 and subsequent growth to PND 11 were all unaffected by treatment of the parent female.

There was no effect of direct dosing of offspring from PND 11 to weaning (PND 21) as assessed by clinical signs, survival, or bodyweight gain to PND 60.

For dams at GD20 erythrocyte cholinesterase activity was inhibited (51%), compared with control; plasma and brain cholinesterase activities were unaffected.

Fetal plasma and erythrocyte cholinesterase activities at GD 20 were inhibited (15% and 19% respectively) compared with Control; brain cholinesterase activity was unaffected.

By PND 4 offspring cholinesterase activities were comparable to control and were unaffected by treatment of the parent female.

Direct treatment of the offspring on PND 11 (1 day of treatment) was associated with a marked inhibition in plasma, erythrocyte and brain cholinesterase activities (approximately 35-36%, 48-55% and 44-48% respectively) compared to the control.

For young adult animals cholinesterase activities were comparable to control following a single dose administration.

Direct treatment of the offspring from PND 11 to PND 21 (11 days of treatment) was associated with a marked inhibition in plasma, erythrocyte and brain cholinesterase activities (24-32%, 67-68% and 16% respectively) compared to the control. Inhibition of plasma and brain cholinesterase activities were lower compared to that observed following one dose on PND 11, but inhibition of erythrocyte cholinesterase activity was increased.

For young adult animals following 11 days of daily administration erythrocyte cholinesterase activity was inhibited (43-48%) compared with control; erythrocyte and brain cholinesterase activities were not considered to have been affected by treatment.

For offspring at PND 60 no effect of treatment on cholinesterase activities were apparent, 39 days after the end of repeat dosing (PND 21).

#### **50 mg/kg/day**

There was no effect of treatment on dams or young adult animals as assessed by clinical signs, bodyweights and bodyweight gain, including those during pregnancy and lactation, and necropsy observations. Litter data on GD 20 or PND 11, fetal weight at GD 20, offspring bodyweight on PND1 and subsequent growth to PND 11 were all unaffected by treatment of the parent female.

There was no effect of direct dosing of offspring from PND 11 to weaning (PND 21) as assessed by clinical signs, survival, or bodyweight gain to PND 60.



For dams at GD20 erythrocyte cholinesterase activity was inhibited (19%), compared with control; plasma and brain cholinesterase activities were unaffected.

Fetal plasma and erythrocyte cholinesterase activities at GD 20 were inhibited (14% and 11% respectively) compared with Control; brain cholinesterase activity was unaffected.

By PND 4 offspring cholinesterase activities were comparable to control and were unaffected by treatment of the parent female.

Direct treatment of the offspring on PND 11 (1 day of treatment) was associated with inhibition of plasma and erythrocyte cholinesterase activities (16-19% and 23-35% respectively) compared to the control; brain cholinesterase activity was unaffected.

For young adult animals cholinesterase activities were comparable to control following a single dose administration.

Direct treatment of the offspring from PND 11 to PND 21 (11 days of treatment) was associated with a inhibition in plasma (19%) and erythrocyte (34-39%) cholinesterase activities compared to the control; brain cholinesterase activity was unaffected. Inhibition of plasma cholinesterase activity was similar to that observed following one dose on PND 11, but inhibition of erythrocyte cholinesterase activity was increased.

For young adult animals following 11 days of daily administration erythrocyte cholinesterase activity was inhibited (20%) compared with control; plasma and brain cholinesterase activities were unaffected.

For offspring at PND 60 no effect of treatment on cholinesterase activities were apparent, 39 days after the end of repeat dosing (PND 21).

#### **5 mg/kg/day**

There was no adverse effect of treatment on dams and young adult animals as assessed by clinical signs, bodyweights and bodyweight gain, including those during pregnancy and lactation, and necropsy observations. Litter data on GD 20 or PND 11, fetal weight at GD 20, offspring bodyweight on PND1 and subsequent growth to PND 11 were all unaffected by treatment of the parent female.

There was no effect of direct dosing of offspring from PND 11 to weaning (PND 21) as assessed by clinical signs, survival, or bodyweight gain to PND 60.

For dams at GD20 cholinesterase activities were comparable to control.

Fetal cholinesterase activities were comparable to control and were unaffected by treatment of the parent female.

PND 4 offspring cholinesterase activities were comparable to control and unaffected by treatment of the parent female.

Cholinesterase activities were not considered to have been adversely affected by direct treatment of the offspring on PND 11 (1 day of treatment).

For young adult animals cholinesterase activities were comparable to control following a single dose administration.

Cholinesterase activities for PND 21 offspring were comparable to control and were not considered to have been adversely affected by direct treatment from PND 11 to PND21.

For young adult animals following 11 days of daily administration cholinesterase activities were comparable to control.

For offspring at PND 60 no effect of treatment on cholinesterase activities were apparent, 39 days after the end of repeat dosing (PND 21).

## Conclusion

Inhibition of erythrocyte cholinesterase activity in dams receiving 150 mg/kg/day was markedly greater than for their fetuses at GD 20. At lower dosages, the magnitude of effects on blood cholinesterase activities was generally similar in both dams and fetuses, with inhibition occurring at 50 mg/kg/day. Brain cholinesterase activity was unaffected at dosages up to 150 mg/kg/day. A dosage of 5 mg/kg/day was considered to be the no-observed-adverse-effect level (NOAEL) for blood cholinesterase activities and 150 mg/kg/day for brain cholinesterase activity.

Cholinesterase activities for PND 4 offspring were unaffected by treatment of the parent female at dosages up to 150 mg/kg/day.

Inhibition of blood cholinesterase activities of PND 11 offspring following a single dose administration was apparent at 450, 150 and 50 mg/kg/day. Brain cholinesterase activity was affected at 450 and 150 mg/kg/day and some offspring at 450 mg/kg/day showed body tremors. A dosage of 5 mg/kg/day was considered to be the no-observed-adverse-effect level (NOAEL) for blood cholinesterase activities and 150 mg/kg/day for brain cholinesterase activity for the PND 11 offspring. The reaction of young adult animals to a single dose was less marked with effects on blood cholinesterase activities only being observed at 450 mg/kg/day, and no inhibition being apparent for brain cholinesterase activity. A dosage of 150 mg/kg/day was considered to be the no-observed-adverse-effect level (NOAEL) for blood cholinesterase activities for young adult rats to a single dose administration, with the NOAEL for brain cholinesterase activity being 450 mg/kg/day.

Inhibition of blood cholinesterase activities of PND 21 offspring (following 11 days of dosing) was apparent at 150 and 50 mg/kg/day, with brain cholinesterase activity also being affected at 150 mg/kg/day. While inhibition of erythrocyte cholinesterase activity compared with values at PND 11 (following a single dose) increased, inhibition of both plasma and brain cholinesterase activities decreased at 150 mg/kg/day. A dosage of 5 mg/kg/day was considered to be the no-observed-adverse-effect level (NOEL) for blood cholinesterase activities for the PND 21 offspring and 50 mg/kg/day the NOAEL for brain cholinesterase activity. For young adults, following 11 days of dosing, effects on cholinesterase activities were again less than observed in similarly treated offspring (PND 21); inhibition being restricted to erythrocyte cholinesterase activity at 150 and 50 mg/kg/day. No inhibition of plasma or brain cholinesterase activities was observed. A dosage of 5 mg/kg/day was considered to be the no-observed-adverse-effect level (NOAEL) for blood cholinesterase activities for young rats following 11 days of dosing, with the NOAEL for brain cholinesterase activity being 150 mg/kg/day.

The overall no-observed-adverse-effect level (NOAEL) was considered to be 5 mg/kg/day for plasma/erythrocyte cholinesterase activities and 50 mg/kg/day for brain cholinesterase activity. The most sensitive period for effects on brain cholinesterase activity was found to be PND 11. No effects of treatment were detected in offspring at PND 4 following maternal treatment at 150 mg/kg/day, or in offspring at PND 60, following 11 days of direct treatment at 150 mg/kg/day and 39 days of recovery.

## TABULAR SUMMARY

MATERNAL RESPONSES DURING GESTATION AND LACTATION				
Parameter	Dosage (mg/kg/day)			
(group mean values)	control	5	50	150
Bodyweight on GD 6 (g)	291	291	288	287
Bodyweight on GD 20 (g)	441	430	429	438
Bodyweight gain GD 6 -20 (g)	151	139	141	151
Bodyweight gain PND 1-21 (g)	18	28	14	27
Plasma CHE on GD 20	1382	1210 (12%)	1297 (6%)	1204 (13%)
Erythrocyte CHE on GD 20	1234	1244 (-1%)	994** (19%)	606** (51%)
Brain CHE on GD 20	13200	13013 (1%)	13100 (1%)	12644 (4%)

LITTER/FETAL RESPONSES ON GD 20				
Parameter	Dosage (mg/kg/day)			
(group mean values)	control	5	50	150
Implantations	17.0	16.9	16.0	16.3
Resorptions	0.9	1.1	0.6	0.6
Live young	16.1	15.8	15.4	15.8
Mean fetal weight (g)	4.01	3.90	3.92	3.97
Plasma CHE	285	265 (7%)	246** (14%)	243** (15%)
Erythrocyte CHE	938	897 (4%)	831* (11%)	756** (19%)
Brain CHE	1606	1656 (-3%)	1519 (5%)	1638 (-2%)

LITTER/OFFSPRING RESPONSES TO PND 11				
Parameter	Dosage (mg/kg/day)			
(group mean values)	control	5	50	150
Total litter size PND 1	15.0	15.7	13.9	14.2
Mean pup weight on PND 1 (g) - males	6.5	6.1	6.7	6.9
Mean pup weight on PND 1 (g) - females	6.2	5.9	6.4	6.4
Offspring viability index (%)	97.9	95.2	99.4	99.2
Bodyweight gain PND 1-11 of age (g) - male offspring	20.5	17.9*	20.7	19.2
Bodyweight gain PND 1-11 of age (g) - female offspring	19.7	18.3	20.0	18.7
Plasma CHE on PND 4 - males	612	594 (3%)	626 (-2%)	620 (-1%)
Plasma CHE on PND 4 - females	622	598 (4%)	606 (3%)	617 (1%)
Erythrocyte CHE on PND 4 - males	1100	1134 (-3%)	1075 (2%)	1017 (8%)
Erythrocyte CHE on PND 4 - females	1147	1125 (2%)	1193 (-4%)	1103 (4%)
Brain CHE on PND 4 - males	3018	3078 (-2%)	2915 (3%)	2867 (5%)
Brain CHE on PND 4 - females	2994	2941 (2%)	2953 (1%)	2967 (1%)

( ) Cholinesterase inhibition (compared with control)

Statistical analysis: \* p<0.05, \*\* p<0.01

## TABULAR SUMMARY

OFFSPRING RESPONSES ON PND 11 (1 DAY OF DOSING)					
Parameter	Dosage (mg/kg/day)				
(group mean values)	control	5	50	150	450
Plasma CHE - males	756	704 (7%)	614** (19%)	482** (36%)	346** (54%)
Plasma CHE - females	737	717 (3%)	620** (16%)	481** (35%)	353** (52%)
Erythrocyte CHE - males	1509	1272* (16%)	1131** (25%)	627** (55%)	428** (72%)
Erythrocyte CHE - females	1319	1228 (7%)	1016** (23%)	688** (48%)	519** (61%)
Brain CHE - males	5756	5688 (1%)	5388 (6%)	3244** (44%)	919** (84%)
Brain CHE - females	5825	5600 (4%)	5249 (10%)	3044** (48%)	1081** (81%)

YOUNG ADULT RESPONSES ON DAY 1 OF TREATMENT						
Parameter	Dosage (mg/kg/day)					
(group mean values)	control (Gp 6)	control (Gp 10)	5	50	150	450@
Plasma CHE - males	342	354	341 (0%)	359 (-5%)	337 (1%)	268** (24%)
Plasma CHE - females	793	624	717 (10%)	822 (-4%)	727 (8%)	558 (11%)
Erythrocyte CHE - males	866	1109	891 (-3%)	975 (-13%)	853 (2%)	831*** (25%)
Erythrocyte CHE - females	950	1069	1013 (-7%)	959 (-1%)	891 (6%)	884*** (17%)
Brain CHE - males	13713	13563	12988 (5%)	13081 (5%)	12744 (7%)	13131 (3%)
Brain CHE - females	12900	13513	13213 (-2%)	13038 (-1%)	13244 (-3%)	12975 (4%)

@ Inhibition compared with Group 10 control

OFFSPRING RESPONSES ON PND 21 (11 DAYS OF DOSING)				
Parameter	Dosage (mg/kg/day)			
(group mean values)	control	5	50	150
Bodyweight gain PND 11-21 (g) - males	28.4	28.7	29.6	28.3
Bodyweight gain PND 11-21 (g) - females	27.7	27.8	28.3	26.6
Plasma CHE - males	393	341 (13%)	320* (19%)	299** (24%)
Plasma CHE - females	374	338 (10%)	304* (19%)	254** (32%)
Erythrocyte CHE - males	1866	1556* (17%)	1144** (39%)	622** (67%)
Erythrocyte CHE - females	1894	1606 (15%)	1250** (34%)	597** (68%)
Brain CHE - males	10500	10363 (1%)	10488 (0%)	8850** (16%)
Brain CHE - females	10356	10250 (1%)	10444 (-1%)	8650** (16%)

( ) Cholinesterase inhibition (compared with control)

Statistical analysis: \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$ , \*  $p < 0.05$

YOUNG ADULT RESPONSES ON DAY 11 OF TREATMENT				
Parameter	Dosage (mg/kg/day)			
(group mean values)	control	5	50	150
Bodyweight gain Days 1-11 (g) - males	81	78	80	74
Bodyweight gain Days 1-11 (g) - females	34	34	35	32
Plasma CHE - males	333	322 (3%)	297* (11%)	289* (13%)
Plasma CHE - females	1028	978 (5%)	871 (15%)	893 (13%)
Erythrocyte CHE - males	1084	1044 (4%)	869** (20%)	616** (43%)
Erythrocyte CHE - females	1094	1069 (2%)	878** (20%)	566** (48%)
Brain CHE - males	13219	13288 (-1%)	13494 (-2%)	13031 (1%)
Brain CHE - females	13731	13463 (2%)	13700 (0%)	13031 (5%)

OFFSPRING RESPONSES ON PND 60				
Parameter	Dosage (mg/kg/day)			
(group mean values)	control	5	50	150
Bodyweight gain PND 11-60 (g) - males	363.2	352.3	355.8	355.9
Bodyweight gain PND 11-60 (g) - females	228.6	214.0	209.8	210.6
Plasma CHE - males	363	377 (-4%)	344 (5%)	317 (13%)
Plasma CHE - females	1090	894 (18%)	913 (16%)	843 (23%)
Erythrocyte CHE - males	903	944 (-5%)	997 (-10%)	1050 (-16%)
Erythrocyte CHE - females	966	988 (-2%)	956 (1%)	994 (-3%)
Brain CHE - males	13231	13269 (0%)	13125 (1%)	12825 (3%)
Brain CHE - females	13513	13313 (1%)	13431 (1%)	13331 (1%)

( ) Cholinesterase inhibition (compared with control)

Statistical analysis: \*\*  $p < 0.01$ , \*  $p < 0.05$

## INTRODUCTION

### Objective

The purpose of this study was to assess the effect in rats of acute and/or repeated dosing with malathion on plasma, erythrocyte and brain acetyl cholinesterase activity in gestating females and their pre-term fetuses (GD (gestation day) 20), in pre-weaning offspring (PND (post natal day) 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.

The study was conducted in support of a Developmental Neurotoxicity study in rats with Malathion (Huntingdon Life Sciences Report No. CHV066/013331).

Groups of animals were dosed by oral gavage at 0 (control) 5, 50 or 150 mg/kg/day at a dosage volume of 5 ml/kg as indicated below. Additional acute investigations were also conducted at 450 mg/kg/day and these investigations are indicated below in square parentheses. Control animals received the vehicle, corn oil, at the same dosage volume and over the same period as treated animals. The dosing regimes used on the study were as follows:

Nine mated females per group were treated from GD 6 to GD 20. Eight dams per group were killed 3 hours after dosing on GD20, litter data were assessed and maternal and fetal plasma, erythrocyte and brain cholinesterase activities determined.

Additionally ten mated females per group were treated from GD 6 to PND 10. These animals were allowed to litter and rear their young (litters culled to 4 males and 4 females on PND 4) to weaning (PND 21). Offspring in eight litters per group were then dosed from PND 11 to PND 21 inclusive to assess effects on survival, weight gain and plasma, erythrocyte and brain cholinesterase activity. Selected offspring from these litters were killed at PND 4, 21 and 60 and plasma, erythrocyte and brain cholinesterase activities determined.

A group of eight undosed pregnant females were allowed to litter and rear their young (litters culled to 5 males and 5 females on PND 4) to PND 11. On PND 11, one male and one female offspring per litter were assigned to each experimental group [including 450 mg/kg/day], giving eight male and eight female offspring per group, and directly dosed. Offspring were then killed 2 hours after dosing and plasma, erythrocyte and brain cholinesterase activities determined.

Eight male and 8 female young adult rats [including 450 mg/kg/day] per group were dosed on one occasion, killed 2 hours after dosing and plasma, erythrocyte and brain cholinesterase activity determined.

Additionally 8 young adult males and 8 young adult females per group [excluding 450 mg/kg/day] were dosed for 11 consecutive days and were killed 2 hours after dosing on day 11 and plasma, erythrocyte and brain cholinesterase activities determined.

### 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 offspring.

Dosages of 5, 50 and 150 mg/kg/day, with additionally 450 mg/kg/day for acute investigations only, were selected by the Sponsor, based on the results from a dose finding study in CD rats (Huntingdon Life Sciences Report No. CHV062/010003). In that study, it was concluded that 150 mg/kg/day would be suitable for use as the highest dosage on a developmental neurotoxicity study. Dosages higher than this were considered likely to lead to adverse clinical signs in the directly dosed offspring which would have the potential to compromise assessment of aspects of neurotoxicity in the offspring, other than reduction in cholinesterase activity.

### Study organisation

Testing facilities:

The principal laboratory was:

Huntingdon Life Sciences Ltd  
Eye  
Suffolk  
IP23 7PX  
England

Measurement of cholinesterase activity was performed at:

Huntingdon Research Centre,  
Huntingdon,  
Cambridgeshire  
PE28 4HS  
England.

Study timing:

Protocol approval	:	27 October 2000
Animals arrived:		
Parental females (Groups 1-5)	:	27 October 2000
Young adult males and females (Groups 6-9)	:	01 November 2000
Young adult males and females (Groups 10-11)	:	15 November 2000
Pairing commenced (Groups 1-5)	:	02 November 2000
Treatment commenced:		
Parental females (Groups 1-4)	:	09 November 2000
PND 11 offspring (Groups 1-4)	:	05 December 2000
PND 11 offspring (Group 5)	:	05 December 2000
Young adult males and females (Groups 6-9)	:	13 November 2000
Young adult males and females (Groups 10-11)	:	30 November 2000
Necropsy completed	:	25 January 2001

## Archives

All experimental data arising from the study (including documentary raw data, specimens, records, and other materials; collectively defined as the “materials”) will remain the property of the Sponsor.

Samples and materials which are unsuitable, by reasons of instability, for long term retention and archiving may be disposed of after the periods defined in Huntingdon Life Sciences Standard Operating Procedures have been completed.

All other samples, specimens and all raw data will be retained by Huntingdon Life Sciences 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. 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 archives indefinitely.



## MATERIALS AND METHODS

### DESIGN CONDITIONS

#### Animals

One hundred and four virgin female rats of the CrI: CD<sup>®</sup> 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. Prior to mating, 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 (GD 0- gestation day 0) the eighty-four animals allocated were in the weight range 214 to 303 g (approximately 10 to 11 weeks of age). These animals were allocated to Groups 1 to 4 and also provided Group 5 females, used to provide litters for cholinesterase investigations on PND 11 (post natal day 11) offspring.

Seventy young adult male and seventy young adult female rats of the CrI: CD<sup>®</sup> BR strain (ordered at 5-6 weeks of age and 120-175 g bodyweight for males and 100-150 g for females) with litter mates identified, 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 223 to 314 g for males and 161 to 231 g for females (approximately 7 to 8 weeks of age). These animals were allocated to Groups 6 to 9.

Twenty young adult male and twenty young adult female rats of the CrI: CD<sup>®</sup> 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 15 days acclimatisation, during which time they were inspected daily to check their physical condition. At commencement of the study (day before treatment commenced) the sixteen young adults of each sex allocated were in the weight range 288 to 319 g for males and 175 to 217 g for females (approximately 7 to 8 weeks of age). These animals were allocated to Groups 10 and 11.

Details relating to Groups can be found in the composition and identity of treatments groups section of these Materials and Methods.

#### 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. 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
	M	F		
F0 generation				
Mating	1	: 1	RB3 modified	Stainless steel grid
Gestation (up to GD 17)	-	1	RB3 modified	Stainless steel grid
Littering (from GD 17 to PND 14-18)	-	1 + litter	RB3	Solid polypropylene
Lactation (from PND 14-18 until weaning)		1 + litter	TR18	Stainless steel grid
F1 generation				
From weaning	Up to 4	Up to 4	TR18	Stainless steel grid
Young adult males and females				
Acclimatisation and treatment period	Up to 4	Up to 4	TR18	Stainless steel grid

### **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.

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 Department of the Environment. Certificates of analysis were routinely received from the supplier.

Certificates of analysis for the batches of diet used on the study, and for the drinking water are presented in Addenda 1 and 2.

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 allocated to the gestation and lactation phases of the study (Groups 1-4 and Group 5) 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. The allocation of females was adjusted to avoid a given male having mated with more than one female in each study group.

Young adult males and females which were not mated and were intended for Groups 6-9 were allocated by sex. After exclusion of animals showing signs of ill-health, males and females were allocated so as to ensure that not more than one offspring from each litter is present in each group. Animals with grossly atypical bodyweights were discarded and a record of littermate relationships is retained in the raw data.

Young adult males and females which were not mated and were intended for Groups 10 and 11 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.

Cage distribution for all animals was arranged to minimise environmental variables.

Four time mated females excess to the study requirements were allowed to litter and were retained as spares until Group 5 dams had littered and their litters had been assessed for adequate size and sex ratio. One animal originally allocated to Group 5, number 82, had a litter which was unable to fulfil the neonate requirements. This animal was therefore replaced with one of the spare litters which was given the study number 1082. The data collected for the replaced animal are retained with the raw data but not reported. Bodyweight data were only recorded for the used spare animal and litter after allocation on PND 4.

#### Composition and identity of treatment groups

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

Group	Treatment	Dosage (mg/kg/day)#	Number of females	Animal numbers
1	control	0	19	1-9A, 10-19B
2	malathion	5	19	20-28A, 29-38B
3	malathion	50	19	39-47A, 48-57B
4	malathion	150	19	58-66A, 67-76B

# Expressed in terms of the test substance as supplied.

Nine adult females (A) in each group were dosed from GD 6 to GD 20 inclusive. Ten adult females (B) in each group 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. These litters were: Group 1: 10, 11, 12, 13, 14, 15, 16 and 17; Group 2: 29, 30, 31, 32, 33, 34, 35 and 37; Group 3: 48, 49, 50, 51, 52, 53, 54 and 56; Group 4: 67, 68, 69, 70, 72, 73, 74 and 75. Offspring from the remaining two litters in each group were not directly dosed.

Group	Treatment	Number of females	Animal numbers
5	Untreated	8	77-84

These adult females were undosed throughout the study. On PND 11 one male and one female offspring within each litter were assigned to each of the following dose groups: control; 5 mg/kg/day; 50 mg/kg/day; 150 mg/kg/day; 450 mg/kg/day. Offspring were dosed on PND 11 only.

The procedure for assigning pups to treatment groups was as follows:

For each litter 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 5 mg/kg/day group. The male and female pup with the third lowest identity numbers were assigned to the 50 mg/kg/day group. The male and female pup with the second highest identity numbers were assigned to the 150 mg/kg/day group. The male and female pup with the highest identity numbers were assigned to the 450 mg/kg/day group.

Group	Treatment	Dosage (mg/kg/day)#	Number of animals/sex	Animal numbers
6	control	0	16F	85-92C, 93-100D
			16M	201-208C, 209-216D
7	malathion	5	16F	101-108C, 109-116D
			16M	217-224C, 225-232D
8	malathion	50	16F	117-124C, 125-132D
			16M	233-240C, 241-248D
9	malathion	150	16F	133-140C, 141-148D
			16M	249-256C, 257-264D

# Expressed in terms of the test substance as supplied.  
F Females.  
M Males.

Eight young adult male and female rats (C) in each group were dosed for one day. The remaining 8 young adult males and females (D) in each group were dosed for 11 consecutive days.

Group	Treatment	Dosage (mg/kg/day)#	Number of animals/sex	Animal numbers
10	control	0	8F	265-272
			8M	281-288
11	malathion	450	8F	273-280
			8M	289-296

# Expressed in terms of the test substance as supplied.  
F Females  
M Males

Eight young adult male and female rats in each group were dosed for one day only.

The decision to investigate acute exposure to malathion at 450 mg/kg/day was taken after the animals for the study had arrived. While it was possible to increase the litter size of the Group 5 dams to allow for an extra dosage, there were insufficient young adult animals to accommodate the additional dosage. Further young adult animals were therefore ordered for the additional dosage (Group 11) and this investigation was conducted after dosing of the other young adults (Groups 6-9) had been completed. In view of this an additional corresponding control group (Group 10) was also employed.

## TREATMENT

### Test material

The malathion (alternative name Fyfanon Technical) used on the study was from batch number 9010501 supplied by the Sponsor. It was supplied as a clear pale pink liquid, 6 x 500 g of which was received at the Huntingdon Research Centre on 07 June 2000. Of this supply, approximately 149 g was used on this study.

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).

The test material was stored refrigerated at approximately 4 °C.

## Formulation

Fresh formulations were prepared each week. For each concentration the required amount of malathion was weighed out and mixed with a small amount of corn oil. Once thoroughly mixed the formulation was made up to the required volume using corn oil and then homogenised using a high shear homogeniser until homogenous. A bulk volume of each formulation 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

Prior to this study, the suitability of the proposed mixing procedure had been determined and, specimen 1 and 250 mg/ml formulations analysed to assess the stability of malathion formulations, as part of a Developmental Neurotoxicity Study (Huntingdon Life Sciences Report No. CHV066/013331). Specimen formulations were shown to be stable for up to 2 days at ambient temperature or 15 days at 4 °C.

Formulations at all concentrations were sampled during their first week of use. Dose formulations at 90 mg/ml were also sampled during the last week of use. Formulations at lower concentration should also have been sampled during their last week of use but these samples were not taken in error. Where formulations were sampled, samples were nominally 1 ml and 2 assays were made from each sample for determination of achieved concentration of malathion. Full details of the results obtained and the methodology employed are presented in the Formulation Chemistry Report (Addendum 4).

## Administration and treatment

Doses were administered to young 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. Controls received the vehicle (corn oil) at the same volume dosage as treated groups.

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

For mated dams, individual dose volumes were calculated from the most recently recorded scheduled bodyweight up to and including GD 17 after mating; thereafter the dosage volume remained constant to PND 1. From PND 1 of lactation 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 PND 1) were weighed and dose volume adjusted accordingly, both bodyweight and adjusted dose volume were recorded. Where PND 1 weighing was scheduled for later on the same day the earlier weight was accepted as the PND 1 weight.

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

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

For offspring, individual dose volumes were calculated from the bodyweight recorded prior to dosing.

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

For young adult males and females, individual dose volumes were calculated from the bodyweight recorded prior to dosing.

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

For the young adult males and females and mated dams, a daily record of the usage of each formulation 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 formulation usage were maintained but records were kept of volumes dosed to individual offspring.

## **SERIAL OBSERVATIONS (YOUNG ADULT ANIMALS 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**

Mated females were weighed on GD0, 3, 6, 10, 14, 17 and 20, then daily until parturition. For gestation, bodyweights are only presented up to GD 20. Weights recorded between GD 20 and parturition are not presented, as weights were not recorded for all animals owing to differences in the time of the start of parturition, but are retained with the raw data for the study. During lactation, females were weighed on PND 1, 4, 7, 11, 14, 17 and 21 (Group 5 females were killed on PND 11).

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

Young adult males and females (Groups 10-11) were weighed on the day before treatment commenced and on the day of treatment.

### **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. 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.

All litters were examined in detail once each day from PND 1 to PND 21 for numbers of live and dead pups and also for general clinical signs, e.g. pups cold to touch, pallor, no milk apparent in stomach, pups scattered in cage. Litters showing poor condition were examined more frequently. The detailed litter observations were performed as soon as possible in the morning after the parturition checks and PND 1 weighing/sexing of pups.

In addition, all dams and litters were subjected to an initial check in the morning and a final check as late as possible in the working day for dam and litter mortality.

#### **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;
- One to two hours after completion of dosing all groups;
- 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 where possible) on PND 4 for animals of Groups 1-4. For parental females in Group 5, litters were culled to 10 (5 males and 5 females) to enable 4 dosages with a control group to be investigated.



## Sex ratio

The sex of the offspring was determined on PND 1, 4 and 21.

## Bodyweight

Dosed offspring were weighed on PND 1, 4, 7, 11-21 and 28, then weekly until termination and on PND 60 (where appropriate). The daily weighing of dosed offspring during the treatment period allowed daily adjustment of the dose volume given, based on bodyweight. Group 5 offspring were weighed on PND 1, 4, 7 and 11. Non-dosed offspring were weighed individually prior to weaning on PND 1, 4, 7, 11, 14, 17 and 21.

## 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 PND 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

Blood and brain samples were obtained as follows:

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

For details relating to the duration of treatment can be found in the treatment groups section of these Materials and Methods.

Blood samples were collected from the retro-orbital sinus under light isoflurane anaesthesia (all adult animals including offspring at PND 21 and 60) or umbilical cord (GD 20 fetuses). For the PND 4 and 11 offspring, blood samples were obtained following decapitation. Lithium heparin was used as the anticoagulant and the sample volume was circa 0.7 ml.

For the adult animals and PND 21/60 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 resulting plasma samples and red cell haemolysates were stored at -80 °C prior to shipment packed in carbon dioxide ice to Huntingdon Research Centre for analysis. For the PND 4 and 11 offspring, 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 resulting plasma samples and red cell haemolysates were stored at -80 °C prior to shipment packed in carbon dioxide ice to Huntingdon Research Centre for analysis. Blood samples were pooled for all fetuses within each litter, providing one sample per litter; this procedure ensuring that sufficient sample was available for analyses from each litter. In a previous study utilising dimethoate (HLS Report No.: CHV068/000129) it had been demonstrated that there were no differences in blood and brain cholinesterase activity between control male and female fetuses at GD20.

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, this procedure ensuring that sufficient sample was available for analyses from each litter.

For the male and female pups that were dosed in each litter, following blood collection, the brains were removed and weighed at necropsy and brain cholinesterase activity measured. One pup, number 3014 in Group 2, was killed on GD 27 and was consequently replaced for assessment of cholinesterase activity by spare pup number 3703 from the same dosage group.

Individual brains (or each pool of brains for GD 20 fetuses) 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 carbon dioxide 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'-Dithiodinitrotic Acid (DTNA) as the colour component on a Hitachi 911 analyser.

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

## **Necropsy procedures**

### **GD 20 - dams and fetuses**

GD 20 the females were killed immediately after blood sampling 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 killed by chilling on a cool plate at approximately 0°C. Fetuses were then weighed and the brains dissected out and weighed as previously described in the section on cholinesterase analysis.

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.

### **Dams allocated for littering**

Dams allowed to litter were killed on PND 11 (Group 5) and PND 21 (Groups 1-4). All animals were killed by inhaled carbon dioxide and subjected to a detailed macroscopic necropsy. The animals were examined externally and internally for macroscopic abnormalities. The number of uterine implantation sites was recorded for the adult females.

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

Offspring at PND 4 and PND 11 (1 day of dosing) were killed by decapitation and a blood sample collected from the torso. Following decapitation for blood sampling the brain was removed, weighed and snap frozen in liquid nitrogen as previously described for cholinesterase investigations.

All 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 (11 days of dosing) 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.

### **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.

### **Young adult males and females on Day 1 or Day 11 of treatment and offspring on PND 60**

The following procedures were performed on young adult animals killed on day 1 (Groups 6-11) or Day 11 (Groups 6-9) of treatment and on offspring killed at 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 for cholinesterase investigations. The animals were subjected to a detailed macroscopic necropsy where they were examined externally and internally for macroscopic abnormalities. Specimens of any abnormal tissues were retained in appropriate fixative.

Offspring at PND 60 not required for cholinesterase investigations were necropsied as detailed above without the collection of blood or brain samples.

### **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 and therefore, it may not always be possible to calculate these values exactly by using values presented in the appendices. Due to an idiosyncrasy of some of the software used for data handling, in some circumstances where the calculated value is exactly half a unit different to that presented, values may be rounded down.

#### **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

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

Where these data are presented in the appendices of this report individual pups are identified using their unique four digit identity. This consists of the two digit dam/litter number followed by a two digit within litter identifier.

### **Bodyweight**

For the adult females in Groups 1-4, group mean values and SD were calculated for bodyweights during gestation and lactation. Group mean weight changes were also calculated and plotted graphically with respect to GD 6 and PND 1. For gestation, group mean values have been calculated for all pregnant animals. Group mean values during lactation were calculated using data for females with live young on PND 21 (Groups 1-4) regardless of whether their offspring were dosed or not.

No group mean bodyweights are presented for Group 5 dams as these animals were not dosed. Individual bodyweight data are presented in Addendum 5.

For all offspring in Groups 1-4, litter mean and SD values were calculated for males and females for each litter up to PND 11. Group mean values were calculated from the individual litter values. Group mean offspring weight change was calculated relative to PND 1.

For the dosed offspring in Groups 1-4, group mean and SD values were calculated for males and females up to PND 60. For the undosed offspring in Groups 1-4, group mean and SD values were calculated for males and females up to PND 21. Group mean values were calculated from the individual values. Group mean offspring weight change was calculated relative to PND 11.

No group mean bodyweights are presented for Group 5 offspring as these data were all recorded prior to dosing. Litter mean bodyweight data are presented in Addendum 5.

For the young adult males and females in Groups 6-9 that received 11 consecutive daily doses, group mean values and SD have been calculated. Group mean weight changes were also calculated and plotted graphically with respect to Day 1 of treatment. For the young adult males and females in Groups 6-11 that received only one dose, group mean values were not calculated as all bodyweights were taken prior to dosing.

### **Gestation index**

For Groups 1-4 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 PND 1 being the day of mating for calculation purposes. Where parturition had started overnight, this value was adjusted by subtracting half of one day.

Individual and group values are presented for Groups 1 to 4. Individual values for Group 5 dams are presented in Addendum 5.

### 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 values. Where the number of implantations exceeded the corpora lutea count, pre-implantation loss was assumed to be zero.

### 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

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

@ after culling

### **Brain weights**

#### **Day 20 dams and fetuses**

Group mean values and SD were calculated for absolute weights (dams) and also for pooled fetal weights.

#### **Brain weights for young adult animals on days 1 or 11 of treatment, and offspring on PND 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.

For offspring of litter 78 assessment of the data for PND 11 indicated that an identification error had occurred within this litter for two male offspring (7802 receiving 5 mg/kg/day and 7805 receiving 450 mg/kg/day). Within each litter used to assess offspring cholinesterase activities at PND 11, one male and one female offspring were present from each dosage. It is believed that the two animals were either wrongly identified during sampling or their samples wrongly identified during processing or analyses, with the end results that activity levels for 7802 were reported as being from 7805 and vice versa. While it was not possible to identify where the error had occurred the results obtained, when compared with the results of other animals at same dosages, showed unequivocally that such an error had been made. In view of this it was considered appropriate by the Study Director to assign these data to the dose level/animals in which it is believed they occurred.

### **Statistical evaluation**

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

Significance tests were performed on the following parameters, and results are presented in relevant tables of this report:

Bodyweight and bodyweight change of adult males and females; bodyweight and bodyweight change of females during gestation and lactation; litter data, fetal weights, litter weights and brain weights for dams and fetuses on GD 20; litter size, offspring survival indices and percentage male sex ratio to PND 11; Male and female offspring bodyweight and bodyweight change up to PND 11; Dosed and undosed male and female offspring bodyweight and bodyweight change; Adult brain weights on day 1 and day 11 of treatment; brain weights for male and female offspring on PND 4, 11, 21 and 60.

These data were analysed using the following criteria, with comparisons being made to concurrent control:

If Bartlett's test for variance homogeneity (Bartlett 1937) was not significant at the 1% level, then parametric analysis was applied. Analysis of variance (Snedecor and Cochran 1967) was first employed followed by F1 test for monotonicity of dose-response (Healey 1999). If the F1 test for monotonicity of dose-response was not significant at the 1% level, Williams' test for a monotonic trend (Williams 1971, 1972) was applied. If the F1 test was significant, suggesting that the dose-response was not monotone, Dunnett's test (Dunnett 1955, 1964) was performed instead.

If Bartlett's test was significant at the 1% level, then logarithmic and square-root transformations were tried. If Bartlett's test was still significant, then non-parametric tests were applied. Kruskal-Wallis test (Snedecor and Cochran 1967) was first employed followed by H1 test for monotonicity of dose-response (Healey 1999). If the H1 test for monotonicity of dose-response was not significant at the 1% level, Shirley's test for a monotonic trend (Shirley 1977) was applied.

Where 75% or more of the values for a given variable were 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.  $p < 0.05$ ) or non-parametric equivalent.

It was noted that variance tended to increase with group mean for erythrocyte cholinesterase for PND 11 and 21 offspring which suggested that transformation of the data may have been appropriate. It was further noted that Bartlett's test for variance homogeneity was generally higher for erythrocyte cholinesterase values following square root transformation, indicating that this analyses following such transformation was more appropriate. With the exception of values for male offspring however, there were no difference between any statistical significance obtained so results from the standard statistical screen have been reported, although the difference for male PND 21 offspring was taken into consideration when interpreting the data.



## RESULTS

### **FORMULATION CHEMISTRY** (Addendum 4)

The mean concentrations for malathion in test formulations analysed during the study were within  $\pm 9\%$  of nominal values confirming accurate formulation.

Full details of the methodology used and the results obtained are presented in the Formulation Chemistry report.

### **GENERAL TOXICITY FINDINGS**

#### **Parent females** (5, 50 or 150 mg/kg/day)

##### **Clinical signs and mortality** (Appendix 1 )

There were no general clinical signs observed that were considered to be related to treatment. Signs were largely 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 1-4, Tables 1-4, Appendices 2-3)

There were no effects of treatment on bodyweight and bodyweight gain during gestation and lactation.

##### **Gestation length and gestation index** (Figure 5, Table 5, Appendix 4)

All animals allocated to the littering phase gave birth to a live litter and there was no effect of treatment on gestation length.

##### **Necropsy findings** (Appendix 5)

Macroscopic examination of females at necropsy on GD 20 or PND 21 revealed no findings that were considered to be related to treatment.

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

##### **Brain weights for dams on GD 20** (Table 8, Appendix 9)

There were no effects of treatment on maternal brain weights at GD 20.

##### **Fetal observations at GD 20** (5, 50 or 150 mg/kg/day)

##### **Litter data** (Tables 6-7, Appendices 7-8)

There was no adverse effect of treatment on litter data, as assessed by the mean number of implantations, early and late fetal deaths, and live young. Sex ratio, as assessed by percentage male, was similar in all groups. Group mean fetal weights and mean litter weights in treated groups were comparable to the control.

**Brain weights (Table 8, Appendix 9)**

There were no effects of treatment on fetal brain weights at GD 20.

**Offspring to PND 11** (5, 50 or 150 mg/kg/day)**Litter data up to PND 11** (Tables 9-11, Appendices 10-12)

There was no effect of treatment of the parent female on implantation count, total litter size at PND 1 and subsequent offspring survival to PND 11 of age.

Offspring sex ratio was essentially similar in all groups.

**Clinical signs for dosed male and female offspring**

There were no general clinical signs observed in offspring to PND 11 that were considered to be related to treatment of the parent females.

**Bodyweights and bodyweight change** (Figures 6-9, Tables 12-15, Appendices 12-14)

There were no effects of treatment of the parent female on bodyweight of the offspring on PND 1 or on subsequent bodyweight gain to PND 11.

**Necropsy findings for offspring on PND 4** (Appendix 15)

Necropsy of offspring at PND 4 revealed no findings that were considered to be related to treatment.

**Brain weights for offspring on PND 4** (Tables 16, Appendices 16)

Mean absolute brain weights of offspring at PND 4 did not indicate any effect of treatment for either sex.

**Directly dosed offspring at PND 11 – 1 day of dosing** (5, 50, 150 or 450 mg/kg/day)**Clinical signs** (Appendix 17)

Clinical observations for these offspring were restricted as animals were killed for cholinesterase investigations 2 hours after dosing.

Post dosing observations were restricted to offspring receiving 450 mg/kg/day. Five of the 16 neonates at this dosage showed tremors between 1-2 hours after dosing, and a further animal was found moribund one hour after dosing and killed early for reasons of animal welfare.

**Necropsy findings** (Appendix 18)

Necropsy of offspring revealed no findings that were considered to be related to treatment.

**Young adult males and females – 1 day of dosing** (5, 50, 150 or 450 mg/kg/day)**Clinical signs**

Clinical observations for these offspring were restricted as animals were killed for cholinesterase investigations 2 hours after dosing.

No clinical signs or post dosing observation considered to be associated with treatment were observed for these animals.

**Necropsy findings** (Appendix 19)

Necropsy of animals on Day 1 of treatment showed no findings that were considered to be related to treatment.

**Directly dosed offspring at PND 21 – 11 days of dosing** (5, 50, or 150 mg/kg/day)**Clinical signs**

There were no general clinical signs observed that were considered to be related to direct treatment of offspring. Survival of these offspring was good and the isolated mortalities that occurred did not indicate any treatment-related effect on survival.

**Bodyweight change** (Figures 10-13, Tables 17-20, Appendices 21-22)

Direct dosing of the offspring from PND 11 to weaning (PND 21) had no obvious adverse effect on bodyweight gain.

**Necropsy findings** (Appendix 25)

Necropsy of offspring at PND 21 revealed no findings that were considered to be related to treatment.

**Brain weights** (Tables 26, Appendices 27)

Mean absolute brain weights of offspring at PND 21 did not indicate any effect of treatment for either sex.

**Young adult males and females – 11 days of dosing** (5, 50, or 150 mg/kg/day)**Clinical signs and mortality** (Appendix 28)

There were no general clinical signs that were considered to be related to treatment and all animals survived to termination.

Post-dosing signs were restricted to transient salivation, occurring principally at 150 mg/kg/day where all animals were affected. At 50 mg/kg/day post-dosing salivation was only observed for two animals, both on one single occasion.

**Bodyweight change** (Figures 18-21, Tables 27-30, Appendices 29-30)

There were no significant effects of treatment on overall bodyweight gain of young adult males at dosages up to 150 mg/kg/day.

For females at 150 mg/kg/day a small, transient reduction in bodyweight gain was observed, compared to control, however subsequent bodyweight gains were comparable with control and overall bodyweight gain was considered to be unaffected by treatment.

At 5 and 50 mg/kg/day bodyweight and bodyweight gain for females were comparable to control.

#### **Necropsy findings (Appendix 31)**

Necropsy of animals on day 11 of treatment showed no findings that were considered to be related to treatment.

#### **Brain weights (Tables 32, Appendices 33)**

There was no effect of treatment on absolute brain weight on day 11 of treatment for either sex.

#### **Offspring to PND 60 (5, 50 or 150 mg/kg/day)**

##### **Clinical signs**

There were no general clinical signs observed in offspring to PND 60 that were considered to be related to either treatment of the parent female or direct treatment of offspring during PND 11 to 21. Survival of these offspring was good and the isolated mortalities that occurred did not indicate any treatment-related effect on survival.

##### **Bodyweight change (Figures 10-13, Tables 17-20, Appendices 21-22)**

Bodyweight gain for offspring to PND 60 was unaffected by treatment of the parent female or direct dosing of the offspring from PND 11 to weaning (PND 21). None of the inter-group differences observed was considered to be of biological significance.

##### **Necropsy findings (Appendix 34)**

Necropsy of offspring at PND 60 revealed no findings that were considered to be related to treatment.

##### **Brain weights (Tables 33, Appendices 35)**

Mean absolute brain weights of offspring at PND 60 did not indicate any effect of treatment for either sex.

#### **EFFECTS ON PLASMA, ERYTHROCYTE AND BRAIN CHOLINESTERASE ACTIVITY**

##### **Dams at GD 20 - 5, 50 or 150 mg/kg/day (Tables 34, Appendices 36)**

Marked inhibition of erythrocyte cholinesterase activity (51%) was apparent at 150 mg/kg/day compared to control, and inhibition was also evident (19%) at 50 mg/kg/day. Plasma and brain cholinesterase activities were apparently unaffected.

Neither plasma, erythrocyte or brain cholinesterase activities were unaffected by treatment at 5 mg/kg/day.

**Fetuses at PND 20 - 5, 50 or 150 mg/kg/day (Tables 35, Appendices 37)**

Among fetuses at 150 and 50 mg/kg/day there was evidence of a slight inhibition (15% and 14% respectively) of plasma cholinesterase activity and inhibition (19% and 11% respectively) of erythrocyte cholinesterase activity, compared with control, while no effect was evident at 5 mg/kg/day. Fetal brain cholinesterase activity was unaffected at all dosages.

**Offspring on PND 4 - 5, 50 or 150 mg/kg/day (Tables 36, Appendix 38)**

By PND 4 offspring plasma, erythrocyte and brain cholinesterase activities were similar in all groups and there were no effects associated with treatment of the parent female.

**Offspring on PND 11 – 1 day of dosing - 5, 50, 150 or 450 mg/kg/day (Tables 37, Appendix 39)**

Treatment of offspring on PND 11 with a single dose of malathion at 450 mg/kg/day was associated with a very marked inhibition in plasma, erythrocyte and brain cholinesterase activities (52-54%, 61-72% and 81-84% respectively) compared with control. At 150 mg/kg/day inhibition of plasma, erythrocyte and brain cholinesterase activities was also apparent (approximately 35-36%, 48-55% and 44-48% respectively). At 50 mg/kg/day plasma and erythrocyte activities were lower than Control (16-19% and 23-35% respectively), although brain cholinesterase activity was unaffected.

Plasma, erythrocyte and brain cholinesterase activities at 5 mg/kg/day were not considered to have been adversely affected by treatment. Although erythrocyte cholinesterase activity for males at this dosage was lower (16%) than control, the majority of values (5 out of 8) were within the control range. It is considered that the mean control value was influenced by one animal (No. 8101) that showed particularly high cholinesterase activity; when these data were analysed with this atypical control value excluded no statistical significance was attained for males at 5 mg/kg/day. When viewed within the context of offspring erythrocyte cholinesterase levels in the study as a whole, and in the absence of any effect in the females at this dosage, this difference was not considered to be of any biological or toxicological significance.

**Young adult males and females - 1 day of dosing - 5, 50, 150 or 450 mg/kg/day (Tables 38, Appendix 40)**

A single administration of malathion at 450 mg/kg/day was associated with inhibition of plasma cholinesterase activity in males (24%) and erythrocyte cholinesterase activity (17-25%) in both sexes, compared with the concurrent control. At lower dosages it was not considered that blood cholinesterase activities were affected by treatment.

Brain cholinesterase activity was unaffected by a single administration of malathion at any of the dosages investigated.

**Offspring on PND 21 – 11 days of dosing - 5, 50 or 150 mg/kg/day (Tables 39, Appendix 41)**

After eleven consecutive doses at 150 mg/kg/day inhibition of plasma cholinesterase activity (24-32%) was slightly less than was observed on PND 11 following a single administration, while inhibition of erythrocyte cholinesterase activity (67-68%) appeared to be increased. Inhibition of brain cholinesterase activity (16%) was also observed for both sexes at 150 mg/kg/day but again at lower level than observed following a single dose at PND 11.

After eleven consecutive doses at 50 mg/kg/day inhibition of plasma cholinesterase activity remained at a similar level (19%) as was observed on PND 11 following a single administration, although again inhibition of erythrocyte cholinesterase activity (34-39%) appeared to be increased. Brain cholinesterase activity was comparable to control and unaffected by treatment.

There was no clear effect of treatment following 11 consecutive doses on plasma, erythrocyte or brain cholinesterase activities at 5 mg/kg/day. Mean erythrocyte cholinesterase activity for males was noted to be lower than control, but only 3 animals were below the concurrent control range. The standard statistical analysis screen employed indicated statistical significance at this dosage, however further assessment of the data showed that the P-level was higher for Bartlett's test for variance homogeneity following square root transformation, indicating that statistical analyses after this transformation was more appropriate. When analysed using this procedure no statistical significance was attained for male offspring at 5 mg/kg/day. When viewed within the context of offspring erythrocyte cholinesterase levels in the study as a whole, and in the absence of any effect in the females at this dosage, this difference was not considered to be of any biological or toxicological significance.

**Young adult males and females - 11 day of dosing - 5, 50 or 150 mg/kg/day (Tables 40, Appendix 42)**

Plasma cholinesterase activity was not considered to have been affected by 11 days of treatment at any of the dosages investigated. Although for males at 150 and 50 mg/kg/day values when compared to control attained statistical significance, the percentage inhibition (11-13%) was small and similar to those of the female and were not considered to be of any biological or toxicological significance.

Inhibition of erythrocyte cholinesterase activity was apparent in both sexes after eleven consecutive doses of malathion at 150 or 50 mg/kg/day (43-48% and 20% respectively) compared with Control. Erythrocyte cholinesterase activity was unaffected by treatment at 5 mg/kg/day.

Brain cholinesterase activity was similar to control and was not considered to have been affected by 11 days of treatment at any of the dosages investigated.

**Offspring on PND 60 - 5, 50 or 150 mg/kg/day (Tables 41, Appendix 43)**

For offspring at PND 60, 39 days after the end of repeat dosing (PND 21), there were no statistically significant differences from control in plasma, erythrocyte and brain cholinesterase activities.

## CONCLUSION

Inhibition of erythrocyte cholinesterase activity in dams receiving 150 mg/kg/day was markedly greater than for their fetuses at GD 20. At lower dosages, the magnitude of effects on blood cholinesterase activities was generally similar in both dams and fetuses, with inhibition occurring at 50 mg/kg/day. Brain cholinesterase activity was unaffected at dosages up to 150 mg/kg/day. A dosage of 5 mg/kg/day was considered to be the no-observed-adverse-effect level (NOAEL) for blood cholinesterase activities and 150 mg/kg/day for brain cholinesterase activity.

Cholinesterase activities for PND 4 offspring were unaffected by treatment of the parent female at dosages up to 150 mg/kg/day.

Inhibition of blood cholinesterase activities of PND 11 offspring following a single dose administration was apparent at 450, 150 and 50 mg/kg/day. Brain cholinesterase activity was affected at 450 and 150 mg/kg/day and some offspring at 450 mg/kg/day showed body tremors. A dosage of 5 mg/kg/day was considered to be the no-observed-adverse-effect level (NOAEL) for blood cholinesterase activities and 150 mg/kg/day for brain cholinesterase activity for the PND 11 offspring. The reaction of young adult animals to a single dose was less marked with effects on blood cholinesterase activities only being observed at 450 mg/kg/day, and no inhibition being apparent for brain cholinesterase activity. A dosage of 150 mg/kg/day was considered to be the no-observed-adverse-effect level (NOAEL) for blood cholinesterase activities for young adult rats to a single dose administration, with the NOAEL for brain cholinesterase activity being 450 mg/kg/day.

Inhibition of blood cholinesterase activities of PND 21 offspring (following 11 days of dosing) was apparent at 150 and 50 mg/kg/day, with brain cholinesterase activity also being affected at 150 mg/kg/day. While inhibition of erythrocyte cholinesterase activity compared with values at PND 11 (following a single dose) increased, inhibition of both plasma and brain cholinesterase activities decreased at 150 mg/kg/day. A dosage of 5 mg/kg/day was considered to be the no-observed-adverse-effect level (NOEL) for blood cholinesterase activities for the PND 21 offspring and 50 mg/kg/day the NOAEL for brain cholinesterase activity. For young adults, following 11 days of dosing, effects on cholinesterase activities were again less than observed in similarly treated offspring (PND 21); inhibition being restricted to erythrocyte cholinesterase activity at 150 and 50 mg/kg/day. No inhibition of plasma or brain cholinesterase activities was observed. A dosage of 5 mg/kg/day was considered to be the no-observed-adverse-effect level (NOAEL) for blood cholinesterase activities for young rats following 11 days of dosing, with the NOAEL for brain cholinesterase activity being 150 mg/kg/day.

The overall no-observed-adverse-effect level (NOAEL) was considered to be 5 mg/kg/day for plasma/erythrocyte cholinesterase activities and 50 mg/kg/day for brain cholinesterase activity. The most sensitive period for effects on brain cholinesterase activity was found to be PND 11. No effects of treatment were detected in offspring at PND 4 following maternal treatment at 150 mg/kg/day, or in offspring at PND 60, following 11 days of direct treatment at 150 mg/kg/day and 39 days of recovery.

## REFERENCES

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FIGURE 1

Bodyweight - group mean values (g) during gestation

Group	:	6	7	8	9
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

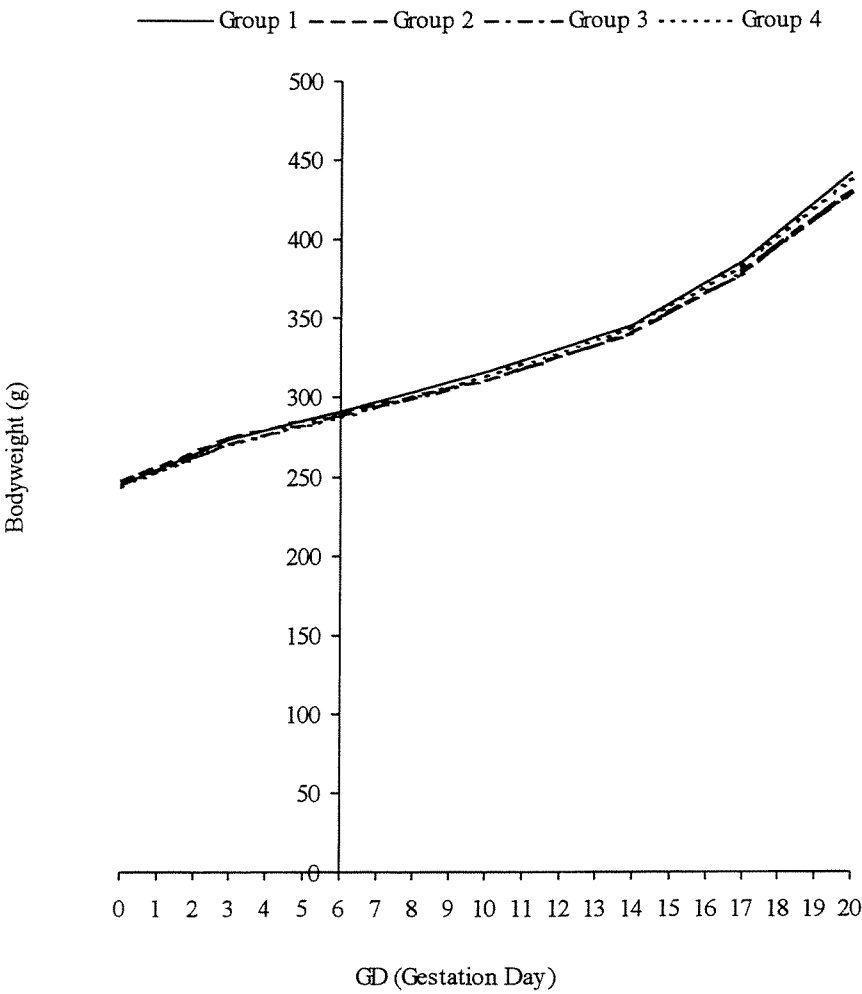


FIGURE 2

Bodyweight change - group mean values (g) during gestation

Group	:	6	7	8	9
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

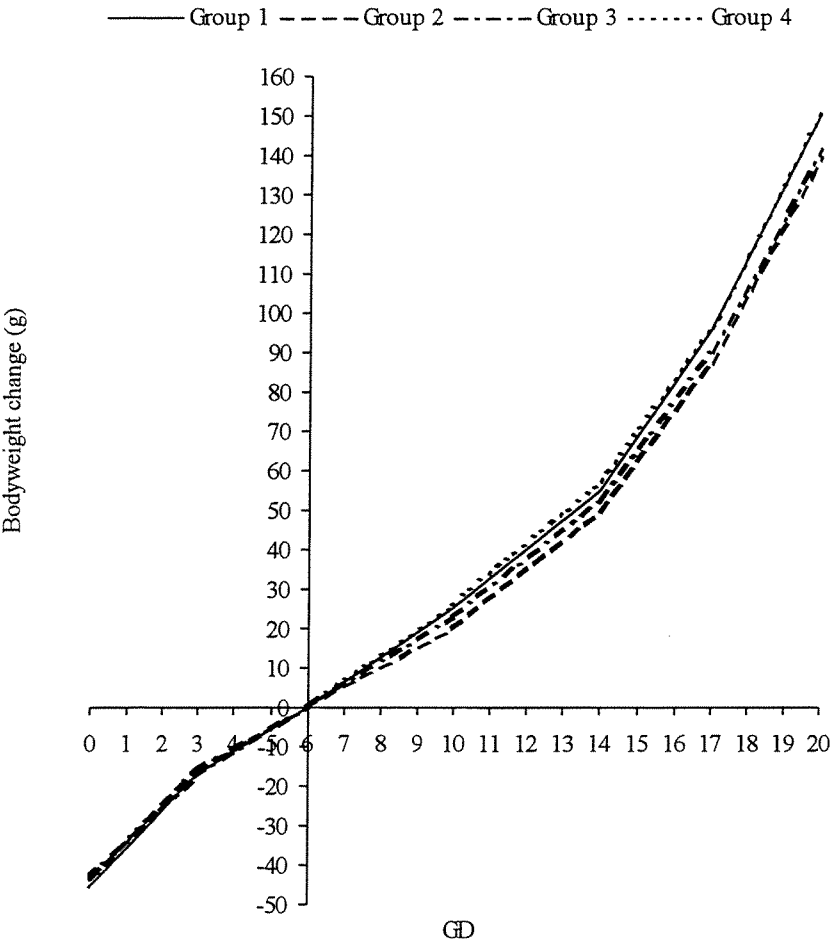


FIGURE 3

Bodyweight – group mean values (g) during lactation

Group	:	1	2	3	4
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

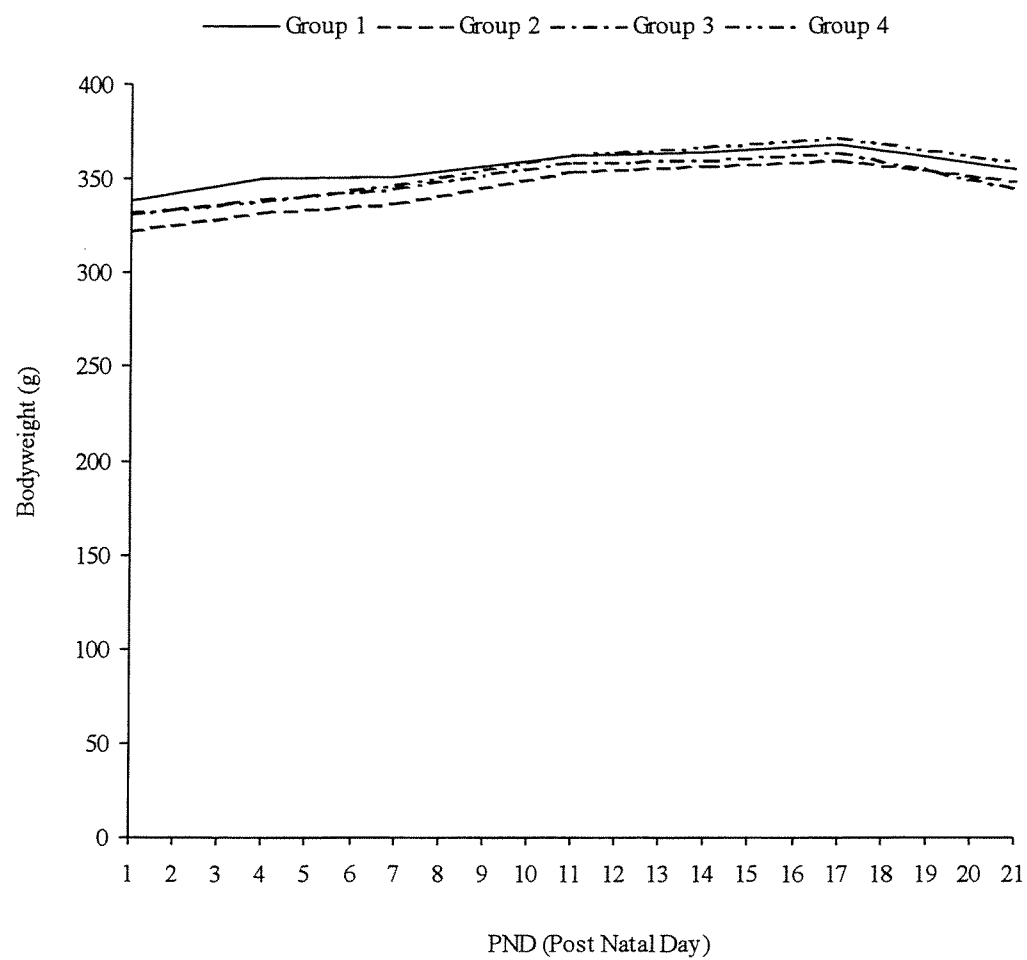


FIGURE 4

Bodyweight change – group mean values (g) during lactation

Group	:	1	2	3	4
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

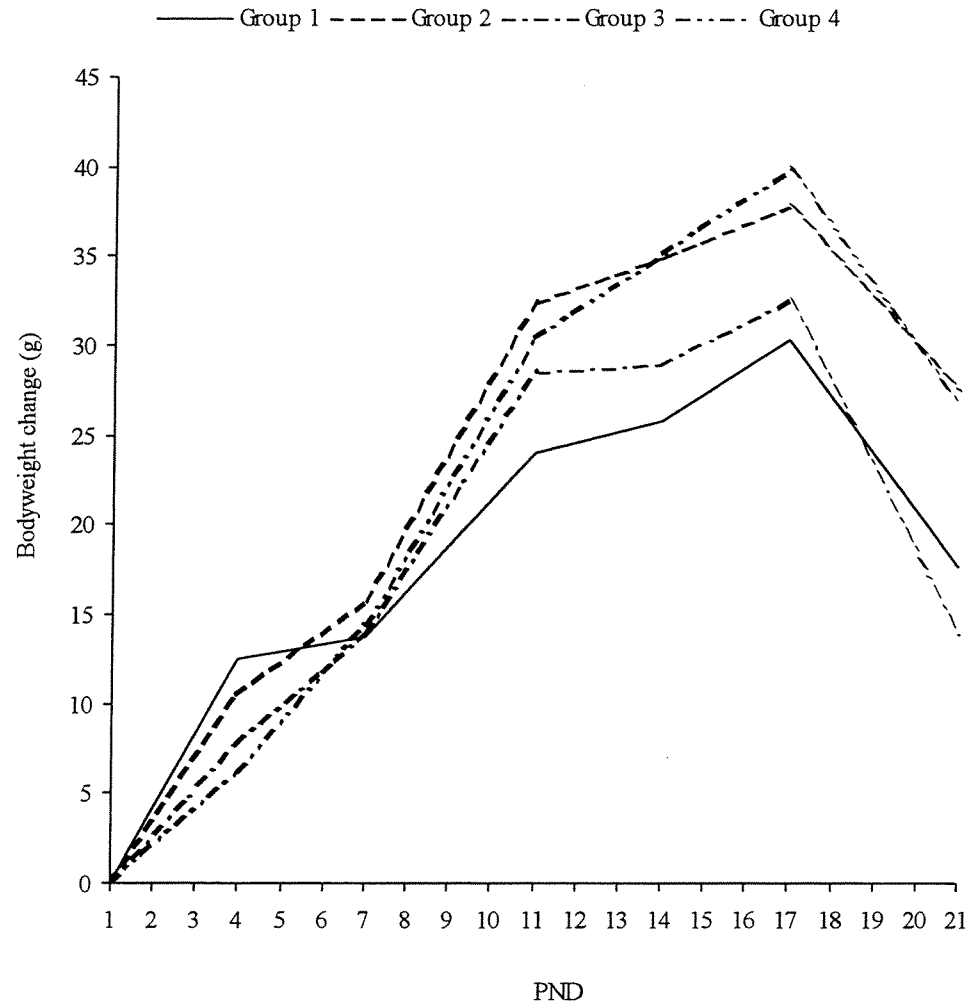


FIGURE 5

Gestation length – group distribution

Group	:	1	2	3	4
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

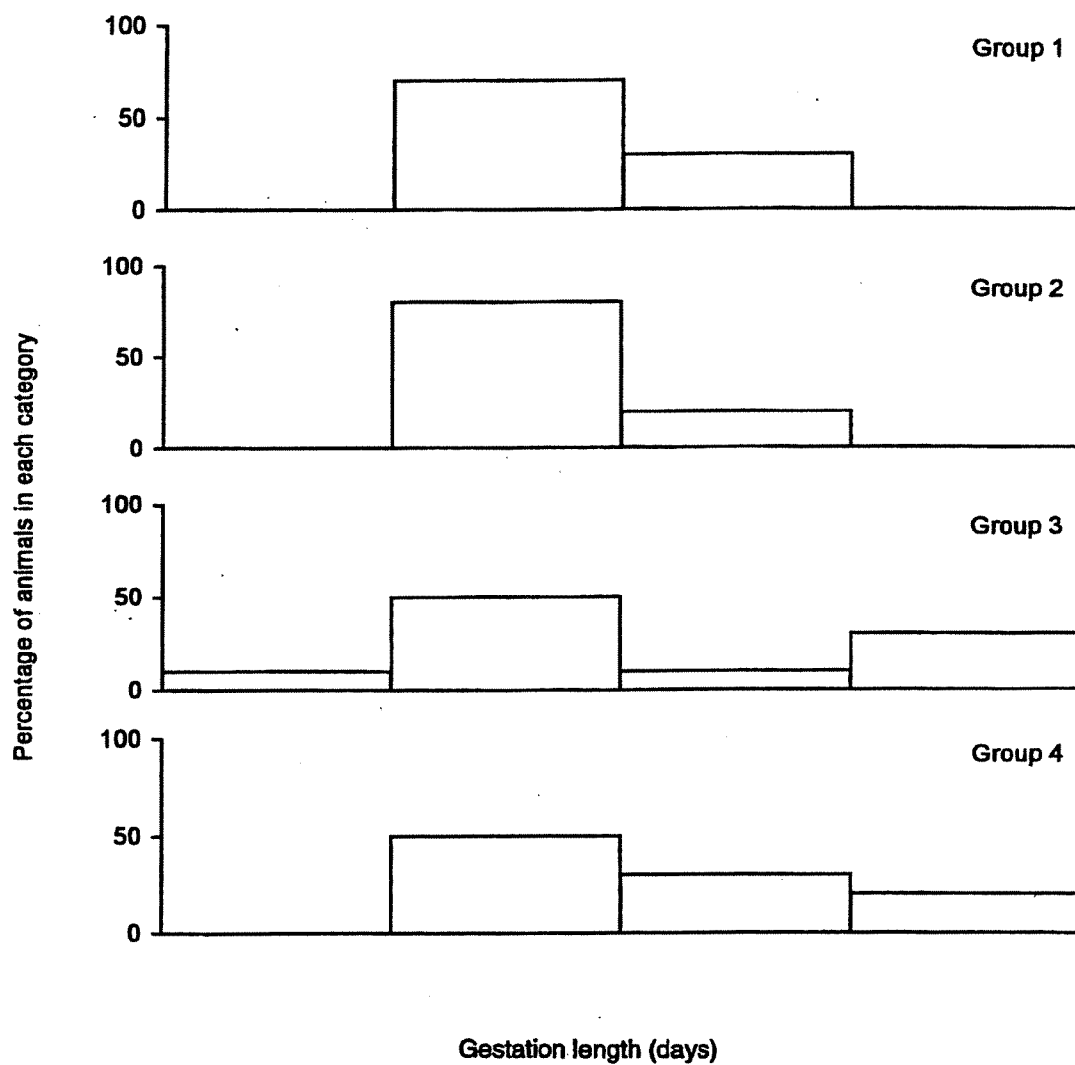


FIGURE 6

Bodyweight up to PND 11– group mean values (g) for male offspring

Group	:	1	2	3	4
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

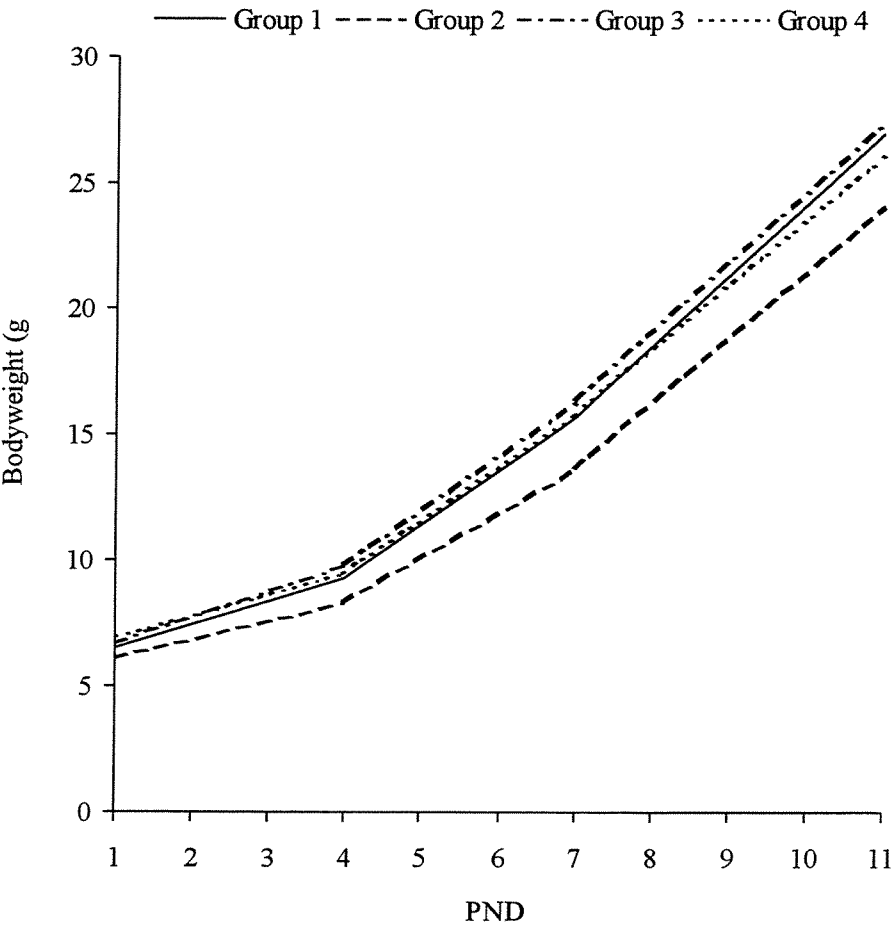


FIGURE 7

Bodyweight change up to PND 11– group mean values (g) for male offspring

Group	:	1	2	3	4
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

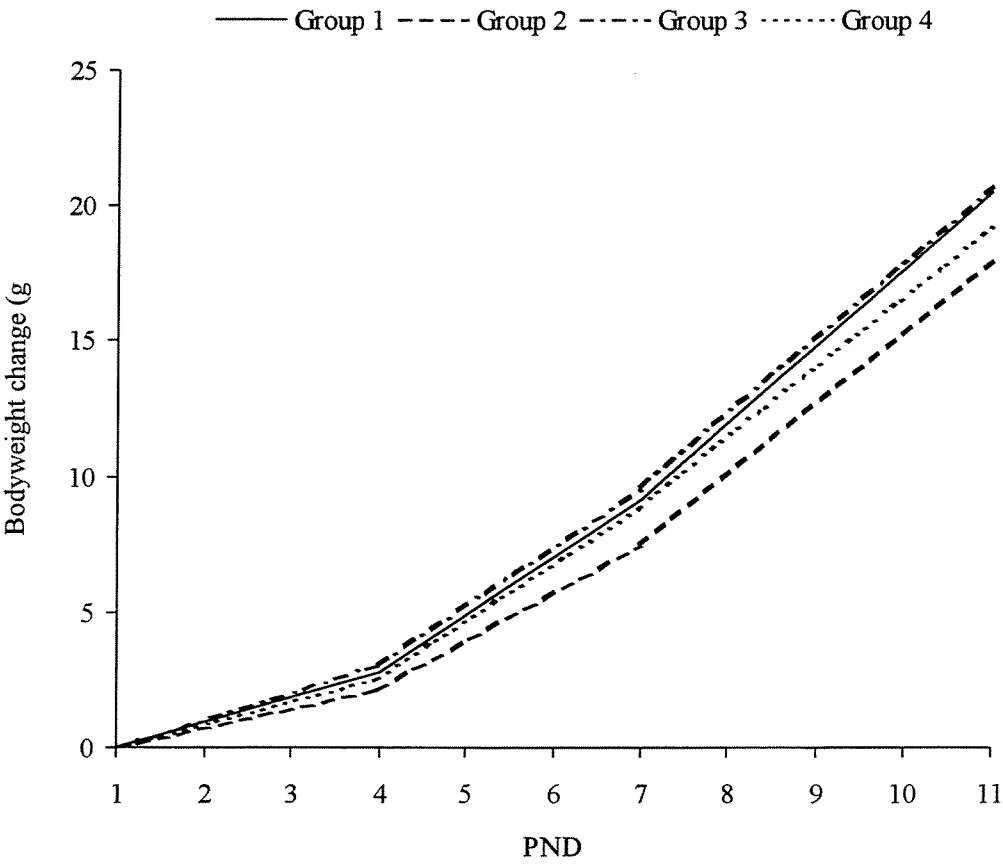


FIGURE 8

Bodyweight up to PND 11– group mean values (g) for female offspring

Group	:	1	2	3	4
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

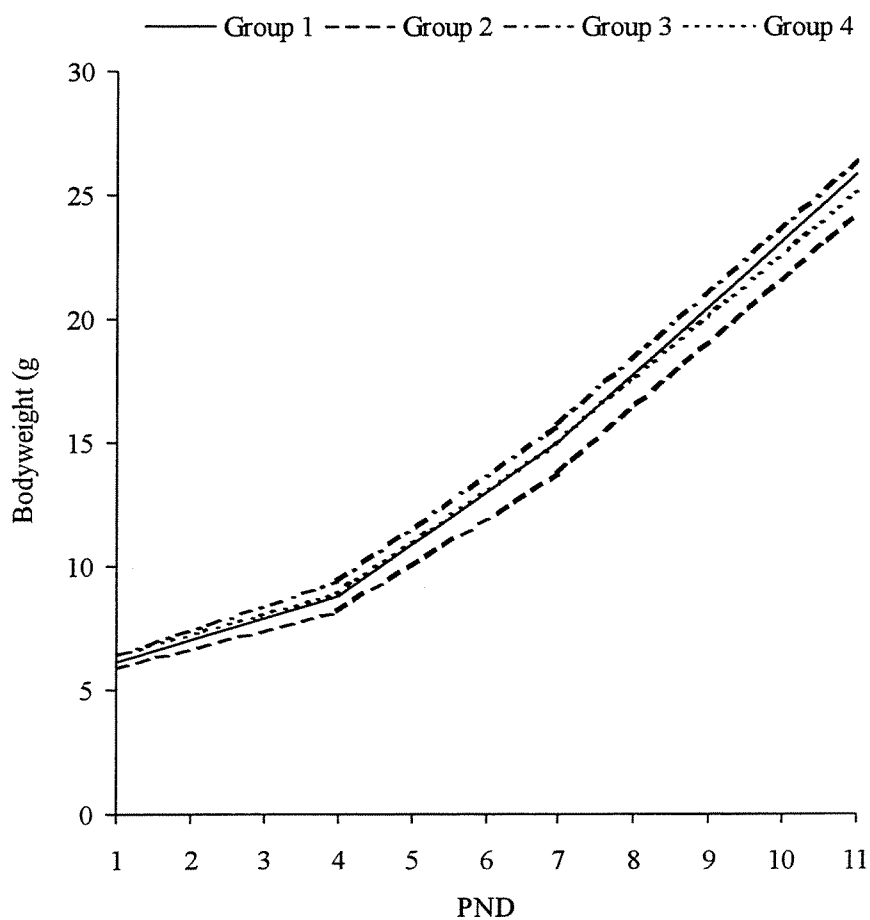




FIGURE 9

Bodyweight change up to PND 11– group mean values (g) for female offspring

Group	:	1	2	3	4
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

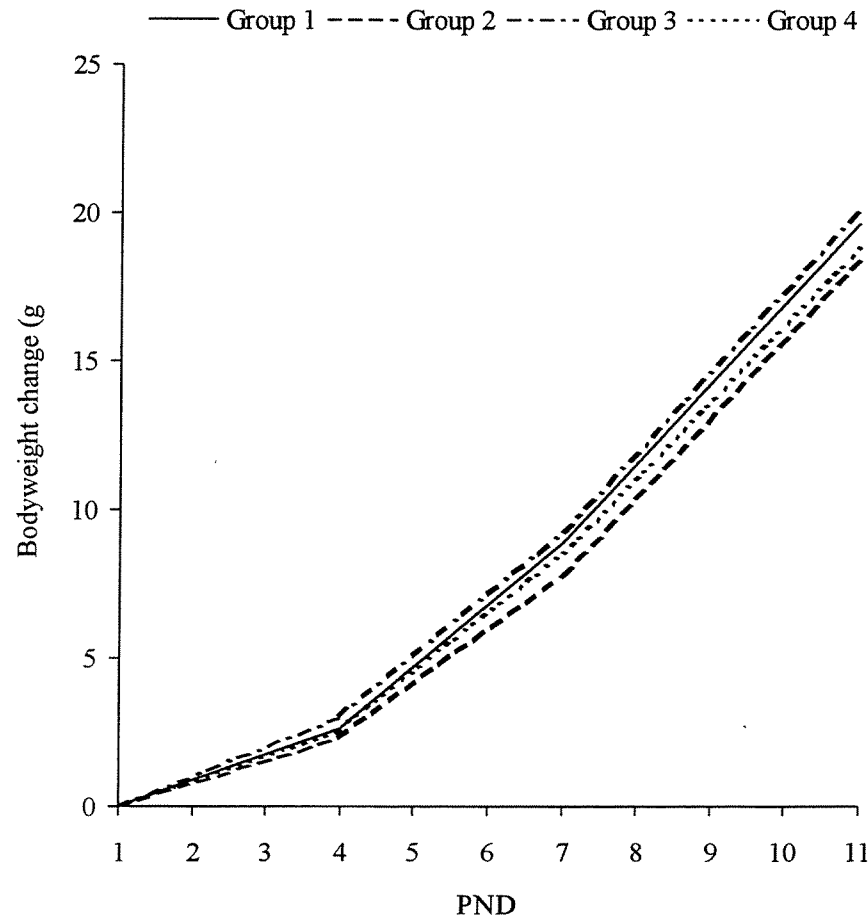


FIGURE 10

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

Group	:	1	2	3	4
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

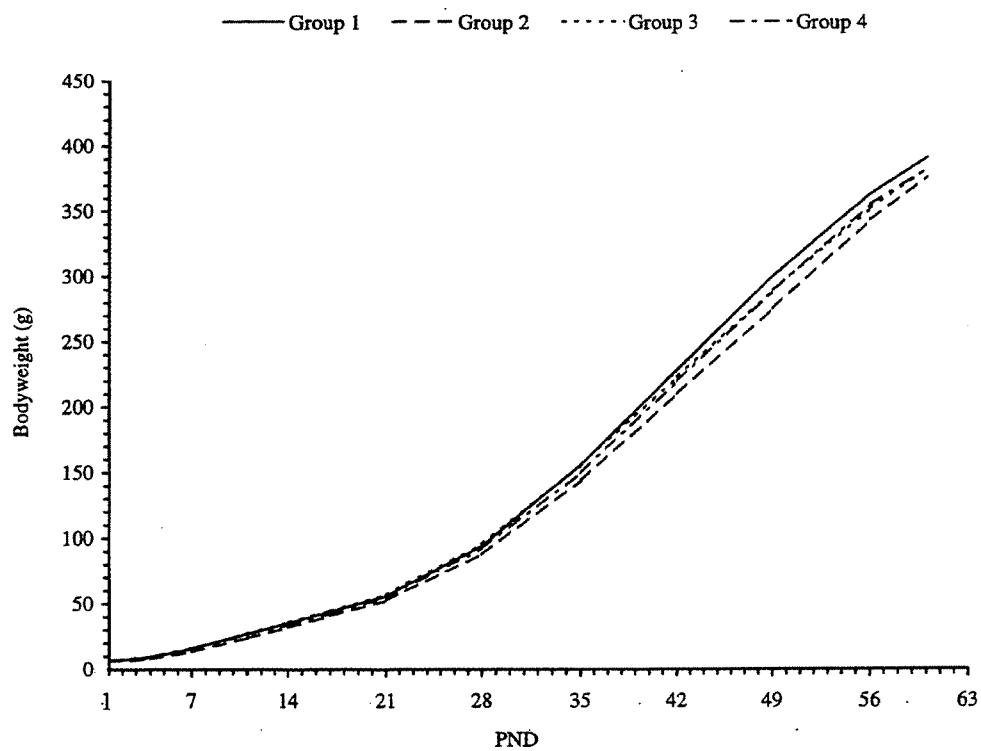


FIGURE 11

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

Group	:	1	2	3	4
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

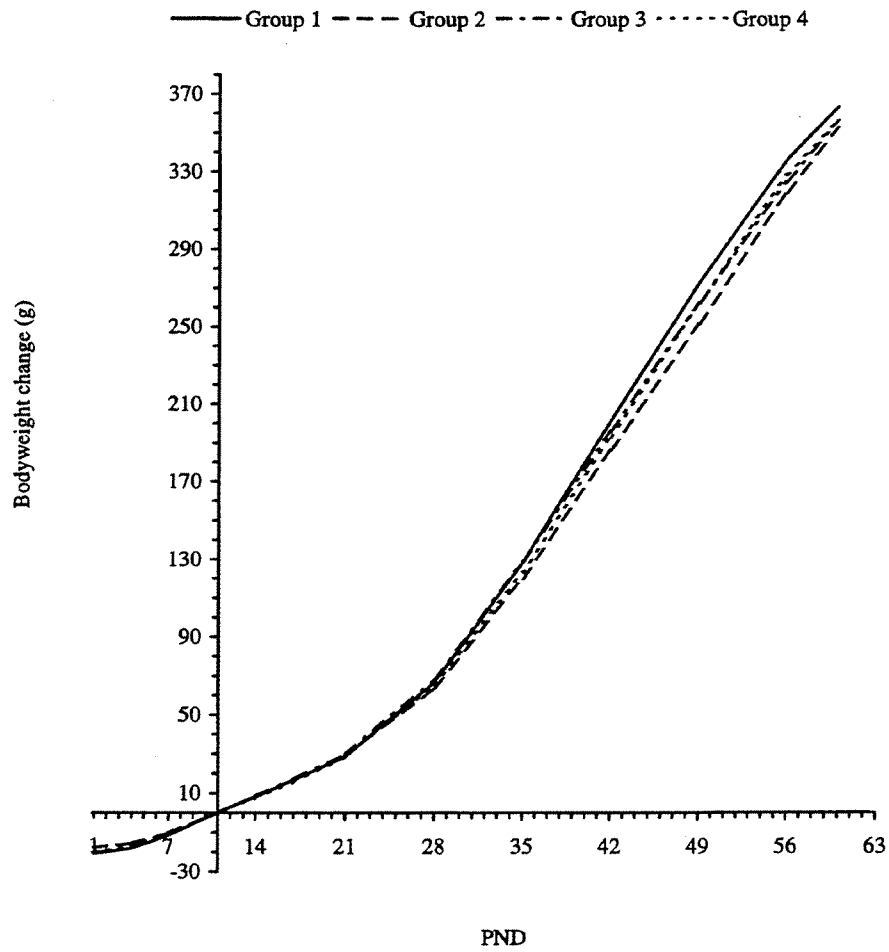


FIGURE 12

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

Group	:	1	2	3	4
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

—— Group 1    - - - - Group 2    ..... Group 3    - . - . - Group 4

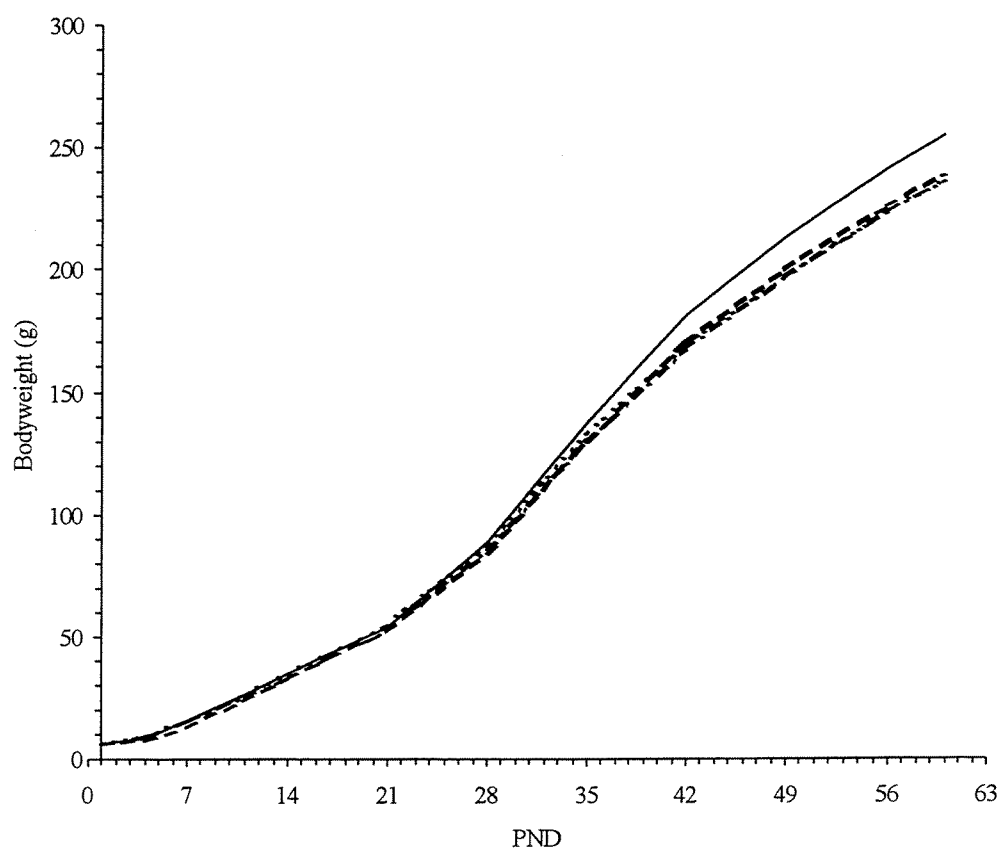


FIGURE 13

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

Group	:	1	2	3	4
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

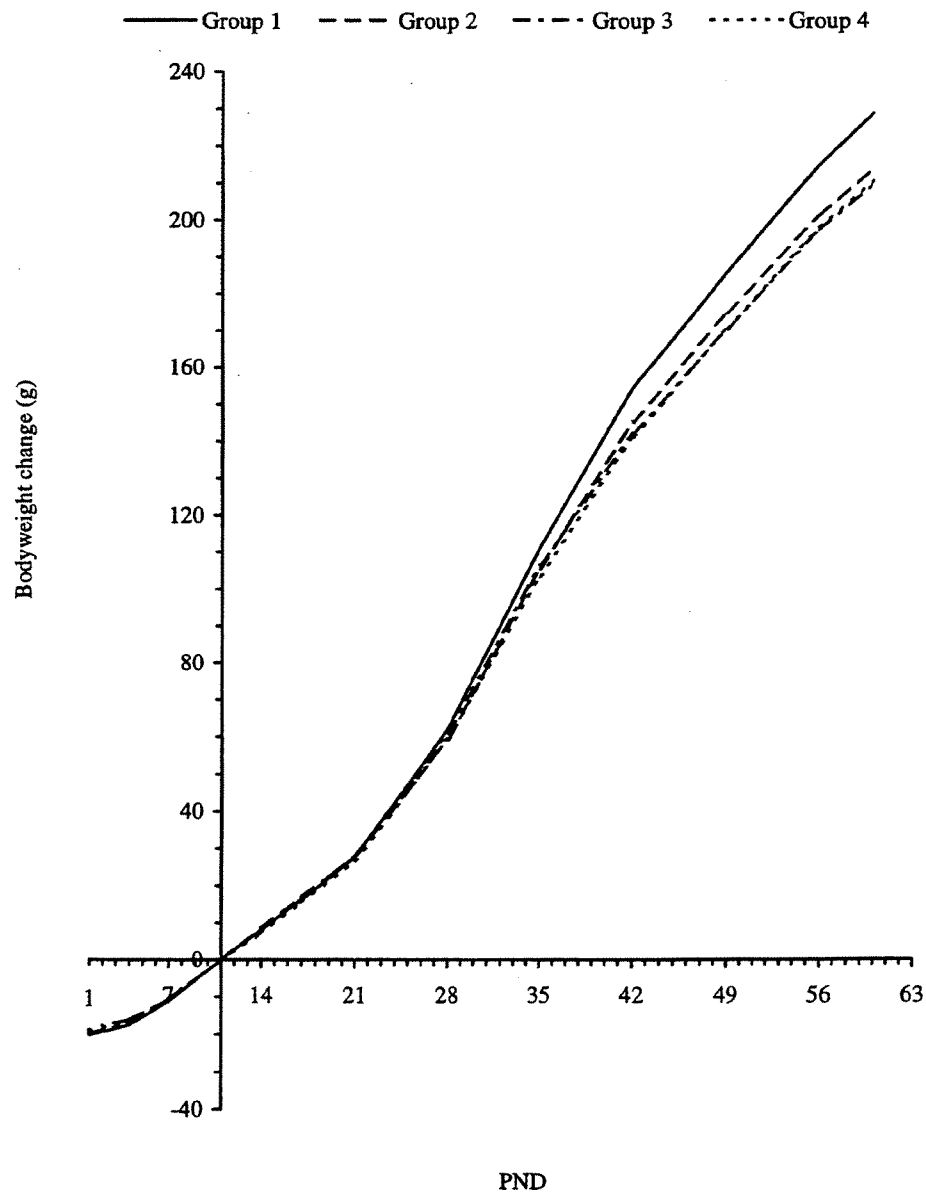


FIGURE 14

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

Group	:	1	2	3	4
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

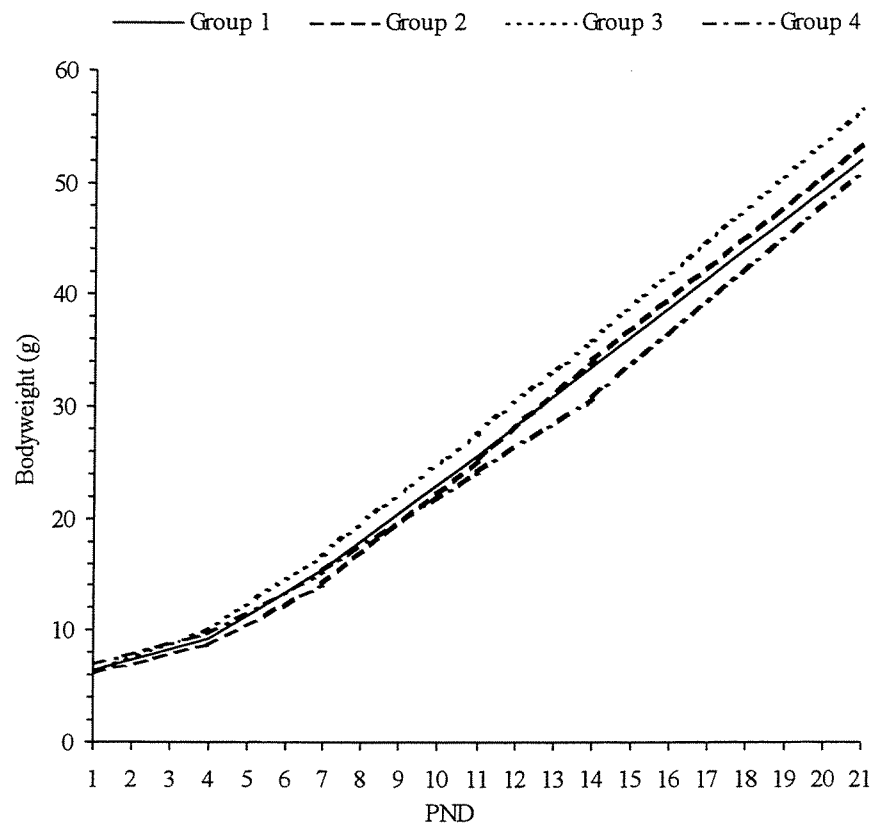


FIGURE 15

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

Group	:	1	2	3	4
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

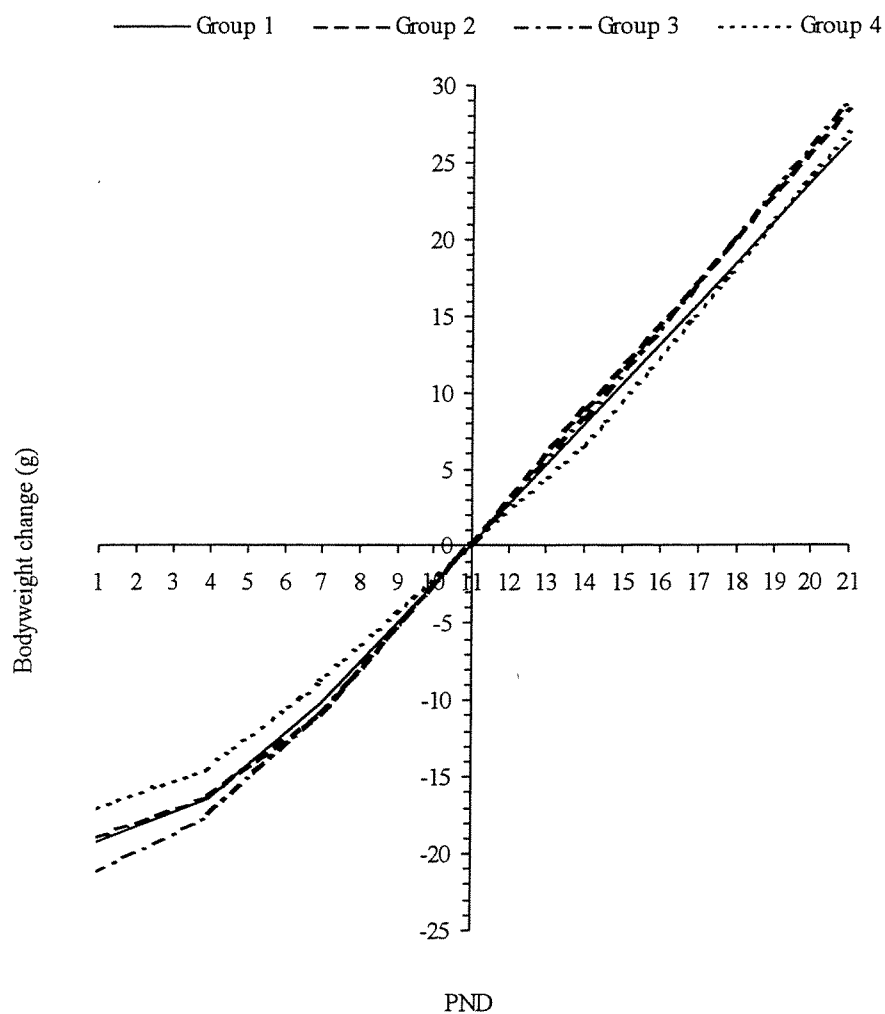


FIGURE 16

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

Group	:	1	2	3	4
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

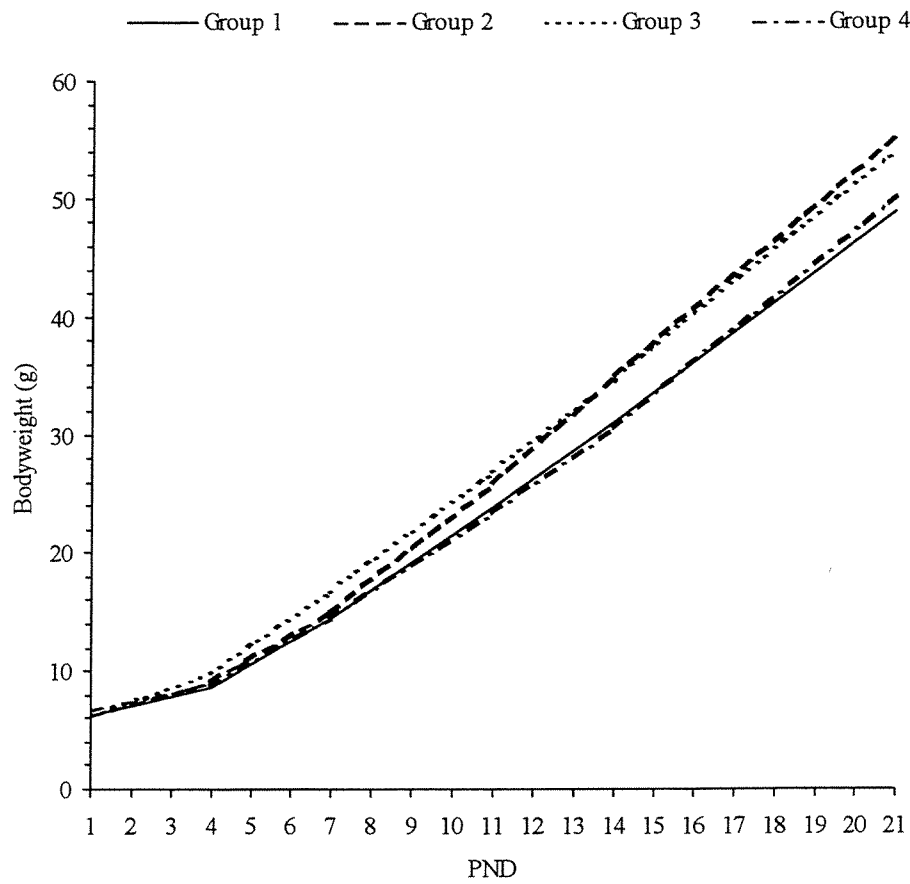




FIGURE 17

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

Group	:	1	2	3	4
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

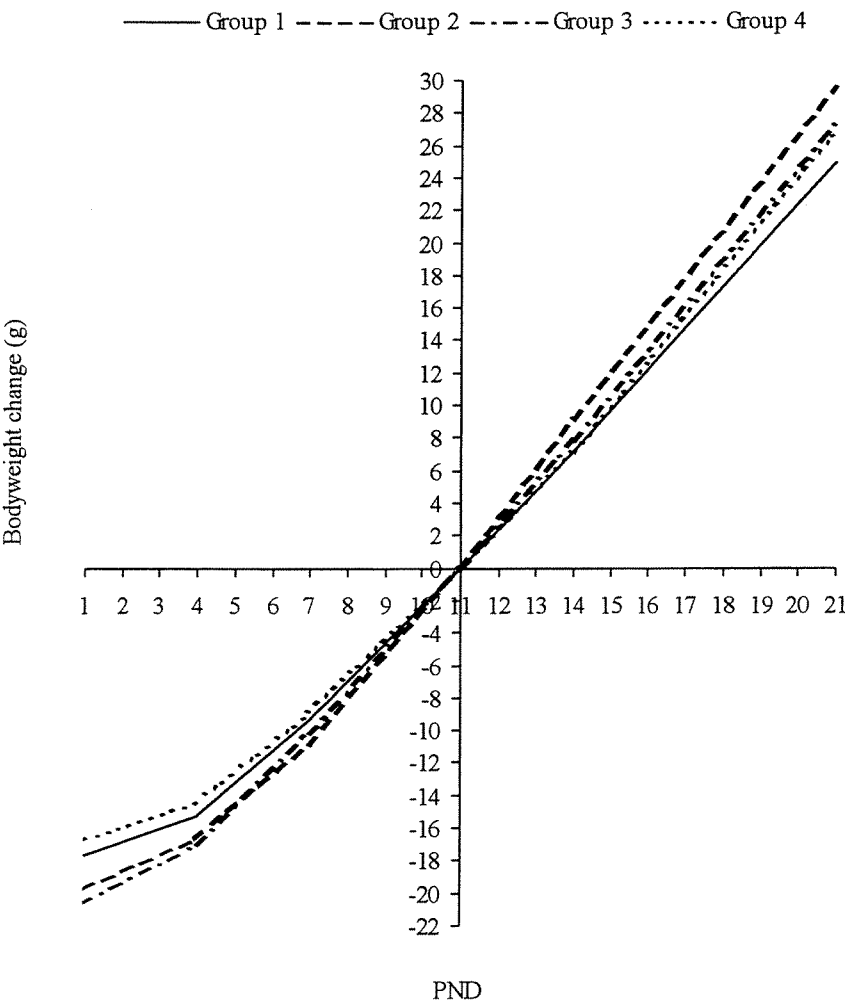


FIGURE 18

Bodyweight – group mean values (g) for young adult males

Group	:	1	2	3	4
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

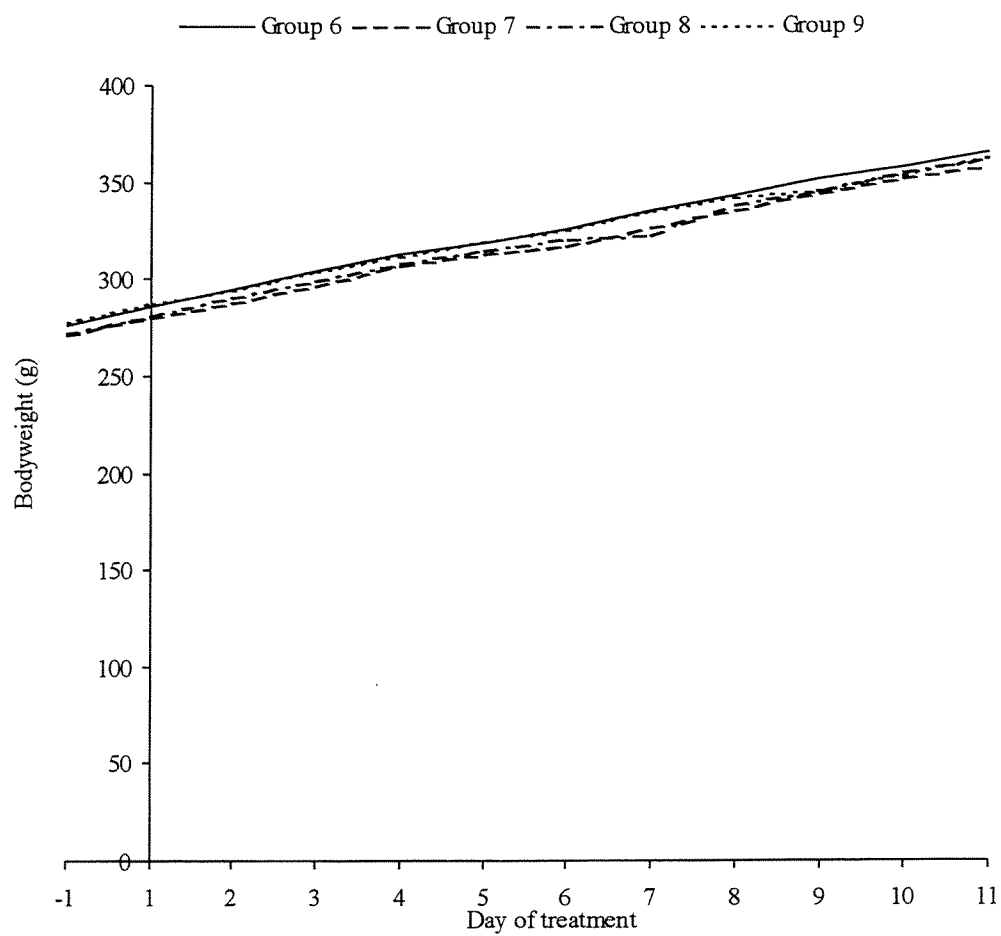


FIGURE 19

Bodyweight change – group mean values (g) for young adult males

Group	:	1	2	3	4
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

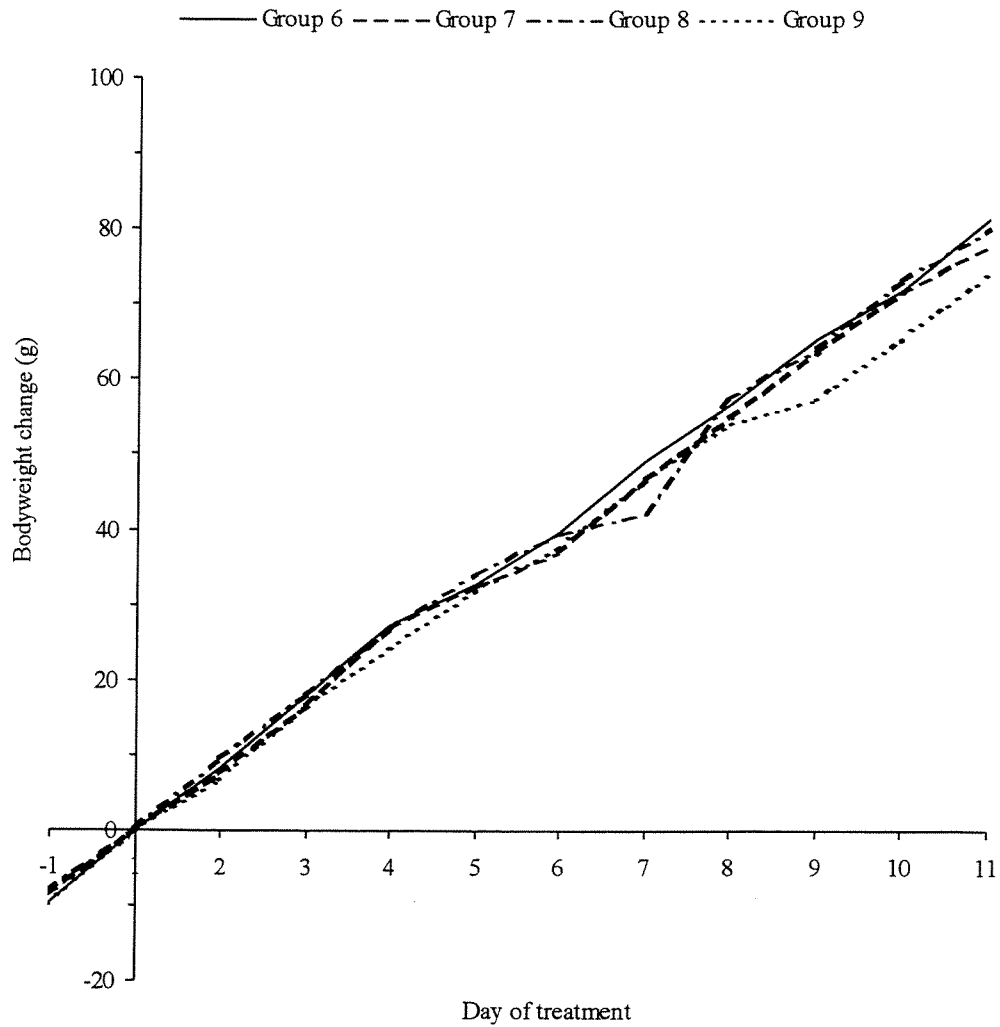


FIGURE 20

Bodyweight – group mean values (g) for young adult females

Group	:	1	2	3	4
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

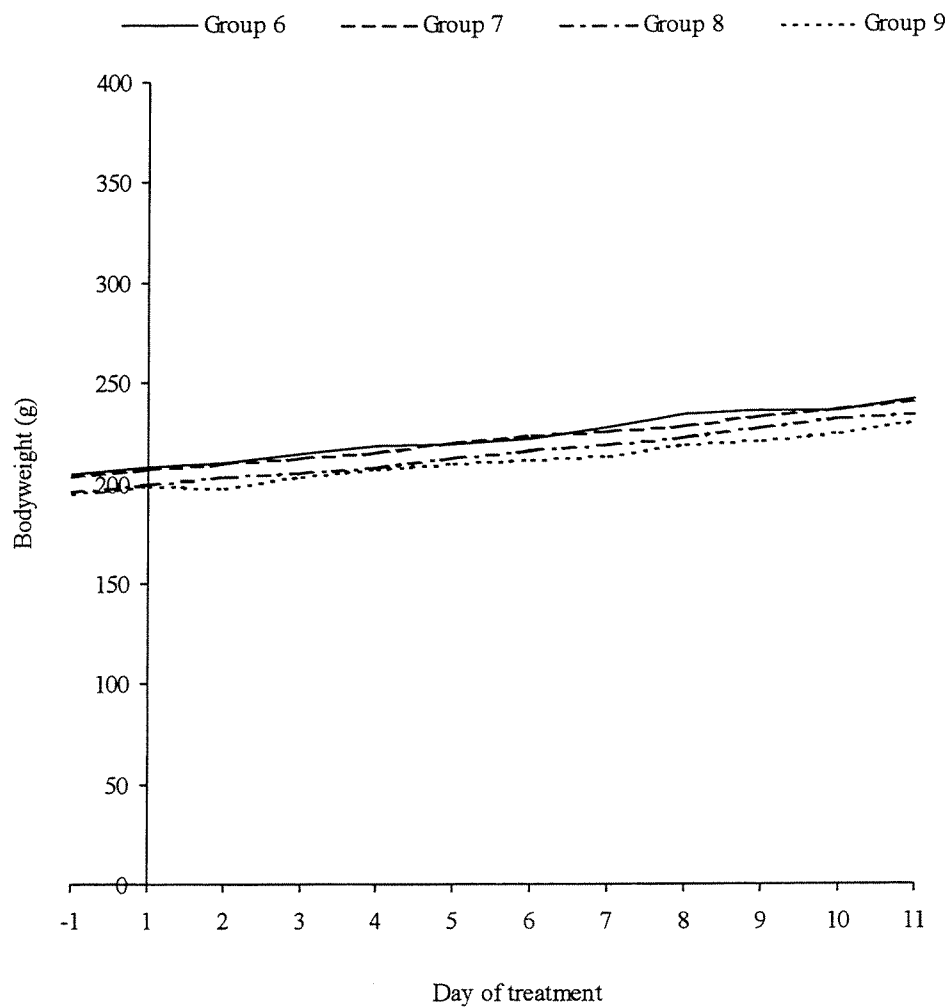


FIGURE 21

Bodyweight change – group mean values (g) for young adult females

Group	:	1	2	3	4
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

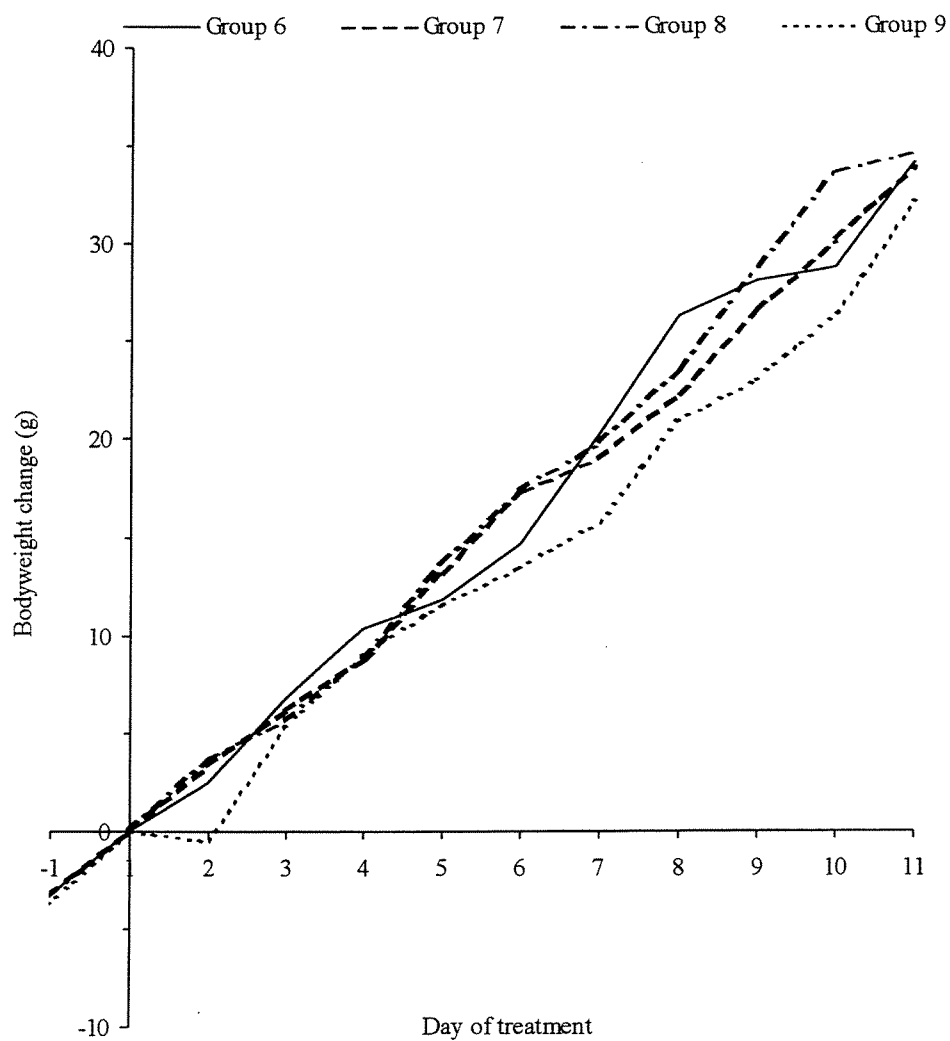


TABLE 1

Bodyweight – group mean values (g) for dams during gestation

Group : 1 2 3 4  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group		GD (gestation day)									
		0	3	6	10	14	17	20			
1	Mean	245	274	291	316	346	386	441			
	SD	19	20	22	23	25	29	33			
	n	19	19	19	19	19	19	19			
2	Mean	247	275	291	311	340	378	430			
	SD	14	14	16	18	20	22	27			
	n	19	19	19	19	19	19	19			
3	Mean	245	271	288	311	341	378	429			
	SD	12	12	12	13	17	20	24			
	n	19	19	19	19	19	19	19			
4	Mean	244	271	287	313	344	384	438			
	SD	12	11	14	17	19	23	26			
	n	19	19	19	19	19	19	19			

Treatment commenced GD 6.

SD Standard deviation.

n Number of animals.

No statistical significance ( $p>0.05$ ).

TABLE 2

Bodyweight change - group mean values (g) for dams during gestation

Group : 1 2 3 4  
Compound : control -----malathion-----  
Dosage (mg/kg/day) : 0 5 50 150

Group		GD							
		0-6	3-6	6-10	6-14	6-17	6-20		
1	Mean	46	17	25	55	96	151		
	SD	6	4	3	6	10	17		
	n	19	19	19	19	19	19		
2	Mean	44	16	20b	49	87	139		
	SD	8	5	6	9	12	17		
	n	19	19	19	19	19	19		
3	Mean	44	18	23	52	90	141		
	SD	5	4	6	8	12	15		
	n	19	19	19	19	19	19		
4	Mean	43	16	26	57	96	151		
	SD	7	4	5	8	12	16		
	n	19	19	19	19	19	19		

Treatment commenced GD 6.

SD Standard deviation.

n Number of animals.

Statistical significance: b-p<0.01.

TABLE 3

Bodyweight – group mean values (g) for dams during lactation

Group Compound Dosage (mg/kg/day)		PND (post natal day)										
		-----malathion-----										
		1	4	7	11	14	17	21	25	28	31	35
1	Mean	338	350	352	362	364	368	356				
	SD	26	27	22	30	30	23	29				
	n	10	10	10	10	10	10	10				
2	Mean	322	332	338	354	357	360	350				
	SD	17	18	18	20	21	22	18				
	n	10	10	10	10	10	10	10				
3	Mean	331	339	345	360	360	364	345				
	SD	24	22	21	25	26	24	24				
	n	10	10	10	10	10	10	10				
4	Mean	332	339	347	363	368	372	359				
	SD	23	21	21	25	28	21	25				
	n	10	10	10	10	10	10	10				

Treatment period: GD 6 to PND10.

SD Standard deviation.

n Number of animals.

No statistical significance ( $p > 0.05$ ).



TABLE 4

Bodyweight change - group mean values (g) for dams during lactation

Group : 1 2 3 4  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group		PND									
		1-4	1-7	1-11	1-14	1-17	1-21				
1	Mean	13	14	24	26	30	18				
	SD	11	9	11	18	12	13				
	n	10	10	10	10	10	10				
2	Mean	11	16	32	35	38	28				
	SD	13	11	10	13	13	11				
	n	10	10	10	10	10	10				
3	Mean	8	14	29	29	33	14				
	SD	7	10	12	9	13	10				
	n	10	10	10	10	10	10				
4	Mean	6	14	30	35	40	27				
	SD	9	8	10	12	11	15				
	n	10	10	10	10	10	10				

Treatment period: GD 6 to PND10.

SD Standard deviation.

n Number of animals.

No statistical significance ( $p>0.05$ ).

TABLE 5

Gestation length and gestation index – group values

Group : 1 2 3 4  
Compound : control -----malathion-----  
Dosage (mg/kg/day) : 0 5 50 150

Group	Number of pregnant animals		Gestation length (days)				Number of live litters born	Gestation index (%)
			21.5	22	22.5	23		
1	10	n (%)	0	7 (70)	3 (30)	0	10	100
2	10	n (%)	0	8 (80)	2 (20)	0	10	100
3	10	n (%)	1 (10)	5 (50)	1 (10)	3 (30)	10	100
4	10	n (%)	0	5 (50)	3 (30)	2 (20)	10	100

Treatment period: GD 6 to PND10.  
n Numbers of animals in category.

TABLE 6

Litter data - group mean values on GD 20

Group Compound Dosage (mg/kg/day) 1 control 2 3 4  
-----malathion-----  
0 5 50 150

Group		Corpora Lutea	Implantations	Resorptions			Live young			Sex ratio (% M)	Implantation loss (%)	
				Early	Late	Total	Male	Female	Total		Pre-	Post-
1	Mean	19.1	17.0	0.9	0.0	0.9	8.2	7.9	16.1	51.3	9.2	5.2
	SD	4.3	1.7				1.9	2.3	2.1			
	n	9	9	9	9	9	9	9	9	9	9	9
2	Mean	18.6	16.9	1.0	0.1	1.1	7.4	8.3	15.8	47.9	8.5	6.7
	SD	2.6	1.1				2.1	2.8	2.0			
	n	9	9	9	9	9	9	9	9	9	9	9
3	Mean	16.0a	16.0	0.6	0.0	0.6	8.2	7.2	15.4	53.4	1.3	3.5
	SD	1.5	1.4				1.7	1.9	1.7			
	n	9	9	9	9	9	9	9	9	9	9	9
4	Mean	16.1a	16.3	0.6	0.0	0.6	7.9	7.9	15.8	50.2	2.0	3.5
	SD	1.3	1.1				1.8	2.0	1.6			
	n	9	9	9	9	9	9	9	9	9	9	9

Treatment commenced GD 6.

SD Standard deviation.

n Number of litters.

Statistical significance: a-p&lt;0.05.

TABLE 7

Fetal and litter weights - group mean values (g) on GD 20

Group : 1 2 3 4  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group		Litter Weight	Fetal weights		
			Males	Females	Overall
1	Mean	64.58	4.10	3.92	4.01
	SD	7.93	0.18	0.15	0.17
	n	9	9	9	9
2	Mean	61.35	3.98	3.84	3.90
	SD	7.37	0.22	0.22	0.22
	n	9	9	9	9
3	Mean	60.36	4.04	3.79	3.92
	SD	5.40	0.26	0.21	0.23
	n	9	9	9	9
4	Mean	62.65	4.09	3.86	3.97
	SD	7.66	0.28	0.26	0.27
	n	9	9	9	9

Treatment commenced GD 6.

SD Standard deviation.

n Number of litters.

No statistical significance ( $p \geq 0.05$ ).

TABLE 8

Brain weights - group mean values (g) for dams and fetuses on GD 20

Group : 1 2 3 4  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group		Brain weight	
		Dam	Fetuses
1	Mean	1.958	0.158
	SD	0.04	0.02
	n	8	8
2	Mean	1.937	0.167
	SD	0.06	0.01
	n	8	8
3	Mean	1.916	0.154
	SD	0.10	0.01
	n	8	8
4	Mean	1.939	0.167
	SD	0.06	0.01
	n	8	8

Treatment commenced GD 6.

SD Standard deviation.

n Number of dams / litters.

No statistical significance ( $p>0.05$ ).

TABLE 9

Litter size up to PND 11 - group mean values

Group : 1 2 3 4  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group		Implants	Total litter size PND 1	Live litter size on PND						
				Before cull			After cull			
				1	4	4	4	7	11	11
1	Mean	16.3	15.0	15.0	14.7	8.0	8.0	8.0	8.0	8.0
	SD	1.8	1.6	1.6	1.8	0.0	0.0	0.0	0.0	0.0
	n	10	10	10	10	10	10	10	10	10
2	Mean	17.0	15.7	15.7	15.0	8.0	8.0	8.0	8.0	8.0
	SD	1.7	1.8	1.8	2.4	0.0	0.0	0.0	0.0	0.0
	n	10	10	10	10	10	10	10	10	10
3	Mean	15.6	13.9	13.9	13.8	8.0	8.0	8.0	8.0	8.0
	SD	1.8	1.6	1.6	1.5	0.0	0.0	0.0	0.0	0.0
	n	10	10	10	10	10	10	10	10	10
4	Mean	15.1	14.2	14.2	14.1	8.0	8.0	8.0	8.0	8.0
	SD	1.9	1.9	1.9	2.0	0.0	0.0	0.0	0.0	0.0
	n	10	10	10	10	10	10	10	10	10

Dams dosed GD6 to PND 10.

SD Standard deviation.

n Number of litters.

No statistical significance ( $p>0.05$ ).

TABLE 10

Offspring survival indices up to PND 11 – group mean values

Group : 1 2 3 4  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group		Post-implantation survival index (%)	Live birth index (%)	Viability index (%)	Lactation index (%)	
					PND 7	PND 11
1	Mean n	92.2 10	100.0 10	97.9 10	100.0 10	100.0 10
2	Mean n	92.3 10	100.0 10	95.2 10	100.0 10	100.0 10
3	Mean n	89.3 10	100.0 10	99.4 10	100.0 10	100.0 10
4	Mean n	94.3 10	100.0 10	99.2 10	100.0 10	100.0 10

Dams dosed GD6 to PND 10.

n Number of litters.

No statistical significance ( $p > 0.05$ ).

TABLE 11

Sex ratio up to PND 11 – group mean values

Group : 1 2 3 4  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group	Total			Live (before cull)						Live (after cull)					
	PND 1			PND 1			PND 4			PND 4			PND 7		
	M	F	%M	M	F	%M	M	F	%M	M	F	%M	M	F	%M
1	Mean	7.5	7.5	50.6	7.5	7.5	50.6	7.2	7.5	49.5	4.0	4.0	50.0	4.0	4.0
	SD	1.6	2.4	12.6	1.6	2.4	12.6	1.7	2.4	12.7	0.0	0.0	0.0	0.0	0.0
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
2	Mean	7.4	8.3	47.3	7.4	8.3	47.3	7.0	8.0	47.3	4.0	4.0	50.0	4.0	4.0
	SD	1.8	2.1	11.1	1.8	2.1	11.1	1.8	2.4	12.3	0.0	0.0	0.0	0.0	0.0
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
3	Mean	6.2	7.7	44.0	6.2	7.7	44.0	6.1	7.7	43.7	3.9	4.1	48.8	3.9	4.1
	SD	2.3	1.7	13.5	2.3	1.7	13.5	2.2	1.7	13.2	0.3	0.3	4.0	0.3	0.3
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10
4	Mean	6.8	7.4	47.1	6.8	7.4	47.1	6.8	7.3	47.4	3.8	4.2	47.5	3.8	4.2
	SD	2.9	2.3	16.4	2.9	2.3	16.4	2.9	2.4	16.4	0.4	0.4	5.3	0.4	0.4
	n	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Dams dosed GD6 to PND 10.

SD Standard deviation.

n Number of litters.

M Males.

F Females.

Statistical analysis of %M: No statistical significance ( $p>0.05$ ).



TABLE 12

Bodyweight up to PND 11 - group mean values (g) for male offspring

Group : 1 2 3 4  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group	Before cull			After cull			
	PND 1	PND 4	PND 4	PND 4	PND 7	PND 11	
1	Mean	6.5	9.2	9.2	15.6	27.0	
	SD	0.3	0.7	0.7	1.3	1.9	
	n	10	10	10	10	10	
2	Mean	6.1	8.4	8.3	13.6a	24.0b	
	SD	0.4	0.9	0.8	1.4	2.1	
	n	10	10	10	10	10	
3	Mean	6.7	9.7	9.7	16.2	27.4	
	SD	0.7	1.1	0.9	1.7	1.9	
	n	10	10	10	10	10	
4	Mean	6.9	9.5	9.5	15.8	26.1	
	SD	0.6	0.8	0.9	1.5	2.1	
	n	10	10	10	10	10	

Dams dosed GD6 to PND 10.

SD Standard deviation.

n Number of litters.

Statistical significance: a-p&lt;0.05, b-p&lt;0.01.

TABLE 13

Bodyweight change up to PND 11 - group mean values (g) for male offspring

Group : 1 2 3 4  
Compound : control ----- malathion -----  
Dosage (mg/kg/day) : 0 5 50 150

Group		Weight change during PND			
		1-4	1-7	1-11	
1	Mean	2.7	9.2	20.5	
	SD	0.6	1.1	1.8	
	n	10	10	10	
2	Mean	2.1	7.5a	17.9a	
	SD	0.6	1.3	2.0	
	n	10	10	10	
3	Mean	3.1	9.5	20.7	
	SD	0.5	1.2	1.3	
	n	10	10	10	
4	Mean	2.6	8.8	19.2	
	SD	0.8	1.4	2.1	
	n	10	10	10	

Dams dosed GD6 to PND 10.  
SD Standard deviation.  
n Number of litters.  
Statistical significance: a-p<0.05.

TABLE 14

Bodyweight up to PND 11 - group mean values (g) for female offspring

Group : 1 2 3 4  
Compound : control -----malathion-----  
Dosage (mg/kg/day) : 0 5 50 150

Group		Before cull		After cull			
		PND 1	PND 4	PND 4	PND 7	PND 11	
1	Mean	6.2	8.8	8.8	15.0	25.8	
	SD	0.4	0.8	0.8	1.3	2.3	
	n	10	10	10	10	10	
2	Mean	5.9	8.3	8.2	13.7	24.2	
	SD	0.4	1.0	1.0	1.8	2.2	
	n	10	10	10	10	10	
3	Mean	6.4	9.2	9.4	15.7	26.4	
	SD	0.6	1.0	1.1	2.1	2.4	
	n	10	10	10	10	10	
4	Mean	6.4	9.0	9.0	15.0	25.2	
	SD	0.5	1.0	1.0	1.4	2.1	
	n	10	10	10	10	10	

Dams dosed GD6 to PND 10.

SD Standard deviation.

n Number of litters.

No statistical significance (p>0.05).

TABLE 15

Bodyweight change up to PND 11 - group mean values (g) for female offspring

Group : 1 2 3 4  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group		Weight change during PND			
		1-4	1-7	1-11	
1	Mean	2.6	8.8	19.7	
	SD	0.6	1.0	2.1	
	n	10	10	10	
2	Mean	2.3	7.8	18.3	
	SD	0.6	1.5	1.9	
	n	10	10	10	
3	Mean	3.0	9.3	20.0	
	SD	0.8	1.7	2.0	
	n	10	10	10	
4	Mean	2.5	8.6	18.7	
	SD	1.0	1.4	2.3	
	n	10	10	10	

Dams dosed GD6 to PND 10

SD Standard deviation.

n Number of litters.

No statistical significance ( $p > 0.05$ ).

TABLE 16

Brain weights - group mean values (g) for male and female offspring on PND 4

Group : 1 2 3 4  
Compound : control -----malathion-----  
Dosage (mg/kg/day) : 0 5 50 150

Group		Brain weight (g)	
		Male	Female
1	Mean	0.407	0.400
	SD	0.043	0.044
	n	17	18
2	Mean	0.396	0.386
	SD	0.043	0.038
	n	16	17
3	Mean	0.414	0.416
	SD	0.060	0.044
	n	13	19
4	Mean	0.421	0.414
	SD	0.036	0.043
	n	15	18

SD Standard deviation.  
n Number of offspring.  
No statistical significance ( $p>0.05$ ).

TABLE 17

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

Group : 1 2 3 4  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group	PND															
	1	4	7	11	14	17	21	28	35	42	49	56	60			
1	Mean	6.5	9.2	15.7	27.3	35.4	44.0	55.7	94.2	155.8	228.4	300.0	363.1	390.8		
	SD	0.5	0.9	1.6	2.3	4.1	5.2	6.7	7.3	11.4	12.6	18.7	23.2	24.8		
	n	32	32	32	32	32	32	32	10	10	10	10	10	10		
2	Mean	6.1a	8.1b	13.4b	23.8b	32.3b	40.7b	52.5	87.8	143.8	210.0	275.3	342.9	376.2		
	SD	0.5	1.0	1.9	2.9	3.8	4.6	5.7	8.2	11.4	16.2	17.0	20.8	26.9		
	n	32	32	32	32	32	32	32	9	9	9	9	9	9		
3	Mean	6.8	9.7	16.1	27.3	35.9	44.7	56.9	95.5	155.7	222.9	289.1	351.7	382.8		
	SD	0.6	1.0	2.0	2.2	2.7	2.8	3.7	7.2	10.9	13.9	15.8	18.7	23.3		
	n	32	32	32	32	32	32	32	10	10	10	10	10	10		
4	Mean	6.8	9.4	15.9	26.6	34.2	42.2	55.0	92.2	149.7	219.6	288.8	354.9	382.6		
	SD	0.8	1.1	2.0	2.4	2.8	4.2	5.6	8.9	12.2	14.5	20.3	24.7	23.7		
	n	31	31	31	31	31	31	31	10	10	10	10	10	10		

Direct dosing of offspring: PND11 to PND21.

SD Standard deviation.

n Number of animals.

Statistical significance: a-p&lt;0.05, b-p&lt;0.01.

TABLE 18

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

Group : 1 2 3 4  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group		Bodyweight change during PND													
		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	20.8	18.1	11.6	8.1	16.7	28.4	66.6	128.2	200.9	272.4	335.6	363.2		
	SD	2.0	1.7	0.9	2.2	3.7	5.4	6.4	10.5	11.8	18.0	22.4	23.9		
	n	32	32	32	32	32	32	10	10	10	10	10	10		
2	Mean	17.7a	15.7b	10.3b	8.5	16.9	28.7	63.9	119.9	186.1	251.4	319.0	352.3		
	SD	2.7	2.2	1.3	1.6	2.3	3.3	5.7	9.1	14.4	15.5	19.6	26.1		
	n	32	32	32	32	32	32	9	9	9	9	9	9		
3	Mean	20.5	17.6	11.2	8.7	17.4	29.6	68.4	128.6	195.9	262.1	324.7	355.8		
	SD	1.8	1.4	0.9	1.3	1.4	2.4	6.2	10.0	13.1	14.9	17.9	22.5		
	n	32	32	32	31	32	32	10	10	10	10	10	10		
4	Mean	19.9	17.2	10.8b	7.6b	15.6	28.3	65.5	123.0	192.9	262.1	328.2	355.9		
	SD	2.2	1.5	0.9	1.3	3.0	3.9	7.4	10.8	13.4	19.3	23.9	23.1		
	n	31	31	31	31	31	31	10	10	10	10	10	10		

Direct dosing of offspring: PND11 to PND21.

SD Standard deviation.

n Number of animals.

Statistical significance: a-p&lt;0.05, b-p&lt;0.01.

TABLE 19

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

Group : 1 2 3 4  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group		PND													
		1	4	7	11	14	17	21	28	35	42	49	56	60	
1	Mean	6.1	8.8	15.2	26.3	34.3	42.6	54.0	88.4	137.5	180.8	212.2	241.2	254.9	
	SD	0.4	0.9	1.5	2.6	4.6	5.7	7.3	8.8	12.1	17.6	21.7	29.7	31.1	
	n	32	32	32	32	32	32	32	10	10	10	10	10	10	
2	Mean	5.8a	7.9b	13.4b	24.0b	32.6	41.1	51.8	83.7	130.0	169.8	199.5	225.6	238.5	
	SD	0.5	0.9	2.0	3.1	3.8	4.9	5.2	7.3	10.3	11.0	12.2	13.1	15.3	
	n	32	32	32	32	32	32	32	10	10	10	10	10	10	
3	Mean	6.5a	9.2	15.4	26.3	34.5	42.9	54.6	87.1	132.7	168.4	196.7	223.4	236.0	
	SD	0.7	1.2	2.3	2.8	3.2	3.4	5.2	9.7	13.0	15.3	16.3	20.3	23.5	
	n	31	31	31	31	31	31	31	10	10	10	10	10	10	
4	Mean	6.5	9.0	15.1	25.5	32.8	41.2	52.2	85.1	129.0	166.9	196.3	223.3	236.2	
	SD	0.7	1.1	1.9	2.7	3.4	4.5	5.7	8.0	10.7	15.0	17.3	19.6	24.4	
	n	33	32	33	33	33	33	33	10	10	10	10	10	10	

Direct dosing of offspring: PND11 to PND21.

SD Standard deviation.

n Number of animals.

Statistical significance: a-p&lt;0.05, b-p&lt;0.01.



TABLE 20

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

Group : 1 2 3 4  
Compound : control -----malathion-----  
Dosage (mg/kg/day) : 0 5 50 150

Group		Bodyweight change during PND											
		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	20.2	17.5	11.2	8.0	16.3	27.7	62.1	111.2	154.6	185.9	214.9	228.6
	SD	2.4	2.0	1.5	2.4	3.8	5.3	7.5	10.9	16.6	20.8	28.9	30.3
	n	32	32	32	32	32	32	10	10	10	10	10	10
2	Mean	18.3	16.1	10.6	8.6	17.1	27.8	59.2	105.6	145.3	175.0	201.1	214.0
	SD	2.8	2.4	1.4	1.3	2.6	2.8	4.7	8.0	9.3	10.5	11.2	13.5
	n	32	32	32	32	32	32	10	10	10	10	10	10
3	Mean	19.7	17.0	10.9	8.2	16.6	28.3	60.9	106.5	142.2	170.5	197.2	209.8
	SD	2.2	1.8	1.0	1.1	1.3	2.8	7.1	10.5	12.8	14.1	18.3	21.3
	n	31	31	31	31	31	31	10	10	10	10	10	10
4	Mean	19.1	16.5	10.4	7.2b	15.6	26.6	59.5	103.4	141.3	170.8	197.8	210.6
	SD	2.7	2.1	1.3	1.1	2.4	3.2	5.3	8.6	13.3	16.2	18.5	23.5
	n	33	32	33	33	33	33	10	10	10	10	10	10

Direct dosing of offspring: PND11 to PND21.  
SD Standard deviation.  
n Number of animals.  
Statistical significance: b-p<0.01.

TABLE 21

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

Group : 1 2 3 4  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group		PND									
		1	4	7	11	14	21				
1	Mean	6.4	9.2	15.5	25.7	33.5	52.0				
	SD	0.5	0.9	1.0	0.5	0.9	2.6				
	n	8	8	8	8	8	8				
2	Mean	6.1	8.8	14.1	25.1	34.0	53.4				
	SD	0.7	1.3	2.0	2.0	2.0	4.1				
	n	8	8	8	8	8	8				
3	Mean	6.4	10.0	16.7	27.6a	35.9	56.5				
	SD	0.5	0.7	0.9	1.3	1.4	2.2				
	n	7	7	7	7	7	7				
4	Mean	7.0a	9.7	15.3	24.1	30.7a	50.9				
	SD	0.4	0.7	0.8	1.9	3.3	8.0				
	n	7	7	7	7	7	7				

SD Standard deviation.

n Number of animals.

Statistical significance: a-p&lt;0.05.

TABLE 22

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

Group : 1 2 3 4  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group		Bodyweight change during PND							
		1-11	4-11	7-11	11-14	11-21			
1	Mean	19.2	16.4	10.2	7.8	26.4			
	SD	0.5	0.9	0.9	0.6	2.3			
	n	8	8	8	8	8			
2	Mean	19.0	16.3	11.0	8.9	28.3			
	SD	1.5	1.1	0.8	1.3	4.1			
	n	8	8	8	8	8			
3	Mean	21.2b	17.6	10.9	8.3	29.0			
	SD	0.9	0.6	0.5	0.6	1.9			
	n	7	7	7	7	7			
4	Mean	17.1b	14.4	8.8a	6.6a	26.8			
	SD	1.5	2.4	1.5	1.4	6.2			
	n	7	7	7	7	7			

SD Standard deviation.

n Number of animals.

Statistical significance: a-p&lt;0.05, b-p&lt;0.01.

TABLE 23

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

Group : 1 2 3 4  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group		PND									
		1	4	7	11	14	21				
1	Mean	6.2	8.6	14.4	23.9	31.0	48.8				
	SD	0.8	1.8	3.0	4.4	4.8	6.8				
	n	8	8	8	8	8	8				
2	Mean	6.1	9.1	14.9	25.8	34.9	55.2				
	SD	1.0	2.0	2.9	3.2	3.4	3.1				
	n	8	8	8	8	8	8				
3	Mean	6.3	9.9	16.7a	26.9	34.7	54.1				
	SD	0.4	0.8	0.8	1.1	0.6	1.4				
	n	9	9	9	9	9	9				
4	Mean	6.7	8.9	14.4	23.3	30.5	50.1				
	SD	0.4	0.7	1.0	1.4	2.3	4.7				
	n	9	9	9	9	9	9				

SD Standard deviation.

n Number of animals.

Statistical significance: a-p&lt;0.05.

TABLE 24

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

Group : 1 2 3 4  
Compound : control -----malathion-----  
Dosage (mg/kg/day) : 0 5 50 150

Group		Bodyweight change during PND							
		1-11	4-11	7-11	11-14	11-21			
1	Mean	17.7	15.3	9.5	7.1	24.9			
	SD	3.9	2.9	1.9	0.9	2.8			
	n	8	8	8	8	8			
2	Mean	19.7	16.7	10.9	9.1b	29.4a			
	SD	2.3	1.3	0.4	0.6	1.9			
	n	8	8	8	8	8			
3	Mean	20.7	17.0	10.2	7.8	27.2a			
	SD	1.0	0.9	0.5	0.9	1.1			
	n	9	9	9	9	9			
4	Mean	16.6	14.4	8.9	7.2	26.8a			
	SD	1.3	1.6	1.0	1.8	3.9			
	n	9	9	9	9	9			

SD Standard deviation.  
n Number of animals.  
Statistical significance: a-p<0.05, b-p<0.01.

TABLE 25

Brain weights - group mean values (g) for dosed male and female offspring on PND 11 (1 day of dosing)

Group	:	1	2	3	4	11
Compound	:	control				
Dosage (mg/kg/day)	:	0	5	50	150	450

Group		Brain weight (g)	
		Male	Female
1	Mean	1.008	0.980
	SD	0.051	0.047
	n	8	8
2	Mean	0.993	0.984
	SD	0.049	0.057
	n	8	8
3	Mean	1.019	0.994
	SD	0.034	0.084
	n	8	8
4	Mean	1.021	0.971
	SD	0.059	0.060
	n	8	8
11	Mean	0.998	1.004
	SD	0.046	0.078
	n	8	8

SD Standard deviation.

n Number of offspring.

No statistical significance ( $p > 0.05$ ).

TABLE 26

Brain weights - group mean values (g) for dosed male and female offspring on PND 21 (11 days of dosing)

Group : 1 2 3 4  
Compound : control -----malathion-----  
Dosage (mg/kg/day) : 0 5 50 150

Group		Brain weight (g)	
		Male	Female
1	Mean	1.493	1.471
	SD	0.063	0.043
	n	8	8
2	Mean	1.457	1.429
	SD	0.069	0.077
	n	8	8
3	Mean	1.520	1.419
	SD	0.080	0.053
	n	8	8
4	Mean	1.479	1.428
	SD	0.057	0.063
	n	8	8

SD Standard deviation.  
n Number of animals.  
No statistical significance (p>0.05).

TABLE 27

Bodyweight - group mean values (g) for young adult males (11 days of dosing)

Group : 6 7 8 9  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group		day of treatment											
		-1	1	2	3	4	5	6	7	8	9	10	11
6	Mean	276	286	294	304	313	319	326	335	342	351	358	365
	SD	27	30	29	30	30	28	29	29	30	30	30	31
	n	8	8	8	8	8	8	8	8	8	8	8	7
7	Mean	271	280	287	297	307	313	317	327	335	344	352	358
	SD	27	26	26	26	26	25	26	26	27	27	29	26
	n	8	8	8	8	8	8	8	8	8	8	8	8
8	Mean	273	281	290	299	308	315	320	323	338	345	354	361
	SD	23	22	23	24	23	22	21	23	21	22	22	22
	n	8	8	8	8	8	8	8	8	8	8	8	8
9	Mean	278	288	295	305	312	320	326	335	342	345	353	362
	SD	28	30	31	30	31	30	32	32	31	30	32	32
	n	8	8	8	8	8	8	8	8	8	8	8	8

SD Standard deviation.

n Number of animals.

No statistical significance ( $p>0.05$ ).



TABLE 28

Bodyweight change - group mean values (g) for young adult males (11 days of dosing)

Group : 6 7 8 9  
 Compound : control ----- malathion -----  
 Dosage (mg/kg/day) : 0 5 50 150

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	10	8	18	27	33	40	49	57	65	72	81
	SD	4	3	2	3	5	4	4	6	5	7	7
	n	8	8	8	8	8	8	8	8	8	8	7
7	Mean	9	7	17	27	33	37	47	55	64	72	78
	SD	3	3	2	3	7	4	6	7	8	9	11
	n	8	8	8	8	8	8	8	8	8	8	8
8	Mean	8	9	18	27	34	40	42	58	64	73	80
	SD	2	3	3	4	6	6	17	8	9	10	10
	n	8	8	8	8	8	8	8	8	8	8	8
9	Mean	10	7	17	24	32	38	47	54	57	65	74
	SD	2	2	3	3	3	4	7	8	7	9	10
	n	8	8	8	8	8	8	8	8	8	8	8

SD Standard deviation.

n Number of animals.

No statistical significance ( $p > 0.05$ ).

TABLE 29

Bodyweight - group mean values (g) for young adult females (11 days of dosing)

Group : 6 7 8 9  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group		day of treatment											
		-1	1	2	3	4	5	6	7	8	9	10	11
6	Mean	204	208	210	214	218	219	222	228	234	236	236	242
	SD	19	22	25	23	21	23	24	22	22	25	26	24
	n	8	8	8	8	8	8	8	8	8	8	8	8
7	Mean	204	207	210	213	216	220	224	226	229	233	237	241
	SD	18	19	18	20	21	19	19	21	21	21	20	22
	n	8	8	8	8	8	8	8	8	8	8	8	8
8	Mean	196	199	203	205	208	213	217	219	223	228	233	234
	SD	9	11	11	9	11	14	12	13	14	15	13	14
	n	8	8	8	8	8	8	8	8	8	8	8	8
9	Mean	195	198	198	204	207	210	212	214	219	221	225	230
	SD	18	19	17	16	19	18	17	19	19	19	20	18
	n	8	8	8	8	8	8	8	8	8	8	8	8

SD Standard deviation.

n Number of animals.

No statistical significance ( $p>0.05$ ).

TABLE 30

Bodyweight change - group mean values (g) for young adult females (11 days of dosing)

Group : 6 7 8 9  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

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	7	10	12	15	20	26	28	29	34
	SD	4	4	4	5	4	6	7	5	5	8	9
	n	8	8	8	8	8	8	8	8	8	8	8
7	Mean	3	3	6	9	13	17	19	22	27	30	34
	SD	3	4	4	4	2	4	4	6	5	8	7
	n	8	8	8	8	8	8	8	8	8	8	8
8	Mean	3	4	6	9	14	17	20	24	29	34	35
	SD	5	2	4	4	3	3	4	3	5	5	5
	n	8	8	8	8	8	8	8	8	8	8	8
9	Mean	4	-1	5	9	12	13	16	21	23	26	32
	SD	3	5	5	4	4	7	6	5	5	8	8
	n	8	8	8	8	8	8	8	8	8	8	8

SD Standard deviation.

n Number of animals.

No statistical significance ( $p>0.05$ ).

TABLE 31

Brain weights - group mean values (g) for young adult animals on day 1 of treatment

Group : 6 and 10 7 8 9 11  
 Compound : control  
 Dosage (mg/kg/day) : 0 5 50 150 450

-----malathion-----

Group		Male brain weight	Female brain weight
6	Mean SD n	1.888 0.036 8	1.876 0.066 8
10	Mean SD n	1.941 0.040 8	1.789 0.055 8
7	Mean SD n	1.937 0.068 8	1.810 0.066 8
8	Mean SD n	1.899 0.094 8	1.833 0.084 8
9	Mean SD n	1.888 0.093 8	1.824 0.082 8
11	Mean SD n	1.946 0.048 8	1.862 0.072 8

SD Standard deviation.

n Number of animals.

No statistical significance ( $p > 0.05$ )

TABLE 32

Brain weights - group mean values (g) for young adult animals on day 11 of treatment

Group	:	6	7	8	9
Compound	:	control		malathion	
Dosage (mg/kg/day)	:	0	5	50	150

Group		Male brain weight	Female brain weight
6	Mean	1.940	1.899
	SD	0.097	0.091
	n	8	8
7	Mean	1.918	1.873
	SD	0.055	0.068
	n	8	8
8	Mean	1.954	1.899
	SD	0.073	0.109
	n	8	8
9	Mean	1.931	1.870
	SD	0.090	0.092
	n	8	8

SD Standard deviation.

n Number of animals.

No statistical significance ( $p > 0.05$ ).

TABLE 33

Brain weights - group mean values (g) for male and female offspring on PND 60

Group : 1 2 3 4  
Compound : control -----malathion-----  
Dosage (mg/kg/day) : 0 5 50 150

Group		Brain weight (g)	
		Male	Female
1	Mean	2.016	1.862
	SD	0.090	0.066
	n	8	8
2	Mean	2.003	1.852
	SD	0.081	0.051
	n	8	8
3	Mean	1.990	1.868
	SD	0.047	0.108
	n	8	8
4	Mean	1.975	1.829
	SD	0.062	0.050
	n	8	8

Offspring directly dosed: PND 11 to PND 21.  
SD Standard deviation.  
n Number of animals.  
No statistical significance ( $p>0.05$ ).

TABLE 34

Plasma, erythrocyte and brain cholinesterase activity - group mean values for dams on GD 20

Group : 1 2 3 4  
Compound : control  
Dosage (mg/kg/day) : 0 5 50 150  
-----malathion-----

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1F	Mean	1382	1234	13200
	SD	257.5	138.2	418.3
	n	8	8	8
2F	Mean	1210 (12)	1244 (-1)	13013 (1)
	SD	133.6	59.4	658.9
	n	8	8	8
3F	Mean	1297 (6)	994b (19)	13100 (1)
	SD	172.5	60.9	516.9
	n	8	8	8
4F	Mean	1204 (13)	606b (51)	12644 (4)
	SD	243.1	75.3	262.5
	n	8	8	8

Dosing commenced GD6.

CHE Cholinesterase (expressed as a percentage inhibition of CHE compared with control).

SD Standard deviation.

n Number of animals.

Statistical significance: b-p<0.01.

TABLE 35

Plasma, erythrocyte and brain cholinesterase activity - group mean values for fetuses on GD 20

Group : 1 2 3 4  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1	Mean SD n	285 23.0 8	938 112.6 8	1606 117.8 8
2	Mean SD n	265 (7) 22.6 8	897 (4) 68.7 8	1656 (-3) 178.2 8
3	Mean SD n	246b (14) 39.3 7	831a (11) 82.1 8	1519 (5) 173.1 8
4	Mean SD n	243b (15) 17.7 8	756b (19) 47.7 8	1638 (-2) 260.2 8

Dosing of dams commenced GD 6.

CHE Cholinesterase (expressed as a percentage inhibition of CHE compared with control).

SD Standard deviation.

n Number of litters.

Statistical significance: a-p&lt;0.05, b-p&lt;0.01.



TABLE 36

Plasma, erythrocyte and brain cholinesterase activity - group mean values for male and female offspring on PND 4

Group : 1 2 3 4  
Compound : control -----malathion-----  
Dosage (mg/kg/day) : 0 5 50 150

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1M	Mean	612	1100	3018
	SD	58.8	170.2	269.8
	n	17	17	17
2M	Mean	594 (3)	1134 (-3)	3078 (-2)
	SD	65.8	155.4	216.8
	n	16	16	16
3M	Mean	626 (-2)	1075 (2)	2915 (3)
	SD	51.9	164.3	326.2
	n	13	13	13
4M	Mean	620 (-1)	1017 (8)	2867 (5)
	SD	67.8	197.0	419.9
	n	15	13	15

CHE Cholinesterase (expressed as a percentage inhibition of CHE compared with control).  
SD Standard deviation.  
n Number of animals.  
No statistical significance ( $p>0.05$ ).

TABLE 36 – continued

Plasma, erythrocyte and brain cholinesterase activity - group mean values for male and female offspring on PND 4

Group : 1 2 3 4  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1F	Mean SD n	622 59.2 18	1147 193.4 18	2994 264.5 18
2F	Mean SD n	598 (4) 61.7 17	1125 (2) 190.2 17	2941 (2) 316.8 17
3F	Mean SD n	606 (3) 51.5 19	1193 (-4) 245.8 19	2953 (1) 547.8 19
4F	Mean SD n	617 (1) 44 18	1103 (4) 227.3 18	2967 (1) 347.7 18

CHE Cholinesterase (expressed as a percentage inhibition of CHE compared with control).

SD Standard deviation.

n Number of animals.

No statistical significance ( $p > 0.05$ ).

TABLE 37

Plasma, erythrocyte and brain cholinesterase activity - group mean values for dosed male and female offspring on PND 11 (1 day of dosing)

Group	:	1	2	3	4	11
Compound	:	control				
Dosage (mg/kg/day)	:	0	5	50	150	450

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1M	Mean SD n	756 73.6 8	1509 256.0 8	5756 224.3 8
2M	Mean SD n	704 (7) 51.6 8	1272a (16) 238.5 8	5688 (1) 216.7 8
3M	Mean SD n	614b (19) 44.2 8	1131b (25) 140.6 8	5388 (6) 280.0 8
4M	Mean SD n	482b (36) 72.4 8	672b (55) 100.4 8	3244b (44) 699.2 8
11M	Mean SD n	346b (54) 62.6 8	428b (72) 94.9 8	919b (84) 435.0 8

CHE Cholinesterase (expressed as a percentage inhibition of CHE compared with control).

SD Standard deviation.

n Number of animals.

Statistical significance: a-p<0.05, b-p<0.01.

TABLE 37 - continued

Plasma, erythrocyte and brain cholinesterase activity - group mean values for dosed male and female offspring on PND 11 (1 day of dosing)

Group : 1 2 3 4 11  
 Compound : control  
 Dosage (mg/kg/day) : 0 5 50 150 450

-----malathion-----

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1F	Mean SD n	737 78.5 8	1319 110.0 8	5825 279.0 8
2F	Mean SD n	717 (3) 47.2 8	1228 (7) 228.5 8	5600 (4) 183.2 8
3F	Mean SD n	620b (16) 79.2 8	1016b (23) 127.4 8	5249 (10) 728.5 8
4F	Mean SD n	481b (35) 46.0 8	688b (48) 58.2 8	3044b (48) 559.6 8
11F	Mean SD n	353b (52) 67.5 8	519b (61) 134.8 8	1081b (81) 512.7 8

CHE Cholinesterase (expressed as a percentage inhibition of CHE compared with control).

SD Standard deviation.

n Number of animals.

Statistical significance: b-p&lt;0.01.

TABLE 38

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

Group : 6 and 10 7 8 9 11  
Compound : control -----malathion-----  
Dosage (mg/kg/day) : 0 5 50 150 450

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
6M	Mean	342	866	13713
	SD	45.4	167.9	853.9
	n	8	8	8
10M	Mean	354	1109	13563
	SD	71.1	86.5	391.7
	n	8	8	8
7M	Mean	341 (0)	891 (-3)	12988 (5)
	SD	35.7	169.5	414.7
	n	8	8	8
8M	Mean	359 (-5)	975 (-13)	13081 (5)
	SD	66.9	83.5	709.6
	n	8	8	8
9M	Mean	337 (1)	853 (2)	12744 (7)
	SD	77.7	60.4	859.2
	n	8	8	8
11M	Mean	268b (24)	831c (25)	13131 (3)
	SD	35.6	85.3	451.1
	n	8	8	8

CHE Cholinesterase (expressed as a percentage inhibition of CHE compared with concurrent control).

n Number of animals.

SD Standard deviation.

Statistical significance: b-p<0.01, c<0.001.

TABLE 38 - continued

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

Group : 6 and 10  
 Compound : control  
 Dosage (mg/kg/day) : 0

-----malathion-----  
 150 450

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
6F	Mean SD n	793 216.1 8	950 66.8 8	12900 470.6 8
10F	Mean SD n	624 85.7 8	1069 65.1 8	13513 501.2 8
7F	Mean SD n	717 (10) 119.3 8	1013 (-7) 108.6 8	13213 (-2) 427.4 8
8F	Mean SD n	822 (-4) 181.5 8	959 (-1) 104.3 8	13038 (-1) 553.4 8
9F	Mean SD n	727 (8) 198.5 8	891 (6) 64 8	13244 (-3) 244.1 8
11F	Mean SD n	558 (11) 83.3 8	884c (17) 96.3 8	12975 (4) 639.2 8

CHE Cholinesterase (expressed as a percentage inhibition of CHE compared with control).

n Number of animals.

SD Standard deviation.

Statistical significance: c-p&lt;0.001.

TABLE 39

Plasma, erythrocyte and brain cholinesterase activity - group mean values for dosed male and female offspring on PND 21 (11 days of dosing)

Group : 1 2 3 4  
Compound : control -----malathion-----  
Dosage (mg/kg/day) : 0 5 50 150

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1M	Mean SD n	393 74.5 8	1866 394.4 8	10500 286.6 8
2M	Mean SD n	341 (13) 75.5 8	1556a (17) 282.1 8	10363 (1) 318.2 8
3M	Mean SD n	320a (19) 42.3 8	1144b (39) 217.8 8	10488 (0) 506.2 8
4M	Mean SD n	299b (24) 47.5 8	622b (67) 207.2 8	8850b (16) 792.8 8

CHE Cholinesterase (expressed as a percentage inhibition of CHE compared with control).

SD Standard deviation.

n Number of animals.

Statistical significance: a-p<0.05, b-p<0.01.

TABLE 39 - continued

Plasma, erythrocyte and brain cholinesterase activity - group mean values for dosed male and female offspring on PND 21 (11 days of dosing)

Group	Compound Dosage (mg/kg/day)		1 control	-----malathion-----		
				2	3	4
			0	5	50	150
Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg		
1F	Mean	374	1894	10356		
	SD	65.1	397.5	252.8		
	n	8	8	8		
2F	Mean	338 (10)	1606 (15)	10250 (1)		
	SD	54.7	484.4	381.7		
	n	8	8	8		
3F	Mean	304a (19)	1250b (34)	10444 (-1)		
	SD	69	159.8	408.3		
	n	8	8	8		
4F	Mean	254b (32)	597b (68)	8650b (16)		
	SD	39.4	185.4	931.2		
	n	8	8	8		

CHE Cholinesterase (expressed as a percentage inhibition of CHE compared with control).

SD Standard deviation.

n Number of animals.

Statistical significance: a-p<0.05, b-p<0.01.



TABLE 40

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

Group : 6 7 8 9  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
6M	Mean	333	1084	13219
	SD	22.2	46.2	601.2
	n	8	8	8
7M	Mean	322 (3)	1044 (4)	13288 (-1)
	SD	27.9	65.1	592.7
	n	8	8	8
8M	Mean	297a (11)	869b (20)	13494 (-2)
	SD	25.5	98.9	390.5
	n	8	8	8
9M	Mean	289a (13)	616b (43)	13031 (1)
	SD	48.0	74.3	723.6
	n	8	8	8

CHE Cholinesterase (expressed as a percentage inhibition of CHE compared with control).

SD Standard deviation.

n Number of animals.

Statistical significance: a-p<0.05, b-p<0.01.

TABLE 40 - continued

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

Group : 6 7 8 9  
Compound : control -----malathion-----  
Dosage (mg/kg/day) : 0 5 50 150

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
6F	Mean	1028	1094	13731
	SD	249.6	92.3	1858.6
	n	8	8	8
7F	Mean	978 (5)	1069 (2)	13463 (2)
	SD	256.5	142.5	319.3
	n	8	8	8
8F	Mean	871 (15)	878b (20)	13700 (0)
	SD	240.3	54.2	463.7
	n	8	8	8
9F	Mean	893 (13)	566b (48)	13031 (5)
	SD	172.5	89.6	441.5
	n	8	8	8

CHE Cholinesterase (expressed as a percentage of control).

SD Standard deviation.

n Number of animals.

Statistical significance: b-p<0.01.

TABLE 41

Plasma, erythrocyte and brain cholinesterase activity - group mean values for male and female offspring on PND 60

Group : 1 2 3 4  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1M	Mean	363	903	13231
	SD	49.2	117.6	523.7
	n	8	8	8
2M	Mean	377 (-4)	944 (-5)	13269 (0)
	SD	65.4	106.7	723.6
	n	8	8	8
3M	Mean	344 (5)	997 (-10)	13125 (1)
	SD	41.8	107.3	580
	n	8	8	8
4M	Mean	317 (13)	1050 (-16)	12825 (3)
	SD	71.8	281.3	1054.6
	n	8	8	8

CHE Cholinesterase (expressed as a percentage inhibition of CHE compared with control).

SD Standard deviation.

n Number of animals.

No statistical significance ( $p>0.05$ ).

TABLE 41 - continued

Plasma, erythrocyte and brain cholinesterase activity - group mean values for male and female offspring on PND 60

Group : 1 2 3 4  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group		Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1F	Mean	1090	966	13513
	SD	293.8	129.5	309.1
	n	8	8	8
2F	Mean	894 (18)	988 (-2)	13313 (1)
	SD	176.2	59.8	1137.6
	n	8	8	8
3F	Mean	913 (16)	956 (1)	13431 (1)
	SD	166.8	82.1	518.9
	n	8	8	8
4F	Mean	843 (23)	994 (-3)	13331 (1)
	SD	98.1	147.4	371.2
	n	8	8	8

CHE Cholinesterase (expressed as a percentage inhibition of CHE compared with control).

SD Standard deviation.

n Number of animals.

No statistical significance ( $p>0.05$ ).

## APPENDIX 1

Clinical signs - individual observations for dams during gestation and lactation

Group 1: control

Animal number	day of kill	Signs observed on days	Post-dose salivation seen on days ('(g)' denotes 'gestation')
1A	GD 20	Brown staining on head on GD 5-20, on both forelimbs on GD 20, and on dorsal body surface on GD 9-11, 16-20.	-
2A	GD 20	Brown staining on upper dorsal thorax on GD 5-20, and on head on GD 14-20.	-
3A	GD 20	Brown staining on upper dorsal thorax on GD 3-11, and on head on GD 5-15, 19-20.	-
4A	GD 20	Dry skin abrasion on head on GD 5-8, with skin encrustations on forelimbs on GD 15-20. Yellow staining on sacral area on GD 12, and brown staining on upper dorsal thorax on GD 20. Hairloss on forelimbs on GD 14-20.	-
5A	GD 20	Hairloss on ventral body surface on GD 20.	-
6A	GD 20	Brown staining on upper dorsal thorax on GD 4-10, on head on GD 12-14, and on sacral area on GD 8-10. Yellow staining on sacral area on GD 11-15. Hairloss on forelimbs on GD 20.	-
7A	GD 20	Brown staining on upper dorsal thorax on GD 2-11, on head on GD 12-20, and on pinna on GD 13-20. Hairloss on forelimbs on GD 14-20.	-
8A	GD 20	Brown staining on upper dorsal thorax on GD 14-20 and on head on GD 20.	-
9A	GD 20	Brown staining on head on GD 2-20, on upper dorsal thorax on GD 14-20, and yellow staining on sacral area on GD 11. Hairloss on dorsal body surface on GD 4-11.	-
10B	PND 21	-	8(g)
11B	PND 21	Brown staining on head on GD 3-15 and from GD 21 to PND 2. Brown staining on upper dorsal thorax on GD 21 and on PND 9-21. Yellow staining on sacral area on GD 12-15.	-
12B	PND 21	Brown staining on upper dorsal thorax from GD 3 to PND 2 and PND 9-21. Brown staining on head from GD 15 to PND 2.	-

A Treated from day 6 to day 20 after mating.

B Treated from day 6 after mating to PND 10 (where litter survived to PND 10).

- No signs observed.

## APPENDIX 1 - continued

Clinical signs - individual observations for dams during gestation and lactation

Group 1: control

Animal number	day of kill	Signs observed on days	Post-dose salivation seen on days ('(g)' denotes 'gestation')
13B	PND 21	Brown staining on head on GD 4-14. Brown staining on upper dorsal thorax on PND 11-21. Yellow staining on sacral area on GD 4-5.	-
14B	PND 21	Brown staining on head from GD 20 to PND 1.	-
15B	PND 21	Brown staining on dorsal body surface on GD 7-10, and on upper dorsal thorax on GD 14-15. Yellow staining on perianal area on GD 8-10, and on sacral area on GD 11-13.	1
16B	PND 21	Brown staining on head on GD 4-17, on sacral area on GD 8-14, on forelimbs on GD 11-14 and from GD 20 to PND 21 and on ventral body surface on PND 8-21. Yellow staining on dorsal body surface on GD 16-17. Hairloss on forelimbs from GD 19 to PND 21.	-
17B	PND 21	Brown staining on head from GD 6 to PND 5 and PND 10-17. Brown staining on forelimbs on GD 7-9.	-
18B	PND 21	Brown staining on head from GD 13 to PND 5 and on PND 17-21. Hairloss on forelimbs on PND 6-21.	21(g)
19B	PND 21	Hairloss on ventral body surface from GD 22 to PND 1 and on forelimbs on PND 9-21. Brown staining on upper dorsal thorax on PND 9-16 and on head on PND 16.	-

A Treated from day 6 to day 20 after mating.

B Treated from day 6 after mating to PND 10 (where litter survived to PND 10).

- No signs observed.

## APPENDIX 1 – continued

Clinical signs - individual observations for dams during gestation and lactation

Group 2: malathion: 5 mg/kg/day

Animal number	day of kill	Signs observed on days	Post-dose salivation seen on days ('(g)' denotes 'gestation')
20A	GD 20	Hairloss on dorsal body surface on GD 3-8.	-
21A	GD 20	-	-
22A	GD 20	Brown staining on upper dorsal thorax on GD 3-20, on head on GD 20 Yellow staining on sacral area on GD 12.	6(g), 7(g)
23A	GD 20	Brown staining on head on GD 5-14, and on upper dorsal thorax on GD 15-20. Partially absent tail from GD 14. Hairloss on forelimbs on GD 17-19.	-
24A	GD 20	-	-
25A	GD 20	Hairloss on forelimbs and ventral body surface on GD 20.	-
26A	GD 20	Brown staining on upper dorsal thorax on GD 14-15.	-
27A	GD 20	Brown staining on head on GD 4-20, on left pinna on GD 4-7, on pinnae on GD 8-20, and on upper dorsal thorax on GD 8-11.	6(g)
28A	GD 20	-	-
29A	PND 21	Brown staining on upper dorsal thorax on GD 12-18, and on head on GD 21-23.	16(g)
30B	PND 21	Yellow staining on sacral area on GD 5-18. Brown staining on forelimbs on GD 5-15, on muzzle on GD 5-11, on head and upper dorsal thorax on GD 12-15, and on pinnae on GD 16-18.	-
31B	PND 21	Brown staining on upper dorsal thorax on GD 3-18, on head on GD 9-15 and PND 19-21.	-

A Treated from day 6 to day 20 after mating.

B Treated from day 6 after mating to PND 10 (where litter survived to PND 10).

- No signs observed.

## APPENDIX 1 – continued

Clinical signs - individual observations for dams during gestation and lactation

Group 2: malathion: 5 mg/kg/day

Animal number	day of kill	Signs observed on days	Post-dose salivation seen on days ('(g)' denotes 'gestation')
32B	PND 21	Brown staining on upper dorsal thorax on GD 2-3, 8-10, on head on GD 4-21 and PND 1, 18-21. Yellow staining on sacral area on GD 11-17. Hairloss on dorsal body surface on GD 8-10.	-
33B	PND 21	Brown staining on upper dorsal thorax on GD 2-10 and from GD 14 to PND 1. Brown staining on left orbital area on GD 18-19.	-
34B	PND 21	Hairloss on right forelimb on PND 18-21.	-
35B	PND 21	Brown staining on head from GD 4 to PND 6 and on PND 18-21, on upper dorsal thorax from GD 4 to PND 1 and on forelimbs on GD 8-11.	2
36B	PND 21	-	-
37B	PND 21	-	-
38B	PND 21	Brown staining on upper dorsal thorax on GD 11-17 and PND 4-5, on head on GD 19-21 and on forelimbs from GD 19 to PND 21.	-

- A Treated from day 6 to day 20 after mating.  
 B Treated from day 6 after mating to PND 10 (where litter survived to PND 10).  
 - No signs observed.



## APPENDIX 1 - continued

Clinical signs - individual observations for dams during gestation and lactation

Group 3: malathion: 50 mg/kg/day

Animal number	day of kill	Signs observed on days	Post-dose salivation seen on days ('(g)' denotes 'gestation')
39A	GD 20	Yellow staining on sacral area on GD 17-18. Brown staining on head, forelimbs, upper dorsal thorax and ventral body surface on GD 20.	-
40A	GD 20	Brown staining on head on GD 20.	6(g)
41A	GD 20	Brown staining on head on GD 12-20. Hairloss on forelimbs on GD 14-20.	-
42A	GD 20	-	-
43A	GD 20	Brown staining on head on GD 20. Hairloss on forelimbs on GD 20.	-
44A	GD 20	Brown staining on upper dorsal thorax on GD 2-20, and on head on GD 8-11.	-
45A	GD 20	Brown staining on upper dorsal thorax on GD 20.	-
46A	GD 20	Brown staining on upper dorsal thorax on GD 2-10, 14-20, on dorsal body surface on GD 8-10, and on head on GD 11-14, 20. Skin encrustation on right forelimb on GD 4-10. Hairloss on forelimbs on GD 14-20.	-
47A	GD 20	Brown staining on head on GD 19-20.	-
48B	PND 21	Yellow staining on sacral area on GD 9-16.	-
49B	PND 21	Brown staining on head on PND 12-21. Hairloss on forelimbs on PND 12.	-
50B	PND 21	Brown staining on head on GD 3-8, from GD 15 to PND 1 and PND 11-21. Hairloss on muzzle on GD 5-12 and on forelimbs on PND 11.	6(g)
51B	PND 21	Brown staining on head from GD 7 to PND 1, on upper dorsal thorax on PND 5-11, 21 and on forelimbs on PND 9-21. Hairloss on forelimbs from GD 14 to PND 21.	-

A Treated from day 6 to day 20 after mating.

B Treated from day 6 after mating to PND 10 (where litter survived to PND 10).

- No signs observed.

## APPENDIX 1 - continued

Clinical signs - individual observations for dams during gestation and lactation

Group 3: malathion: 50 mg/kg/day

Animal number	day of kill	Signs observed on days	Post-dose salivation seen on days ('(g)' denotes 'gestation')
52B	PND 21	Partially absent left pinna from GD 4. Hairloss on forelimbs from GD 8 to PND 21. Brown staining on head on GD 20-21.	16(g)
53B	PND 21	Yellow staining on sacral area on GD 4-11. Brown staining on sacral area on GD 12-19. Hairloss on dorsal body surface on GD 4-10.	15(g)
54B	PND 21	Hairloss on forelimbs on GD 11-21 and PND 3-21, on dorsal body surface on PND 1-21 and on hindlimbs on PND 8-21. Brown staining on head on PND 4-6, 21 and on left orbital on PND 17.	-
55B	PND 21	Hairloss on dorsal body surface on GD 3-9 and on forelimbs from GD 10 to PND 21. Brown staining on head on GD 10-13 and on PND 21, on forelimbs on GD 11-13 and on upper dorsal thorax from GD 14 of gestation to PND 20. Encrustation on forepaws on GD 15-16 and on forelimbs on GD 19-21.	-
56B	PND 21	Brown staining on head from GD 19 to PND 5 and on PND 17-21.	-
57B	PND 21	-	12(g)

A Treated from day 6 to day 20 after mating.

B Treated from day 6 after mating to PND 10 (where litter survived to PND 10).

- No signs observed.

## APPENDIX 1 - continued

Clinical signs - individual observations for dams during gestation and lactation

Group 4: malathion: 150 mg/kg/day

Animal number	day of kill	Signs observed on days	Post-dose salivation seen on days ('(g)' denotes 'gestation')
58A	GD 20	Brown staining on upper dorsal thorax on GD 3-20, and on head on GD 12-20.	13(g), 14(g), 16(g), 18(g), 19(g)
59A	GD 20	Brown staining on head on GD 20.	8(g), 12(g)-14(g), 16(g), 18(g), 19(g)
60A	GD 20	Yellow staining on sacral area on GD 5-20. Brown staining on head on GD 5-20, and on upper dorsal thorax and pinnae on GD 11-20.	12(g), 14(g), 16(g), 18(g), 19(g)
61A	GD 20	-	16(g), 18(g)
62A	GD 20	Yellow staining on sacral area on GD 11-14. Brown staining on upper dorsal thorax on GD 14-19, on pinnae on GD 15-19, and on forepaws and head on GD 20.	11(g), 13(g), 17(g), 18(g)
63A	GD 20	Brown staining on upper dorsal thorax on GD 2-10, and on head on GD 11-20. Yellow staining on sacral area on GD 11-20. Hairloss on forelimbs on GD 16-20, and ungroomed on GD 20.	13(g), 15(g), 18(g)
64A	GD 20	Yellow staining on sacral area on GD 4-10, and on dorsal body surface on GD 11-19. Brown staining on head on GD 4-20, on forelimbs on GD 11-19, and on upper dorsal thorax on GD 20. Hairloss on forelimbs on GD 13-20, and on hindlimbs on GD 16-20.	13(g), 15(g)
65A	GD 20	Brown staining on head on GD 4-20, and on upper dorsal thorax on GD 4-19.	12(g), 13(g), 15(g), 17(g), 18(g)
66A	GD 20	-	10(g), 12(g), 14(g), 16(g), 17(g)
67B	PND 21	Brown staining on head on GD 3-11 and on muzzle on GD 19. Hairloss on forelimbs from GD 21 to PND 12.	18(g), 4

A Treated from day 6 to day 20 after mating.

B Treated from day 6 after mating to PND 10 (where litter survived to PND 10).

- No signs observed.

## APPENDIX 1 - continued

Clinical signs - individual observations for dams during gestation and lactation

Group 4: malathion: 150 mg/kg/day

Animal number	day of kill	Signs observed on days	Post-dose salivation seen on days ('(g)' denotes 'gestation')
68B	PND 21	Brown staining on muzzle on PND 11-18 and on upper dorsal thorax from GD 21 to PND 2. Hairloss on forelimbs from GD 14 to PND 21.	7(g), 12(g)-14(g), 16(g), 18(g), 2, 8
69B	PND 21	Brown staining on upper dorsal thorax on GD 2-3, and on head on GD 5-11. Hairloss on forelimbs on PND 11-21.	11(g)-13(g), 15(g), 17(g), 18(g), 1, 7
70B	PND 21	Brown staining on upper dorsal thorax on GD 4-10 and from GD 14 to PND 21. Brown staining on head on PND 21. Hairloss on forelimbs from GD 12 to PND 21.	11(g)-13(g), 15(g), 17(g), 18(g), 3, 6, 10
71B	PND 21	Brown staining on head on GD 20-21.	13(g), 17(g)
72B	PND 21	Brown staining on head on GD 15-21 and on muzzle on PND 17-21. Hairloss on forelimbs from GD 19 to PND 21, and on hindlimbs on PND 17. Reddening of left forelimb on PND 1. Encrustation on right forelimb on PND 4-9, and on both forelimbs on PND 17.	11(g)-13(g), 15(g), 17(g), 3, 6, 7
73B	PND 21	Brown staining on head on GD 12-13.	11(g), 13(g), 17(g), 7, 8
74B	PND 21	-	10(g), 12(g), 14(g), 17(g), 8
75B	PND 21	Brown staining on head on GD 2-10, and on head and forelimbs on PND 10.	10(g)-12(g), 2, 7, 10
76B	PND 21	Brown staining on upper dorsal thorax from GD 14 to PND 3 and on PND 21.	11(g), 12(g), 14(g), 17(g), 2, 3, 4, 7

A Treated from day 6 to day 20 after mating.

B Treated from day 6 after mating to PND 10 (where litter survived to PND 10).

- No signs observed.

## APPENDIX 2

Bodyweight - individual values (g) for dams after mating

Group 1: control

Animal number	day after mating									
	0	3	6	10	14	17	20			
1	257	287	304	335	358	399	448			
2	242	276	296	318	350	393	441			
3	236	269	286	311	344	384	437			
4	231	254	266	292	317	355	417			
5	260	286	307	334	363	413	465			
6	266	295	311	336	371	415	466			
7	246	278	291	316	336	386	439			
8	247	273	285	305	337	376	425			
9	303	336	355	385	418	473	538			
10	236	262	286	312	348	389	449			
11	234	264	285	310	341	378	440			
12	221	254	268	292	321	357	404			
13	238	264	283	310	333	374	436			
14	240	267	277	293	324	360	404			
15	238	264	287	316	357	400	480			
16	234	259	280	306	337	379	446			
17	262	290	307	330	357	387	432			
18	248	281	298	321	351	389	433			
19	215	241	250	276	302	333	384			

## APPENDIX 2 - continued

Bodyweight - individual values (g) for dams after mating

Group 2: malathion: 5 mg/kg/day

Animal number	day after mating									
	0	3	6	10	14	17	20			
20	249	283	310	335	368	405	460			
21	247	271	291	313	340	374	414			
22	237	267	285	309	341	383	429			
23	230	266	288	311	343	380	433			
24	270	299	320	342	374	426	487			
25	256	277	286	306	330	363	407			
26	249	274	286	308	337	375	424			
27	263	298	320	343	381	412	466			
28	277	304	320	337	369	411	472			
29	244	272	287	289	315	348	399			
30	231	258	270	289	322	362	420			
31	226	257	273	288	321	360	415			
32	245	271	292	321	353	404	465			
33	240	261	273	295	326	357	412			
34	235	263	280	300	330	368	424			
35	238	263	275	297	323	361	411			
36	258	280	294	307	329	359	397			
37	258	289	300	318	336	374	429			
38	242	269	281	302	331	367	408			

## APPENDIX 2 - continued

Bodyweight - individual values (g) for dams after mating

Group 3: malathion: 50 mg/kg/day

Animal number	day after mating									
	0	3	6	10	14	17	20			
39	261	291	311	338	377	419	485			
40	244	269	286	302	330	361	412			
41	244	270	286	312	341	377	433			
42	236	266	288	318	350	385	435			
43	266	289	303	322	355	389	438			
44	255	284	307	325	358	388	448			
45	253	279	297	312	340	380	426			
46	250	275	291	317	345	387	437			
47	258	283	295	317	349	394	453			
48	236	260	280	295	321	352	401			
49	234	265	286	316	346	383	432			
50	219	249	261	283	305	336	386			
51	249	274	291	316	341	382	431			
52	244	269	285	319	349	398	459			
53	225	250	266	286	310	343	392			
54	239	259	283	308	337	380	420			
55	252	278	294	318	350	394	441			
56	249	267	288	303	331	367	413			
57	239	269	282	306	339	376	418			

## APPENDIX 2 - continued

Bodyweight - individual values (g) for dams after mating

Group 4: malathion: 150 mg/kg/day

Animal number	day after mating									
	0	3	6	10	14	17	20			
58	254	289	309	342	376	413	464			
59	245	276	297	322	355	397	451			
60	240	268	292	321	357	405	470			
61	234	260	273	307	333	366	429			
62	258	282	296	324	355	392	447			
63	256	285	305	342	380	430	486			
64	250	278	299	326	358	389	436			
65	248	268	286	310	343	382	435			
66	254	280	299	318	353	401	467			
67	224	262	278	304	330	360	419			
68	225	254	266	291	312	344	393			
69	253	272	284	303	330	368	412			
70	248	274	287	320	353	397	446			
71	243	271	293	313	348	388	450			
72	228	254	266	287	320	349	391			
73	236	260	272	294	329	369	418			
74	264	290	306	334	359	404	466			
75	242	266	273	292	317	359	414			
76	232	258	276	303	337	376	428			



## APPENDIX 3

Bodyweight – individual values (g) for dams during lactation

Group 1: control

Animal number	PND									
	1	4	7	11	14	17	21			
10	359	381	376	398	384	386	383			
11	351	353	364	372	356	374	350			
12	316	338	328	331	324	358	335			
13	331	350	352	359	376	372	376			
14	328	325	329	332	328	336	351			
15	369	395	378	384	398	388	378			
16	332	333	344	351	348	355	347			
17	363	366	367	398	400	395	385			
18	348	359	363	383	391	390	362			
19	282	304	315	311	332	328	288			

## APPENDIX 3 - continued

Bodyweight – individual values (g) for dams during lactation

Group 2: malathion: 5 mg/kg/day

Animal number	PND									
	1	4	7	11	14	17	21			
29	308	316	314	344	334	334	329			
30	333	316	327	339	338	344	340			
31	318	338	335	353	341	352	345			
32	360	373	382	404	402	403	382			
33	307	322	330	337	344	350	336			
34	333	346	348	364	375	383	378			
35	308	334	327	344	345	341	333			
36	308	332	339	344	357	348	346			
37	326	332	332	358	366	368	346			
38	318	315	342	356	366	374	360			

## APPENDIX 3 - continued

Bodyweight – individual values (g) for dams during lactation

Group 3: malathion: 50 mg/kg/day

Animal number	PND									
	1	4	7	11	14	17	21			
48	308	324	328	336	325	343	326			
49	348	358	351	360	371	377	366			
50	296	305	307	322	316	322	316			
51	336	348	360	381	381	391	366			
52	374	379	380	403	396	401	379			
53	300	315	330	333	335	350	304			
54	334	344	361	384	368	375	345			
55	336	341	341	357	374	360	360			
56	330	325	336	354	363	346	338			
57	349	349	356	366	371	371	350			

## APPENDIX 3 - continued

Bodyweight – individual values (g) for dams during lactation

Group 4: malathion: 150 mg/kg/day

Animal number	PND									
	1	4	7	11	14	17	21			
67	318	338	345	362	362	374	375			
68	298	305	315	328	319	351	340			
69	318	320	328	339	350	346	335			
70	353	360	369	396	405	400	374			
71	353	364	373	396	401	398	384			
72	319	322	335	339	351	358	338			
73	331	344	353	359	358	359	348			
74	374	361	371	388	389	396	395			
75	316	321	323	344	348	356	321			
76	344	351	356	377	392	385	381			

## APPENDIX 4

Gestation length – individual values for dams

Group	:	1	2	3	4
Compound	:	control			
Level (mg/kg/day)	:	0	5	50	150

-----malathion-----

Group 1		Group 2		Group 3		Group 4	
Female number	Gestation length (days)	Female number	Gestation Length (days)	Female number	Gestation length (days)	Female number	Gestation length (days)
10	22	29	22	48	22	67	22.5
11	22	30	22	49	22	68	22
12	22	31	22	50	23	69	22
13	22	32	22	51	22	70	23
14	22	33	22	52	23	71	22.5
15	22	34	22	53	21.5	72	23
16	22	35	22.5	54	23	73	22
17	22.5	36	22.5	55	22.5	74	22.5
18	22.5	37	22	56	22	75	22
19	22.5	38	22	57	22	76	22

## APPENDIX 5

Necropsy observations for adult females on GD 20

Group	:	1	2	3	4
Compound	:	control		-----malathion-----	
Dosage (mg/kg/day)	:	0	5	50	150

Group	Animal number	Necropsy observations
1	1	Brown staining on head, forelimbs and dorsal body surface
	2	Brown staining on head and upper dorsal thorax
	3	Slight brown staining on head and upper dorsal thorax
	4	Slight brown staining on upper dorsal thorax
		Hairloss on forelimbs and forepaws
	5	Hairloss on ventral body surface
	6	Hairloss on forelimbs
	7	Slight brown staining on head and pinna
		Hairloss on forelimbs
2	8	Slight brown staining on head and upper dorsal thorax
	9	Brown staining on head and dorsal surfaces
	20	NAD
	21	NAD
	22	Slight brown staining on head, forelimbs and upper dorsal thorax
	23	Brown staining on upper dorsal thorax
	24	<b>Liver:</b> raised area on median liver lobe 3x3x2mm adhered to thin area on diaphragm
	25	Hairloss on ventral abdomen and forelimbs
		<b>Liver:</b> accentuated lobular markings on all lobes
	26	NAD
	27	Brown staining on head and pinnae
		<b>Liver:</b> accentuated lobular markings on all lobes
	28	NAD

Animals dosed from GD 6.

NAD No abnormalities detected.

## APPENDIX 5 – continued

Necropsy observations for adult females on GD 20

Group	:	1	2	3	4
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

Group	Animal number	Necropsy observations
3	39	Brown staining on head, forelimbs, ventral body surface and upper dorsal thorax
	40	Brown staining on head
	41	Slight brown staining on head
		Slight hairloss on both forelimbs
	42	NAD
	43	Brown staining on head
		Slight hairloss on both forelimbs
	44	Brown staining on upper dorsal thorax
	45	Slight brown staining on upper dorsal thorax
	46	Brown staining on head and upper dorsal thorax
4		Slight hairloss on both forelimbs
	47	Brown staining on head
	58	Brown staining on head and upper dorsal thorax
	59	Brown staining on head
	60	Brown staining on head, dorsal body surface and upper dorsal thorax
		Yellow staining on sacral area
	61	NAD
	62	Brown staining on head and both forelimbs
	63	Brown staining on head
		Yellow staining and wet fur on urinogenital area
		Hairloss on both forelimbs
		Slightly ungroomed coat
	64	Brown staining on head and upper dorsal thorax
		Hairloss on all limbs
	65	Brown staining on head
	66	Kidneys: bilateral renal cavitation

Animals dosed from GD 6.

NAD No abnormalities detected.

## APPENDIX 6

## Necropsy observations for adult females on PND 21

Group	:	1	2	3	4
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

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

Animals dosed from GD 6.

NAD No abnormalities detected.



## APPENDIX 6 – continued

Necropsy observations for adult females on PND 21

Group	:	1	2	3	4
Compound	:	control	-----	malathion-----	
Dosage (mg/kg/day)	:	0	5	50	150

Group	Animal number	Necropsy observations
3	48	NAD
	49	Brown staining on head and upper dorsal thorax
	50	Slight brown staining on head
	51	Brown staining on forelimbs and upper dorsal thorax
		Slight hairloss on lateral surfaces of forelimbs
	52	Partially absent left pinna
		Hairloss on both forepaws
	53	NAD
	54	Brown staining on head and upper dorsal thorax
		Hairloss on all limbs, dorsal and ventral body surfaces
	55	Brown staining on head, muzzle, forelimbs and upper dorsal thorax
4		Hairloss on both forelimbs
	56	Brown staining on head and upper dorsal thorax
	57	NAD
	67	NAD
	68	Hairloss on both forelimbs
	69	Slight hairloss on both forelimbs
	70	Brown staining on head and upper dorsal thorax
		Hairloss on both forelimbs
	71	Brown staining on muzzle
	72	Brown staining on muzzle
		Hairloss on both forelimbs
	73	NAD
	74	NAD
	75	NAD
	76	Brown staining on head and upper dorsal thorax

Animals dosed from GD 6.

NAD No abnormalities detected.

## APPENDIX 7

Litter data – individual values on GD 20

Group 1: control

Animal number	Corpora lutea	Implants	Resorptions		Total	Live young		Sex ratio (% M)	Implantation loss (%)	
			Early	Late		Male	Female		Pre-	Post-
1	19	14	1	0	1	7	6	53.8	26.3	7.1
2	30	18	1	0	1	9	8	52.9	40.0	5.6
3	18	18	4	0	4	7	7	50.0	0.0	22.2
4	18	16	0	0	0	10	6	62.5	11.1	0.0
5	17	17	0	0	0	11	6	64.7	0.0	0.0
6	17	17	2	0	2	8	7	53.3	0.0	11.8
7	18	19†	0	0	0	6	13	31.6	0.0	0.0
8	15	15	0	0	0	6	9	40.0	0.0	0.0
9	20	19	0	0	0	10	9	52.6	5.0	0.0

† Total number of implants exceeds corpora lutea, therefore pre-implantation loss assumed to be zero. Dams dosed from GD 6.

## APPENDIX 7 – continued

Litter data – individual values on GD 20

Group 2: malathion: 5 mg/kg/day

Animal number	Corpora lutea	Implants	Resorptions		Total	Male	Live young		Sex ratio (% M)	Implantation loss (%)	
			Early	Late			Female	Total		Pre-	Post-
20	18	17	0	0	0	5	12	17	29.4	5.6	0.0
21	16	16	1	1	2	5	9	14	35.7	0.0	12.5
22	21	16	0	0	0	9	7	16	56.3	23.8	0.0
23	23	17	1	0	1	8	8	16	50.0	26.1	5.9
24	20	19	0	0	0	8	11	19	42.1	5.0	0.0
25	16	16	2	0	2	6	8	14	42.9	0.0	12.5
26	19	18	0	0	0	9	9	18	50.0	5.3	0.0
27	19	17	4	0	4	11	2	13	84.6	10.5	23.5
28	15	16†	1	0	1	6	9	15	40.0	0.0	6.3

† Total number of implants exceeds corpora lutea, therefore pre-implantation loss assumed to be zero.  
Dams dosed from GD 6.

## APPENDIX 7 - continued

Litter data – individual values on GD 20

Group 3: malathion: 50 mg/kg/day

Animal number	Corpora lutea	Implants	Resorptions		Live young		Sex ratio (%M)	Implantation loss (%)	
			Early	Late	Male	Female		Pre-	Post-
39	19	18	0	0	11	7	61.1	5.3	0.0
40	15	16†	2	0	8	6	57.1	0.0	12.5
41	16	17†	1	0	10	6	62.5	0.0	5.9
42	15	14	1	0	8	5	61.5	6.7	7.1
43	17	17	0	0	8	9	47.1	0.0	0.0
44	16	16	1	0	5	10	33.3	0.0	6.3
45	14	14	0	0	9	5	64.3	0.0	0.0
46	15	15	0	0	7	8	46.7	0.0	0.0
47	17	17	0	0	8	9	47.1	0.0	0.0

† Total number of implants exceeds corpora lutea, therefore pre-implantation loss assumed to be zero.  
Dams dosed from GD 6.

## APPENDIX 7 - continued

Litter data – individual values on GD 20

Group 4: malathion: 150 mg/kg/day

Animal number	Corpora lutea	Implants	Resorptions		Total	Male	Live young		Sex ratio (% M)	Implantation loss (%)	
			Early	Late			Female	Total		Pre-	Post-
58	14	16†	2	0	2	8	6	14	57.1	0.0	12.5
59	17	16	2	0	2	6	8	14	42.9	5.9	12.5
60	17	17	0	0	0	9	8	17	52.9	0.0	0.0
61	16	16	0	0	0	9	7	16	56.3	0.0	0.0
62	16	15	1	0	1	9	5	14	64.3	6.3	6.7
63	14	15†	0	0	0	7	8	15	46.7	0.0	0.0
64	17	16	0	0	0	5	11	16	31.3	5.9	0.0
65	17	18†	0	0	0	7	11	18	38.9	0.0	0.0
66	17	18†	0	0	0	11	7	18	61.1	0.0	0.0

† Total number of implants exceeds corpora lutea, therefore pre-implantation loss assumed to be zero.  
Dams dosed from GD 6.

APPENDIX 8

Fetal and litter weights – individual values (g) on GD 20

Group 1: control

Animal number	Litter weight	Fetal weights					
		Males		Females		Total	
		Mean	SD	Mean	SD	Mean	SD
1	51.98	4.10	0.37	3.88	0.30	4.00	0.34
2	65.09	3.91	0.15	3.74	0.20	3.83	0.19
3	55.82	4.05	0.20	3.92	0.18	3.99	0.19
4	66.79	4.21	0.14	4.11	0.13	4.17	0.14
5	73.69	4.43	0.28	4.16	0.23	4.33	0.29
6	60.01	4.14	0.11	3.84	0.24	4.00	0.23
7	72.01	3.89	0.16	3.74	0.16	3.79	0.17
8	61.54	4.22	0.15	4.02	0.20	4.10	0.21
9	74.28	3.95	0.18	3.87	0.27	3.91	0.23

Dams dosed from GD 6.  
SD Standard deviation.

## APPENDIX 8 - continued

Fetal and litter weights – individual values (g) on GD 20

Group 2: malathion: 5 mg/kg/day

Animal number	Litter weight	Fetal weights (g)					
		Males		Females		Total	
		Mean	SD	Mean	SD	Mean	SD
20	62.10	3.84	0.27	3.58	0.31	3.65	0.32
21	53.36	3.91	0.29	3.75	0.11	3.81	0.20
22	61.36	3.93	0.20	3.72	0.27	3.84	0.25
23	65.40	4.11	0.28	4.07	0.18	4.09	0.23
24	76.12	4.14	0.13	3.91	0.18	4.01	0.20
25	58.89	4.31	0.07	4.13	0.13	4.21	0.14
26	66.58	3.74	0.19	3.66	0.23	3.70	0.21
27	53.98	4.16	0.19	4.13	0.16	4.15	0.18
28	54.40	3.65	0.21	3.61	0.20	3.63	0.20

Dams dosed from GD 6.

SD Standard deviation.

## APPENDIX 8 - continued

Fetal and litter weights – individual values (g) on GD 20

Group 3: malathion: 50 mg/kg/day

Animal number	Litter weight	Fetal weights (g)					
		Males		Females		Total	
		Mean	SD	Mean	SD	Mean	SD
39	70.18	3.96	0.18	3.81	0.27	3.90	0.22
40	51.67	3.82	0.15	3.52	0.32	3.69	0.27
41	63.55	4.01	0.26	3.90	0.14	3.97	0.22
42	55.33	4.47	0.19	3.92	0.22	4.26	0.34
43	60.35	3.59	0.27	3.51	0.20	3.55	0.23
44	62.24	4.32	0.04	4.06	0.29	4.15	0.26
45	56.88	4.09	0.16	4.02	0.14	4.06	0.15
46	59.53	4.16	0.20	3.80	0.16	3.97	0.25
47	63.47	3.91	0.30	3.58	0.15	3.73	0.28

Dams dosed from GD 6.

SD Standard deviation.



## APPENDIX 8 - continued

Fetal and litter weights – individual values (g) on GD 20

Group 4: malathion: 150 mg/kg/day

Animal number	Litter weight	Fetal weights (g)					
		Males		Females		Total	
		Mean	SD	Mean	SD	Mean	SD
58	55.71	4.11	0.16	3.81	0.19	3.98	0.23
59	50.76	3.81	0.13	3.49	0.21	3.63	0.24
60	71.78	4.43	0.33	3.99	0.31	4.22	0.38
61	67.65	4.32	0.21	4.11	0.14	4.23	0.21
62	58.74	4.24	0.24	4.12	0.09	4.20	0.20
63	60.52	4.13	0.26	3.95	0.24	4.03	0.26
64	61.07	3.99	0.21	3.74	0.19	3.82	0.22
65	74.86	4.26	0.08	4.09	0.49	4.16	0.39
66	62.75	3.51	0.21	3.45	0.17	3.49	0.19

Dams dosed from GD 6.

SD Standard deviation.

## APPENDIX 9

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

Group	Animal Number	Dam brain weight	Fetuses		
			Pooled brain weight	Number of fetuses	Mean brain weight (g)
1	1	1.936	1.761	13	0.135
	2	1.897	2.908	17	0.171
	3	1.990	2.111	14	0.151
	4	1.956	2.247	16	0.140
	5	1.956	2.768	17	0.163
	6	2.036	2.930	15	0.195
	7	1.945	2.817	19	0.148
	8	1.944	2.347	15	0.156
2	20	1.947	2.684	17	0.158
	21	1.879	2.316	14	0.165
	22	2.037	2.624	16	0.164
	23	1.975	2.853	16	0.178
	24	1.890	2.980	19	0.157
	25	1.963	2.587	14	0.185
	26	1.929	2.738	18	0.152
	27	1.877	2.306	13	0.177

Dams dosed from GD 6.

## APPENDIX 9 - continued

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

Group	Animal Number	Dam brain weight	Fetuses			
			Pooled brain weight	Number of fetuses	Mean brain weight (g)	
3	39	1.993	2.895	18	0.161	
	40	1.829	2.166	14	0.155	
	41	1.991	2.755	16	0.172	
	42	1.950	2.172	13	0.167	
	43	1.710	2.198	17	0.129	
	44\$	1.909	2.185	15	0.146	
	45	2.029	2.126	14	0.152	
	46	1.916	2.200	15	0.147	
	58	2.009	2.353	14	0.168	
	59	1.936	2.286	14	0.163	
4	60	1.845	2.725	17	0.160	
	61	1.900	2.732	16	0.171	
	62	1.964	2.518	14	0.180	
	63	1.920	2.499	15	0.167	
	64	2.020	2.616	16	0.164	
	65	1.916	2.972	18	0.165	

Dams dosed from GD 6.

\$ Brain of dam weighed post-freezing.

APPENDIX 10

Litter size up to PND 11 – individual values

Group 1 : control

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

Dams treated from GD 6 to PND 11.

## APPENDIX 10 - continued

Litter size up to PND 11 – individual values

Group 2: malathion: 5 mg/kg/day

Animal number	Implants	Total litter size Day 1	Live litter size on PND						
			Before cull			After cull			
			1	4	4	4	7	11	
29	16	16	16	16	16	8	8	8	
30	16	14	14	11	11	8	8	8	
31	17	16	16	16	16	8	8	8	
32	19	18	18	18	18	8	8	8	
33	17	16	16	15	15	8	8	8	
34	16	15	15	13	13	8	8	8	
35	17	17	17	17	17	8	8	8	
36	14	12	12	12	12	8	8	8	
37	20	18	18	18	18	8	8	8	
38	18	15	15	14	14	8	8	8	

Dams treated from GD 6 to PND 11.

## APPENDIX 10 - continued

Litter size up to PND 11 – individual values

Group 3: malathion: 50 mg/kg/day

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

Dams treated from GD 6 to PND 11.

APPENDIX 10 - continued

Litter size up to PND 11 – individual values

Group 4: malathion: 150 mg/kg/day

Animal number	Implants	Total litter size Day 1	Live litter size on PND						
			Before cull		After cull				
			1	4	4	7	11		
67	17	17	17	17	8	8	8		
68	17	15	15	15	8	8	8		
69	11	11	11	11	8	8	8		
70	15	15	15	15	8	8	8		
71	17	16	16	16	8	8	8		
72	15	13	13	12	8	8	8		
73	13	13	13	13	8	8	8		
74	16	15	15	15	8	8	8		
75	15	15	15	15	8	8	8		
76	15	12	12	12	8	8	8		

Dams treated from GD 6 to PND 11.

## APPENDIX 11

Offspring survival indices up to PND 11 - individual values

Group 1: control

Animal number	Post-implantation Survival index (%)	Live birth Index (%)	Viability index (%)	Lactation index (%)	
				PND 7	PND 11
10	93	100	100	100	100
11	88	100	86	100	100
12	94	100	100	100	100
13	94	100	94	100	100
14	92	100	100	100	100
15	89	100	100	100	100
16	95	100	100	100	100
17	83	100	100	100	100
18	94	100	100	100	100
19	100	100	100	100	100

Dams treated from GD 6 to PND 11.



## APPENDIX 11 - continued

Offspring survival indices up to PND 11 - individual values

Group 2: malathion: 5 mg/kg/day

Animal number	Post-implantation Survival index (%)	Live birth Index (%)	Viability index (%)	Lactation index (%)	
				PND 7	PND 11
29	100	100	100	100	100
30	88	100	79	100	100
31	94	100	100	100	100
32	95	100	100	100	100
33	94	100	94	100	100
34	94	100	87	100	100
35	100	100	100	100	100
36	86	100	100	100	100
37	90	100	100	100	100
38	83	100	93	100	100

Dams treated from GD 6 to PND 11.

## APPENDIX 11 - continued

Offspring survival indices up to PND 11 - individual values

Group 3: malathion: 50 mg/kg/day

Animal number	Post-implantation Survival index (%)	Live birth Index (%)	Viability index (%)	Lactation index (%)	
				PND 7	PND 11
48	93	100	100	100	100
49	89	100	100	100	100
50	100	100	94	100	100
51	88	100	100	100	100
52	82	100	100	100	100
53	93	100	100	100	100
54	86	100	100	100	100
55	76	100	100	100	100
56	93	100	100	100	100
57	92	100	100	100	100

Dams treated from GD 6 to PND 11.

APPENDIX 11 - continued

Offspring survival indices up to PND 11 - individual values

Group 4: malathion: 150 mg/kg/day

Animal number	Post-implantation Survival index (%)	Live birth Index (%)	Viability index (%)	Lactation index (%)	
				PND 7	PND 11
67	100	100	100	100	100
68	88	100	100	100	100
69	100	100	100	100	100
70	100	100	100	100	100
71	94	100	100	100	100
72	87	100	92	100	100
73	100	100	100	100	100
74	94	100	100	100	100
75	100	100	100	100	100
76	80	100	100	100	100

Dams treated from GD 6 to PND 11.

## APPENDIX 12

Sex ratio up to PND 11 – individual litter values

Group 1: control

Animal number	Total PND 1			Live (before cull)						Live (after cull)					
	PND 1			PND 4			PND 4			PND 7			PND 11		
	M	F	%M	M	F	%M	M	F	%M	M	F	%M	M	F	%M
10	8	6	57.1	8	6	57.1	8	6	57.1	4	4	50.0	4	4	50.0
11	7	7	50.0	7	7	50.0	5	7	41.7	4	4	50.0	4	4	50.0
12	9	6	60.0	9	6	60.0	9	6	60.0	4	4	50.0	4	4	50.0
13	10	6	62.5	10	6	62.5	9	6	60.0	4	4	50.0	4	4	50.0
14	8	4	66.7	8	4	66.7	8	4	66.7	4	4	50.0	4	4	50.0
15	8	8	50.0	8	8	50.0	8	8	50.0	4	4	50.0	4	4	50.0
16	6	12	33.3	6	12	33.3	6	12	33.3	4	4	50.0	4	4	50.0
17	8	7	53.3	8	7	53.3	8	7	53.3	4	4	50.0	4	4	50.0
18	4	11	26.7	4	11	26.7	4	11	26.7	4	4	50.0	4	4	50.0
19	7	8	46.7	7	8	46.7	7	8	46.7	4	4	50.0	4	4	50.0

Dams treated from GD 6 to PND 11.

M Males.

F Females.

## APPENDIX 12 - continued

Sex ratio up to PND 11 – individual litter values

Group 2: malathion: 5 mg/kg/day

Animal number	Total on Day			Live (before cull)						Live (after cull)					
	PND 1			PND 1			PND 4			PND 4			PND 7		
	M	F	%M	M	F	%M	M	F	%M	M	F	%M	M	F	%M
29	5	11	31.3	5	11	31.3	5	11	31.3	4	4	50.0	4	4	50.0
30	9	5	64.3	9	5	64.3	7	4	63.6	4	4	50.0	4	4	50.0
31	8	8	50.0	8	8	50.0	8	8	50.0	4	4	50.0	4	4	50.0
32	9	9	50.0	9	9	50.0	9	9	50.0	4	4	50.0	4	4	50.0
33	9	7	56.3	9	7	56.3	9	6	60.0	4	4	50.0	4	4	50.0
34	9	6	60.0	9	6	60.0	8	5	61.5	4	4	50.0	4	4	50.0
35	8	9	47.1	8	9	47.1	8	9	47.1	4	4	50.0	4	4	50.0
36	5	7	41.7	5	7	41.7	5	7	41.7	4	4	50.0	4	4	50.0
37	7	11	38.9	7	11	38.9	7	11	38.9	4	4	50.0	4	4	50.0
38	5	10	33.3	5	10	33.3	4	10	28.6	4	4	50.0	4	4	50.0

Dams treated from GD 6 to PND 11.

M Males.

F Females.

## APPENDIX 12 - continued

Sex ratio up to PND 11 – individual litter values

Group 3: malathion: 50 mg/kg/day

Animal number	Total on Day			Live (before cull)						Live (after cull)					
	PND 1			PND 1			PND 4			PND 4			PND 7		
	M	F	%M	M	F	%M	M	F	%M	M	F	%M	M	F	%M
48	6	8	42.9	6	8	42.9	6	8	42.9	4	4	50.0	4	4	50.0
49	10	6	62.5	10	6	62.5	10	6	62.5	4	4	50.0	4	4	50.0
50	9	7	56.3	9	7	56.3	8	7	53.3	4	4	50.0	4	4	50.0
51	5	10	33.3	5	10	33.3	5	10	33.3	4	4	50.0	4	4	50.0
52	6	8	42.9	6	8	42.9	6	8	42.9	4	4	50.0	4	4	50.0
53	5	9	35.7	5	9	35.7	5	9	35.7	4	4	50.0	4	4	50.0
54	4	8	33.3	4	8	33.3	4	8	33.3	4	4	50.0	4	4	50.0
55	3	10	23.1	3	10	23.1	3	10	23.1	3	5	37.5	3	5	37.5
56	9	5	64.3	9	5	64.3	9	5	64.3	4	4	50.0	4	4	50.0
57	5	6	45.5	5	6	45.5	5	6	45.5	4	4	50.0	4	4	50.0

Dams treated from GD 6 to PND 11.

M Males.

F Females.

## APPENDIX 12 - continued

Sex ratio up to PND 11 – individual litter values

Group 4: malathion: 150 mg/kg/day

Animal number	Total on Day			Live (before cull)						Live (after cull)					
	PND 1			PND 1			PND 4			PND 4			PND 7		
	M	F	%M	M	F	%M	M	F	%M	M	F	%M	M	F	%M
67	12	5	70.6	12	5	70.6	12	5	70.6	4	4	50.0	4	4	50.0
68	6	9	40.0	6	9	40.0	6	9	40.0	4	4	50.0	4	4	50.0
69	3	8	27.3	3	8	27.3	3	8	27.3	3	5	37.5	3	5	37.5
70	9	6	60.0	9	6	60.0	9	6	60.0	4	4	50.0	4	4	50.0
71	3	13	18.8	3	13	18.8	3	13	18.8	3	5	37.5	3	5	37.5
72	6	7	46.2	6	7	46.2	6	6	50.0	4	4	50.0	4	4	50.0
73	6	7	46.2	6	7	46.2	6	7	46.2	4	4	50.0	4	4	50.0
74	8	7	53.3	8	7	53.3	8	7	53.3	4	4	50.0	4	4	50.0
75	10	5	66.7	10	5	66.7	10	5	66.7	4	4	50.0	4	4	50.0
76	5	7	41.7	5	7	41.7	5	7	41.7	4	4	50.0	4	4	50.0

Dams treated from GD 6 to PND 11.

M Males.

F Females.

## APPENDIX 13

Bodyweight up to PND 11 – individual litter mean values (g) for male offspring

Group 1: control

Animal Number	Before cull				After cull			
	PND 1		PND 4		PND 4		PND 7	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
10	6.5	0.4	9.5	0.4	9.4	0.5	15.6	0.8
11	6.4	0.6	10.4	0.3	10.4	0.3	17.3	0.6
12	6.6	0.2	9.5	0.3	9.3	0.3	16.4	0.5
13	6.0	0.4	8.7	0.6	8.9	0.7	15.2	0.8
14	6.2	0.5	8.1	0.7	7.9	1.0	12.9	1.8
15	6.6	0.3	9.0	0.4	9.3	0.3	17.0	0.9
16	6.4	0.5	8.8	0.8	8.7	0.6	15.3	1.1
17	7.1	0.4	9.8	0.4	9.9	0.3	15.8	1.4
18	6.1	0.3	8.5	0.3	8.5	0.3	14.7	0.5
19	6.8	0.4	10.0	0.6	10.0	0.6	16.3	0.7

Dams treated from GD 6 to PND 11.

SD Standard deviation.



## APPENDIX 13 - continued

Bodyweight up to PND 11 – individual litter mean values (g) for male offspring

Group 2: malathion: 5 mg/kg/day

Animal Number	Before cull				After cull			
	PND 1		PND 4		PND 4		PND 7	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
29	6.0	0.9	8.8	1.5	8.6	1.5	15.5	2.5
30	6.0	0.4	8.2	1.0	7.9	1.1	12.0	2.3
31	6.2	0.4	7.9	0.3	8.0	0.3	13.5	0.5
32	6.7	0.3	9.0	0.7	8.7	0.7	14.1	0.9
33	6.2	0.3	9.2	0.4	9.1	0.5	14.8	0.7
34	6.0	0.3	7.1	1.0	7.3	0.7	11.5	1.1
35	6.3	0.3	8.1	0.8	7.8	0.9	12.4	1.6
36	6.9	0.8	10.2	1.4	9.8	1.1	15.4	2.0
37	5.7	0.2	8.1	0.7	7.7	0.7	14.2	1.0
38	5.4	0.5	7.8	0.3	7.8	0.3	12.9	1.0
							24.1	1.7

Dams treated from GD 6 to PND 11.

SD Standard deviation.

## APPENDIX 13 - continued

Bodyweight up to PND 11 – individual litter mean values (g) for male offspring

Group 3: malathion: 50 mg/kg/day

Animal Number	Before cull				After cull			
	PND 1		PND 4		PND 4		PND 7	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
48	6.5	0.4	9.2	0.4	9.1	0.4	15.0	0.6
49	5.9	0.3	7.5	0.7	8.0	0.4	13.1	0.7
50	6.9	0.4	9.6	0.6	9.6	0.7	15.9	1.2
51	7.0	0.4	9.8	0.4	9.7	0.5	16.1	0.4
52	8.1	0.1	11.7	0.8	11.4	0.7	19.7	1.4
53	6.3	0.3	9.4	0.3	9.5	0.3	15.7	0.6
54	7.1	0.5	10.4	0.6	10.4	0.6	17.6	0.6
55	6.8	0.1	10.7	0.3	10.7	0.3	17.3	0.8
56	6.6	0.3	9.3	0.3	9.5	0.3	15.9	0.5
57	5.8	0.5	9.3	0.5	9.5	0.4	16.3	0.8
							25.8	1.0
							25.3	1.3
							26.7	1.3
							28.2	0.7
							31.2	2.3
							26.1	1.1
							29.2	1.1
							28.7	0.7
							26.1	0.4
							26.7	0.7

Dams treated from GD 6 to PND 11.

SD Standard deviation.

## APPENDIX 13 - continued

Bodyweight up to PND 11 – individual litter mean values (g) for male offspring

Group 4: malathion: 150 mg/kg/day

Animal Number	Before cull				After cull			
	PND 1		PND 4		PND 4		PND 7	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
67	6.5	0.3	8.2	0.6	7.8	0.5	13.2	1.0
68	5.8	0.3	8.2	0.6	8.4	0.3	14.2	0.5
69	7.7	0.3	9.8	0.4	9.8	0.4	15.3	0.4
70	7.4	0.3	10.5	0.7	10.1	0.7	17.1	1.8
71	7.4	0.4	9.1	0.5	9.1	0.5	15.6	0.7
72	7.3	0.4	10.8	0.2	10.8	0.3	18.3	0.1
73	6.8	1.0	9.5	1.6	10.2	1.3	17.5	2.7
74	7.2	0.4	9.5	0.3	9.6	0.3	15.6	0.8
75	6.3	0.4	9.6	0.7	9.1	0.8	15.7	0.8
76	6.8	0.2	10.1	0.4	10.1	0.5	15.0	0.9

Dams treated from GD 6 to PND 11.

SD Standard deviation.

## APPENDIX 14

Bodyweight up to PND 11 – individual litter mean values (g) for female offspring

Group 1: control

Animal Number	Before cull				After cull			
	PND 1		PND 4		PND 4		PND 7	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
10	6.1	0.3	8.7	0.1	8.7	0.1	14.9	0.2
11	6.2	0.2	10.0	0.5	9.8	0.5	16.2	0.9
12	6.2	0.2	8.8	0.4	9.0	0.1	15.5	0.5
13	6.0	0.2	8.7	0.4	8.8	0.3	14.8	0.6
14	5.6	0.2	7.4	0.4	7.4	0.4	12.6	0.7
15	6.3	0.3	8.6	1.1	8.8	0.6	16.2	1.4
16	6.0	0.3	8.4	1.0	8.4	1.0	14.8	1.6
17	6.7	0.3	9.7	0.7	9.6	0.9	16.4	1.8
18	5.8	0.4	7.9	1.2	7.5	1.9	12.9	3.6
19	6.8	0.5	9.5	1.0	9.7	0.7	16.0	1.4
							25.0	1.8

Dams treated from GD 6 to PND 11.

SD Standard deviation.

## APPENDIX 14 - continued

Bodyweight up to PND 11 – individual litter mean values (g) for female offspring

Group 2: malathion: 5 mg/kg/day

Animal Number	Before cull				After cull			
	PND 1		PND 4		PND 4		PND 7	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
29	5.8	0.7	8.7	1.2	8.1	1.9	14.7	3.3
30	5.6	0.5	7.9	0.8	7.9	0.8	12.5	1.3
31	5.6	0.2	7.5	0.4	7.7	0.3	13.3	0.8
32	6.3	0.4	8.6	0.8	8.2	0.9	13.4	1.8
33	5.9	0.3	8.6	0.2	8.6	0.1	14.9	0.4
34	5.5	0.3	6.7	0.3	6.7	0.4	10.3	0.6
35	6.2	0.3	8.4	0.6	8.3	0.6	13.5	0.9
36	6.9	0.4	10.6	1.0	10.6	1.4	17.0	2.0
37	5.7	0.2	8.0	0.5	8.0	0.6	14.8	0.9
38	5.5	0.4	7.6	0.6	7.6	1.0	12.8	2.0

Dams treated from GD 6 to PND 11.

SD Standard deviation.

## APPENDIX 14 - continued

Bodyweight up to PND 11 – individual litter mean values (g) for female offspring

Group 3: malathion: 50 mg/kg/day

Animal Number	Before cull				After cull			
	PND 1		PND 4		PND 4		PND 7	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
48	6.3	0.2	9.0	0.5	9.3	0.4	14.9	0.6
49	5.7	0.2	7.5	0.2	7.6	0.2	12.3	0.3
50	6.7	0.3	9.0	0.6	9.4	0.4	15.5	0.6
51	6.6	0.3	8.9	0.7	8.3	0.7	14.5	1.7
52	7.5	0.4	10.8	0.9	11.2	0.4	19.3	0.7
53	5.8	0.3	8.5	0.5	8.8	0.4	14.7	0.6
54	7.1	0.5	10.5	0.4	10.7	0.3	18.1	0.8
55	6.3	0.5	10.0	0.8	10.4	0.7	17.2	0.9
56	6.2	0.4	8.9	0.7	8.7	0.5	14.2	0.8
57	6.0	0.1	9.3	0.2	9.3	0.3	16.2	0.4

Dams treated from GD 6 to PND 11.

SD Standard deviation.

## APPENDIX 14 - continued

Bodyweight up to PND 11 – individual litter mean values (g) for female offspring

Group 4: malathion: 150 mg/kg/day

Animal Number	Before cull				After cull			
	PND 1		PND 4		PND 4		PND 7	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
67	6.1	0.5	7.3	1.1	6.9	1.1	12.8	2.2
68	5.6	0.3	7.8	0.6	8.3	0.4	14.4	0.4
69	6.9	0.7	8.9	0.9	8.9	1.2	13.6	2.0
70	7.1	0.6	9.9	1.0	9.5	0.8	16.1	1.5
71	6.8	0.5	8.6	0.7	8.4	0.3	14.4	0.8
72	6.9	0.2	10.5	0.5	10.3	0.4	17.4	1.0
73	6.0	0.6	8.8	1.0	9.4	0.5	16.2	0.9
74	6.4	0.5	8.9	0.5	9.0	0.5	14.5	1.2
75	6.0	0.2	9.5	0.4	9.5	0.5	16.2	0.6
76	6.4	0.4	9.6	0.6	9.5	0.7	14.4	1.3
							23.2	3.1
							25.9	0.5
							22.4	3.0
							26.5	1.9
							23.7	1.4
							27.5	1.6
							27.9	1.3
							24.1	1.8
							27.6	0.8
							22.9	1.5

Dams treated from GD 6 to PND 11.

SD Standard deviation.

## APPENDIX 15

Necropsy findings – individual observations for offspring on PND 4

Group 1: control

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
1005	NAD	1013	NAD
1006	NAD	1014	NAD
1106	NAD	1112	NAD
1205	<b>Kidneys and ureter:</b> bilateral renal cavitation and bilateral hydroureter	1113	NAD
1206	<b>Kidneys and ureter:</b> bilateral renal cavitation and bilateral hydroureter	1214	<b>Kidneys and ureter:</b> bilateral renal cavitation and bilateral hydroureter
1306	<b>Kidneys and ureter:</b> unilateral renal cavitation and unilateral hydroureter	1215	<b>Kidneys and ureter:</b> bilateral renal cavitation and bilateral hydroureter
1307	NAD	1315	NAD
1405	NAD	1316	<b>Kidneys and ureter:</b> unilateral renal cavitation and unilateral hydroureter
1406	NAD	1513	NAD
1505	NAD	1514	NAD
1506	<b>Kidneys and ureter:</b> bilateral renal cavitation and bilateral hydroureter	1611	<b>Kidneys and ureter:</b> bilateral renal cavitation and bilateral hydroureter
1605	<b>Kidneys and ureter:</b> unilateral renal cavitation and unilateral hydroureter	1612	NAD
1606	NAD	1713	NAD
1705	NAD	1714	NAD
1706	NAD	1809	NAD
1905	NAD	1810	NAD
1906	NAD	1912	NAD
		1913	NAD

NAD No abnormalities detected.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

Dams treated from GD 6.



## APPENDIX 15 - continued

Necropsy findings – individual observations for offspring on PND 4

Group 2: malathion: 5 mg/kg/day

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
2905	NAD	2910	<b>Kidneys and ureter:</b> bilateral renal cavitation and bilateral hydroureter
3005	NAD	2911	<b>Kidneys and ureter:</b> unilateral renal cavitation and unilateral hydroureter
3007	NAD	3113	NAD
3105	NAD	3114	NAD
3106	NAD	3214	NAD
3205	NAD	3215	NAD
3206	NAD	3315	NAD
3305	NAD	3316	NAD
3306	NAD	3415	NAD
3405	NAD	3513	NAD
3406	NAD	3514	NAD
3506	NAD	3610	NAD
3507	NAD	3611	NAD
3605	NAD	3712	NAD
3705	NAD	3713	NAD
3706	NAD	3810	NAD
		3811	NAD

NAD No abnormalities detected.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

Dams treated from GD 6.

## APPENDIX 15 - continued

Necropsy findings – individual observations for offspring on PND 4

Group 3: malathion: 50 mg/kg/day

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
4805	NAD	4811	NAD
4806	NAD	4812	NAD
4905	NAD	4915	NAD
4906	NAD	4916	NAD
5006	NAD	5014	NAD
5007	NAD	5015	NAD
5105	NAD	5110	NAD
5205	NAD	5111	NAD
5206	NAD	5211	NAD
5305	<b>Kidneys and ureter:</b> bilateral renal cavitation and bilateral hydroureter	5212	NAD
5605	NAD	5310	<b>Kidneys and ureter:</b> unilateral renal cavitation and unilateral hydroureter
5606	NAD	5311	NAD
5705	NAD	5409	NAD
		5410	NAD
		5509	NAD
		5510	NAD
		5614	NAD
		5710	NAD
		5711	NAD

NAD No abnormalities detected.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

Dams treated from GD 6.

## APPENDIX 15 - continued

Necropsy findings – individual observations for offspring on PND 4

Group 4: malathion: 150 mg/kg/day

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
6705	NAD	6717	NAD
6706	NAD	6811	<b>Kidneys and ureter:</b> bilateral renal cavitation and bilateral hydroureter
6805	NAD	6812	NAD
6806	NAD	6909	NAD
7005	NAD	6910	NAD
7006	<b>Kidneys:</b> unilateral renal cavitation	7014	NAD
7205	NAD	7015	NAD
7206	NAD	7109	NAD
7305	NAD	7110	NAD
7306	NAD	7212	NAD
7405	NAD	7213	NAD
7406	<b>Kidneys and ureter:</b> unilateral renal cavitation and unilateral hydroureter	7311	<b>Kidneys and ureter:</b> bilateral renal cavitation and bilateral hydroureter
7505	<b>Kidneys and ureter:</b> bilateral renal cavitation and bilateral hydroureter	7312	<b>Kidneys and ureter:</b> unilateral renal cavitation and unilateral hydroureter
7506	NAD	7413	<b>Kidneys and ureter:</b> unilateral renal cavitation and unilateral hydroureter
7605	NAD	7414	<b>Kidneys and ureter:</b> bilateral renal cavitation and bilateral hydroureter
		7515	NAD
		7610	NAD
		7611	NAD

NAD No abnormalities detected.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

Dams treated from GD 6.

## APPENDIX 16

Brain weights – individual values (g) for male and female offspring on PND 4

## Group 1: control

Sex	Animal number	Brain weight (g)	Sex	Animal number	Brain weight (g)
M	1005	0.393	F	1013	0.380
	1006	0.383		1014	0.371
	1106	0.431		1112	0.429
	1205	0.401		1113	0.450
	1206	0.427		1214	0.403
	1306	0.345		1215	0.392
	1307	0.392		1315	0.336
	1405	0.360		1316	0.392
	1406	0.375		1513	0.325
	1505	0.382		1514	0.400
	1506	0.392		1611	0.408
	1605	0.399		1612	0.404
	1606	0.436		1713	0.411
	1705	0.415		1714	0.398
	1706	0.383		1809	0.345
	1905	0.488		1810	0.389
	1906	0.513		1912	0.504
				1913	0.470

Last two digits of animal numbers denote pup number. Other digits denote litter numbers.  
Dams dosed from GD 6.

## APPENDIX 16 - continued

Brain weights – individual values (g) for male and female offspring on PND 4

Group 2: malathion: 5 mg/kg/day

Sex	Animal number	Brain weight (g)	Sex	Animal number	Brain weight (g)
M	2905	0.413	F	2910	0.385
	3005	0.385		2911	0.378
	3007	0.398		3113	0.368
	3105	0.395		3114	0.363
	3106	0.343		3214	0.371
	3205	0.418		3215	0.389
	3206	0.413		3315	0.416
	3305	0.426		3316	0.408
	3306	0.427		3415	0.328
	3405	0.344		3513	0.358
	3406	0.336		3514	0.374
	3506	0.371		3610	0.470
	3507	0.383		3611	0.473
	3605	0.516		3712	0.370
	3705	0.390		3713	0.390
	3706	0.382		3810	0.346
				3811	0.373

Last two digits of animal numbers denote pup number. Other digits denote litter numbers.  
Dams dosed from GD 6.

## APPENDIX 16 - continued

Brain weights – individual values (g) for male and female offspring on PND 4

Group 3: malathion: 50 mg/kg/day

Sex	Animal number	Brain weight (g)	Sex	Animal number	Brain weight (g)
M	4805	0.443	F	4811	0.403
	4806	0.422		4812	0.404
	4905	0.356		4915	0.347
	4906	0.337		4916	0.340
	5006	0.462		5014	0.455
	5007	0.491		5015	0.456
	5105	0.434		5110	0.398
	5205	0.502		5111	0.393
	5206	0.482		5211	0.462
	5305	0.357		5212	0.478
	5605	0.370		5310	0.378
	5606	0.394		5311	0.397
	5705	0.337		5409	0.469
				5410	0.455
				5509	0.470
				5510	0.444
				5614	0.407
				5710	0.370
				5711	0.378

Last two digits of animal numbers denote pup number. Other digits denote litter numbers.  
Dams dosed from GD 6.

## APPENDIX 16 - continued

Brain weights – individual values (g) for male and female offspring on PND 4

Group 4: malathion: 150 mg/kg/day

Sex	Animal number	Brain weight (g)	Sex	Animal number	Brain weight (g)
M	6705	0.394	F	6717	0.383
	6706	0.392		6811	0.352
	6805	0.390		6812	0.384
	6806	0.384		6909	0.373
	7005	0.468		6910	0.417
	7006	0.469		7014	0.469
	7205	0.480		7015	0.503
	7206	0.470		7109	0.464
	7305	0.383		7110	0.451
	7306	0.380		7212	0.450
	7405	0.428		7213	0.416
	7406	0.401		7311	0.384
	7505	0.428		7312	0.334
	7506	0.414		7413	0.418
	7605	0.430		7414	0.404
				7515	0.420
				7610	0.413
				7611	0.420

Last two digits of animal numbers denote pup number. Other digits denote litter numbers.  
Dams dosed from GD 6.

## APPENDIX 17

Post-dose signs observed for PND 11 offspring following a single dose at 450 mg/kg/day

Dam No.	Pup No.	Clinical signs observed
77	5	Slight tremors approximately 1 hour after dosing
	12	Slight tremors approximately 1 hour after dosing
78	5	No signs observed
	12	No signs observed
79	5	No signs observed
	10	No signs observed
80	5	Moribund, killed early approximately 1 hour after dosing
	11	Spasmodic period of marked head nodding approximately 1 $\frac{3}{4}$ hours after dosing, tremors approximately 2 hours after dosing
81	5	No signs observed
	13	No signs observed
1082	5	No signs observed
	10	Slight tremors approximately 1 hour after dosing
83	5	No signs observed
	13	No signs observed
84	5	No signs observed
	11	Tremors approximately 2 hours after dosing

No clinical signs were observed at lower dosages.



## APPENDIX 18

Necropsy findings – individual observations for offspring on PND 11 (1 day of dosing)

Group 1: control

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
7701	NAD	7708	NAD
7801	NAD	7808	NAD
7901	NAD	7906	NAD
8001	NAD	8007	NAD
8101	NAD	8109	NAD
8301	NAD	8309	NAD
8401	NAD	8407	NAD
108201	NAD	108206	NAD

Group 2: malathion: 5 mg/kg/day

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
7702	NAD	7709	NAD
7802	NAD	7809	NAD
7902	NAD	7907	NAD
8002	NAD	8008	NAD
8102	NAD	8110	NAD
8302	NAD	8310	NAD
8402	NAD	8408	NAD
108202	NAD	108207	NAD

NAD No abnormalities detected.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

## APPENDIX 18 – continued

Necropsy findings – individual observations for offspring on PND 11 (1 day of dosing)

Group 3: malathion: 50 mg/kg/day

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
7703	NAD	7710	NAD
7803	NAD	7810	NAD
7903	<b>Kidneys and ureters:</b> bilateral renal cavitation, bilateral hydroureter	7908	NAD
8003		8009	NAD
8103		8111	NAD
8303		8311	NAD
8403	NAD	8409	NAD
108203	NAD	108208	NAD

Group 4: malathion: 150 mg/kg/day

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
7704	NAD	7711	NAD
7804	NAD	7811	NAD
7904	NAD	7909	NAD
8004	NAD	8010	NAD
8104	NAD	8112	NAD
8304	NAD	8312	NAD
8404	NAD	8410	NAD
108204	NAD	108209	NAD

NAD No abnormalities detected.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

## APPENDIX 18 – continued

Necropsy findings – individual observations for offspring on PND 11 (1 day of dosing)

Group 5: malathion: 450 mg/kg/day

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
7705	NAD	7712	NAD
7805	NAD	7812	NAD
7905	<b>Kidneys and ureter:</b> bilateral renal cavitation, bilateral hydroureter	7910	NAD
8005	NAD	8011	NAD
8105	NAD	8113	NAD
8305	NAD	8313	NAD
8405	NAD	8411	NAD
108205	NAD	108210	NAD

NAD No abnormalities detected.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

## APPENDIX 19

Necropsy findings – individual observations for young adult animals on day 1 of treatment

Group	:	6 and 10	7	8	9	11
Compound	:	control		-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150	450

Group	Male number	Necropsy observations	Female number	Necropsy observations
6	201	NAD	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 19 - continued

Necropsy findings – individual observations for young adult animals on day 1 of treatment

Group	:	6 and 10	7	8	9	11
Compound	:	control		-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150	450

Group	Male number	Necropsy observations	Female number	Necropsy observations
8	233	NAD	117	NAD
	234	NAD	118	NAD
	235	NAD	119	Hairloss dorsal body surface
	236	NAD	120	NAD
	237	NAD	121	NAD
	238	<b>Kidneys:</b> unilateral renal cavitation	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 19 - continued

Necropsy findings – individual observations for young adult animals on day 1 of treatment

Group	:	6 and 10	7	8	9	11
Compound	:	control		-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150	450

Group	Male number	Necropsy observations	Female number	Necropsy observations
10	281	NAD	265	NAD
	282	NAD	266	NAD
	283	NAD	267	NAD
	284	NAD	268	<b>Kidneys:</b> bilateral renal cavitation
	285	NAD	269	NAD
	286	NAD	270	NAD
	287	NAD	271	NAD
	288	NAD	272	NAD
11	289	<b>Kidneys:</b> bilateral renal cavitation	273	NAD
	290	NAD	274	NAD
	291	NAD	275	NAD
	292	NAD	276	NAD
	293	NAD	277	NAD
	294	NAD	278	NAD
	295	NAD	279	NAD
	296	<b>Testes:</b> enlarged right side <b>Epididymides:</b> reduced right side	280	NAD

NAD No abnormalities detected.

## APPENDIX 20

Brain weights – individual values (g) for young adult animals on day 1 of treatment

Group : 6 and 10                      7                      8                      9                      11  
 Compound : control                      -----malathion-----  
 Dosage (mg/kg/day) : 0                      5                      50                      150                      450

Group	Sex	Animal number	Brain weight	Sex	Animal number	Brain weight
6	M	201	1.899	F	85	1.811
		202	1.928		86	1.996
		203	1.888		87	1.889
		204	1.879		88	1.804
		205	1.885		89	1.818
		206	1.846		90	1.858
		207	1.943		91	1.916
		208	1.836		92	1.913
7	M	217	1.902	F	101	1.681
		218	1.940		102	1.806
		219	1.843		103	1.808
		220	1.925		104	1.840
		221	2.067		105	1.844
		222	2.000		106	1.807
		223	1.912		107	1.915
		224	1.910		108	1.779
8	M	233	1.728	F	117	1.744
		234	2.005		118	1.770
		235	1.907		119	1.956
		236	1.988		120	1.958
		237	1.929		121	1.759
		238	1.944		122	1.794
		239	1.893		123	1.830
		240	1.794		124	1.850
9	M	249	1.771	F	133	1.677
		250	1.960		134	1.832
		251	1.939		135	1.798
		252	1.963		136	1.884
		253	2.000		137	1.768
		254	1.869		138	1.807
		255	1.847		139	1.882
		256	1.752		140	1.945
10	M	281	1.966	F	265	1.726
		282	1.875		266	1.702
		283	1.957		267	1.795
		284	1.886		268	1.776
		285	1.976		269	1.789
		286	1.930		270	1.835
		287	1.970		271	1.870
		288	1.969		272	1.819
11	M	289	1.932	F	273	1.846
		290	1.871		274	1.815
		291	2.012		275	1.779
		292	2.016		276	1.893
		293	1.922		277	1.835
		294	1.954		278	2.020
		295	1.926		279	1.836
		296	1.931		280	1.871

## APPENDIX 21

Bodyweight - individual values (g) for dosed male offspring

Group 1: control

Animal number	PND												
	1	4	7	11	14	17	21	28	35	42	49	56	60
1001	6.3	9.0	14.8	26.8	36.8	46.0	56.1						
1002	5.7	9.3	15.2	27.8	37.9	49.3	58.5						
1003	7.1	10.1	16.6	29.8	39.9	48.8	59.4						
1004	6.5	9.3	15.7	28.0	38.4	47.9	59.1	92.3	156.3	230.6	303.0	373.7	402.0
1101	6.6	10.3	17.0	29.7	39.8	45.9	52.7						
1102	6.3	10.0	16.6	29.1	38.4	45.0	56.0						
1103	6.7	10.6	17.7	29.4	38.1	45.2	57.3						
1104	6.7	10.6	17.8	29.8	39.0	45.6	54.6	91.0	149.4	221.2	285.7	349.2	373.0
1201	6.6	9.5	16.8	28.3	34.7	42.0	54.0						
1202	6.4	9.2	15.8	27.8	33.9	39.9	54.6						
1203	6.7	8.9	16.2	27.0	34.0	42.2	54.0						
1204	6.6	9.5	16.8	28.8	37.1	43.6	58.2	97.1	169.6	248.7	334.4	408.0	439.0
1301	5.9	8.3	14.1	24.7	32.8	40.6	54.6						
1302	6.4	9.7	15.8	27.0	34.9	43.4	56.5						
1304	6.4	9.1	15.4	26.7	35.8	43.3	57.8						
1305	6.1	8.4	15.6	26.9	35.6	43.7	56.8	94.2	153.6	222.2	291.1	354.1	388.0
1401	5.2	6.6	10.5	19.6	22.5	29.8	36.7						
1402	6.3	8.1	12.8	22.6	25.5	32.9	41.3						
1403	6.7	9.1	14.9	25.5	28.4	34.1	42.9						
1404	6.3	7.9	13.4	24.2	27.4	33.2	42.7	76.1	129.0	202.3	267.4	327.0	352.0
1501	6.9	9.8	18.1	30.2	37.9	47.6	60.4						
1502	6.9	9.0	16.8	29.1	37.7	47.1	61.2						
1503	6.3	9.2	15.8	27.0	35.3	45.1	57.5						
1504	7.0	9.2	17.1	28.5	37.1	45.9	54.3	100.3	159.0	230.9	305.4	370.7	398.0
1601	6.2	8.4	14.8	27.0	36.1	45.5	57.1						
1602	5.8	8.1	14.1	25.1	35.2	43.4	55.1						
1603	6.4	8.7	15.6	27.5	36.9	46.1	56.0	94.2	151.7	221.6	285.3	335.8	362.0
1604	6.8	9.5	16.7	29.4	38.5	47.3	57.9	98.5	162.6	232.4	305.0	367.4	400.0
1701	7.2	10.1	16.9	29.5	38.8	53.0	68.8						
1702	7.5	9.5	15.8	27.8	36.7	50.6	66.3						
1703	6.5	10.2	13.8	24.6	33.9	46.4	60.2	95.6	160.0	233.8	305.0	364.8	387.0
1704	7.2	9.7	16.6	27.9	37.9	48.1	64.3	102.7	166.6	240.4	317.4	380.5	407.0

Last two digits of animal number denote pup number. Other digits denote litter number.  
 Direct treatment of offspring: PND 11 to PND 21.



## APPENDIX 21 - continued

Bodyweight - individual values (g) for dosed male offspring

Group 2: malathion: 5 mg/kg/day

Animal number	PND												
	1	4	7	11	14	17	21	28	35	42	49	56	60
2901	6.1	9.1	16.3	28.2	35.0	44.4	57.4						
2902	4.4	6.3	11.7	20.1	27.3	34.1	43.1						
2903	6.4	9.4	17.0	27.2	35.9	43.8	55.7						
2904	6.2	9.4	16.8	27.8	36.8	46.0	60.2	99.5	154.9	219.1	277.3	333.3	352.0
3001	5.7	6.8	9.3	17.3	24.6	31.7	40.6						
3002	5.9	7.9	13.5	22.6	26.8	38.5	48.2						
3003	6.8	9.4	14.2	23.8	31.3	40.6	56.6						
3004	5.5	7.4	10.8	20.5	27.3	35.6	45.8	79.0	130.8	196.2	260.6	318.6	342.0
3101	6.5	8.1	14.1	25.0	32.3	38.4	52.7						
3102	5.9	7.6	13.2	23.1	30.5	38.9	48.5						
3103	6.2	8.3	13.7	24.5	30.8	38.1	49.9						
3104HK	6.2	7.9	13.1	21.9	28.7	37.7	49.0						
3201	6.6	8.7	13.3	26.5	35.8	45.0	51.8						
3202	7.0	9.7	15.3	27.5	37.6	48.4	59.1						
3203	6.4	8.1	13.5	24.5	34.6	43.7	52.9						
3204	6.6	8.4	14.1	25.8	35.2	46.3	54.6	85.8	142.2	210.9	271.9	342.9	379.0
3301	6.1	9.4	14.6	25.5	35.9	45.1	59.7						
3302	5.9	8.6	15.1	26.8	37.3	46.9	61.1						
3303	6.1	8.8	13.9	25.0	34.2	44.7	58.4						
3304	6.8	9.7	15.6	26.8	36.1	45.9	60.8	99.5	164.3	244.2	312.2	387.0	431.0
3401	5.4	6.4	10.2	19.6	27.6	35.2	45.2						
3402	6.5	8.2	12.9	22.8	31.6	39.6	52.0						
3403	6.2	7.5	11.5	22.4	29.6	33.8	47.8						
3404	6.2	7.2	11.2	20.8	28.6	37.9	47.0	79.6	130.2	189.1	253.3	317.5	355.0
3501	6.1	6.4	10.3	18.0	26.1	33.7	43.5						
3502	6.3	8.3	12.9	22.2	34.3	38.4	52.1						
3504	5.9	8.0	12.5	20.2	28.1	35.9	48.4	80.3	138.8	211.0	279.3	350.8	393.0
3505	6.3	8.3	14.0	24.7	34.1	41.7	56.3	91.1	147.6	212.0	275.7	340.2	369.0
3701	5.7	8.2	15.1	26.0	35.5	43.6	56.9						
3702	5.8	8.3	14.7	25.6	35.5	45.6	57.1						
3703	5.4	6.8	12.8	23.4	32.5	40.6	51.0	82.8	135.6	195.5	263.3	341.6	374.0
3704	5.6	7.4	14.3	24.9	35.1	43.2	56.4	92.4	149.4	212.0	283.8	354.0	391.0

Last two digits of animal number denote pup number. Other digits denote litter number.

Direct treatment of offspring: PND 11 to PND 21.

HK Killed for reasons of animal welfare PND 27.

## APPENDIX 21 - continued

Bodyweight - individual values (g) for dosed male offspring

Group 3: malathion: 50 mg/kg/day

Animal number	PND												
	1	4	7	11	14	17	21	28	35	42	49	56	60
4801	6.3	9.3	14.7	25.9	34.4	41.9	55.0						
4802	6.2	8.6	14.6	24.6	32.1	40.4	54.5						
4803	6.2	8.9	14.8	25.5	33.8	42.5	56.4						
4804	6.3	9.5	15.8	27.1	36.8	46.4	58.7	98.7	156.9	221.9	281.2	331.4	350.0
4901	6.4	7.6	12.8	24.2	32.7	42.1	52.8						
4902	6.3	8.6	14.1	27.0	35.1	44.8	56.3						
4903	5.9	7.8	12.7	25.3	34.4	43.7	57.3						
4904	6.2	8.0	12.7	24.5	32.9	42.5	52.9	82.3	134.0	193.8	257.6	320.2	351.0
5002	6.9	9.3	15.2	26.5	33.9	41.9	53.6						
5003	7.2	9.4	15.4	26.4	34.7	44.2	59.8						
5004	7.1	10.6	17.7	28.4	37.3	45.3	60.4						
5005	6.3	9.2	15.1	25.3	32.8	43.1	58.4	97.8	159.9	232.6	294.5	355.6	390.0
5101	7.0	9.7	15.9	27.9	37.3	45.3	57.0						
5102	6.4	9.3	15.6	27.7	37.0	45.8	57.7						
5103	6.9	9.5	16.3	28.0	36.8	45.2	57.4						
5104	7.1	10.4	16.5	29.2	38.1	46.8	59.5	104.2	165.3	237.0	310.3	375.6	414.0
5201	8.1	12.1	21.3	33.7	43.5	53.6	67.3						
5202	7.9	11.8	19.8	31.3	40.4	49.6	62.9						
5203	8.1	11.3	19.7	31.5	40.9	49.8	63.9						
5204	8.0	10.5	17.8	28.1	36.8	43.4	53.3	94.3	156.5	222.2	290.3	348.1	378.0
5301	6.3	9.3	14.8	25.1	32.7	41.8	50.4						
5302	6.5	9.7	16.0	26.7	35.7	43.9	53.0						
5303	6.7	9.2	16.0	25.2	33.8	41.4	53.1						
5304	6.2	9.7	16.1	27.4	35.1	43.9	56.4	94.9	155.6	224.0	282.5	344.0	373.0
5401	7.8	11.0	18.2	30.0	38.7	46.0	59.9						
5402	7.1	10.7	17.6	29.9	33.3	43.3	55.4						
5403	7.0	10.4	17.7	29.2	40.8	48.5	62.2	106.9	173.4	237.7	309.0	378.6	417.0
5404	6.6	9.6	16.8	27.7	37.2	44.7	54.9	87.8	142.6	205.2	275.5	341.1	370.0
5601	6.7	9.7	15.9	26.1	35.2	44.5	54.6						
5602	6.8	9.1	15.9	26.2	35.4	45.2	54.3						
5603	6.7	9.5	15.3	25.5	34.6	43.2	53.9	92.0	154.0	224.6	292.2	355.0	385.0
5604	7.2	9.7	16.6	26.4	35.5	44.9	57.3	95.7	158.6	230.3	297.9	367.5	400.0

Last two digits of animal number denote pup number. Other digits denote litter number.  
 Direct treatment of offspring: PND 11 to PND 21.

## APPENDIX 21 - continued

Bodyweight - individual values (g) for dosed male offspring

Group 4: malathion: 150 mg/kg/day

Animal number	PND												
	1	4	7	11	14	17	21	28	35	42	49	56	60
6701	6.1	7.7	12.2	23.1	31.7	40.4	50.9						
6702	6.3	7.3	12.5	22.3	30.8	40.3	50.3						
6703	6.8	7.7	13.8	23.0	33.0	42.5	53.7						
6704	6.8	8.4	14.3	26.5	35.0	45.0	57.5	89.7	147.4	219.2	292.8	366.4	396.0
6801	6.0	8.3	14.2	25.9	32.2	39.7	51.6						
6802	5.3	8.2	14.2	26.1	30.7	39.4	49.1						
6803	5.9	8.9	14.9	25.7	32.4	39.6	51.5						
6804	5.8	8.3	13.6	25.6	32.7	39.8	51.8	91.1	151.8	218.9	283.9	349.3	382.0
6901	8.0	10.1	15.8	25.1	32.7	39.5	52.0						
6902	7.4	9.4	15.0	24.8	30.2	36.8	51.3						
6903	7.8	10.0	15.1	24.7	33.1	38.7	52.0	88.0	142.7	207.1	264.4	326.8	369.0
7301	7.7	11.2	19.8	30.8	39.7	49.0	62.9						
7302	7.7	11.1	19.2	30.6	39.1	48.1	63.1						
7303	7.5	10.2	17.2	29.0	38.9	47.7	59.7						
7304	6.0	8.3	13.9	24.3	31.9	29.4	41.0	73.7	124.9	190.6	256.6	319.6	347.0
7001	7.2	9.9	16.0	27.1	35.4	39.5	61.6						
7002	7.7	10.9	19.1	29.5	38.0	45.3	61.0						
7003	7.3	9.2	15.3	25.5	33.1	44.1	53.3						
7004	7.5	10.3	18.1	29.6	38.0	48.6	65.7	109.3	172.6	242.6	318.1	388.5	413.0
7201	6.9	10.5	18.2	29.4	35.3	43.0	58.6						
7202	7.1	11.2	18.4	30.7	37.5	46.1	61.1						
7203	6.8	10.7	18.3	28.9	36.1	45.3	62.0						
7204	7.4	10.6	18.1	29.3	36.6	45.5	60.3	98.1	158.0	221.9	287.2	344.9	369.0
7401	7.6	9.3	14.9	24.7	30.2	36.6	47.8						
7402	7.5	10.0	16.6	26.0	31.7	40.1	49.7						
7403	6.7	9.3	15.2	25.8	32.8	41.0	50.8	92.6	154.1	235.6	317.9	394.0	421.0
7404	7.5	9.7	15.8	25.9	32.3	41.2	51.5	92.2	148.5	221.3	293.3	361.7	383.0
7501	6.0	9.5	15.5	25.4	34.3	43.9	55.4						
7502	5.4	8.1	14.7	25.2	33.9	42.4	52.9						
7503	6.0	8.7	15.8	26.9	34.6	43.9	55.1	91.3	143.9	212.2	274.1	334.7	355.0
7504	6.5	10.0	16.6	28.3	36.3	46.3	58.9	96.1	153.4	226.9	299.2	363.0	391.0

Last two digits of animal number denote pup number. Other digits denote litter number.  
 Direct treatment of offspring: PND 11 to PND 21.

## APPENDIX 22

Bodyweight - individual values (g) for dosed female offspring

Group 1: control

Animal number	PND												
	1	4	7	11	14	17	21	28	35	42	49	56	60
1009	6.1	8.6	14.8	27.3	36.7	48.1	55.7						
1010	6.4	8.8	15.1	27.8	38.3	47.3	60.9						
1011	5.7	8.7	14.9	27.1	37.3	46.7	57.9						
1012	6.1	8.8	14.7	26.5	36.6	45.3	55.0	89.5	143.3	189.0	227.4	263.7	282.0
1108	6.4	9.8	16.1	28.2	36.0	43.0	52.8						
1109	5.9	9.9	15.6	27.2	35.5	42.9	53.7						
1110	6.5	10.3	17.5	29.5	38.2	42.6	54.2						
1111	6.2	9.1	15.4	27.6	36.1	43.1	53.7	95.0	151.9	211.4	251.9	298.8	314.0
1210	6.1	9.1	15.7	26.4	34.5	39.8	51.2						
1211	6.2	8.8	14.9	25.4	32.7	39.3	51.2						
1212	6.2	9.0	16.0	26.9	35.2	42.7	55.3						
1213	6.1	9.1	15.5	27.0	36.1	41.2	56.2	85.1	127.0	162.2	193.8	220.7	237.0
1310	6.1	9.1	15.1	25.2	35.5	45.0	55.8						
1311	6.2	8.9	15.4	25.9	33.5	41.0	53.7						
1312	6.2	8.5	14.7	26.2	34.5	43.9	54.9						
1314	5.9	8.8	14.0	27.3	33.4	41.2	52.6	86.0	132.2	168.3	186.3	205.5	213.0
1409	5.5	7.1	12.2	20.4	23.2	29.2	36.6						
1410	5.7	7.8	13.2	24.2	26.5	32.6	42.9						
1411	5.4	6.9	11.8	16.8	19.6	25.7	35.0						
1412	5.8	7.7	13.1	23.0	25.0	32.6	36.8	67.2	111.0	150.0	180.7	203.3	214.0
1509	6.6	9.1	17.4	29.0	37.8	46.3	59.9						
1510	6.3	9.3	16.9	28.2	36.6	43.5	57.0						
1511	5.8	8.0	14.3	25.7	33.5	41.2	52.1						
1512	6.2	8.6	16.2	28.7	37.5	46.0	58.7	96.5	147.9	197.1	228.5	259.8	272.0
1607	5.8	8.8	15.2	27.7	37.2	45.4	59.2						
1608	6.2	9.5	16.7	28.3	36.9	47.1	57.3						
1609	6.0	8.0	14.0	25.3	33.8	43.5	53.5	86.6	137.2	182.8	213.5	237.8	253.0
1610	5.6	7.3	13.1	23.1	32.9	41.7	52.0	86.5	134.8	176.1	206.8	230.5	245.0
1709	7.1	10.7	18.5	29.7	39.9	51.6	68.1						
1710	6.2	8.4	14.2	25.9	34.4	46.5	59.6						
1711	6.7	9.8	16.5	27.2	36.2	48.4	60.9	93.9	143.7	184.5	222.8	261.4	272.0
1712	6.9	9.5	16.3	27.1	38.0	49.7	62.2	97.4	146.1	187.0	210.4	230.0	247.0

Last two digits of animal number denote pup number. Other digit(s) denote litter number.  
 Direct treatment of offspring: PND 11 to PND 21.

## APPENDIX 22 - continued

Bodyweight - individual values (g) for dosed female offspring

Group 2: malathion: 5 mg/kg/day

Animal number	PND												
	1	4	7	11	14	17	21	28	35	42	49	56	60
2906	3.8	5.3	9.8	16.2	24.3	32.6	40.9						
2907	5.8	8.8	16.3	26.3	34.3	42.6	56.7						
2908	6.1	9.0	16.0	27.1	34.8	43.0	57.6						
2909	5.9	9.3	16.6	27.8	35.5	44.1	56.9	85.8	129.2	163.4	196.9	225.7	235.0
3011	5.1	7.1	11.0	20.5	27.4	34.8	47.0						
3012	5.9	7.4	11.7	20.3	26.9	35.7	46.2						
3013	5.9	8.3	13.3	22.5	30.7	38.5	51.7						
3014	6.2	8.8	13.8	24.9	32.8	40.2	52.6	85.4	135.2	167.7	196.7	226.2	238.0
3109	5.7	7.6	12.7	23.8	33.1	39.5	53.6						
3110	5.6	8.1	14.4	25.1	31.1	37.8	48.2						
3111	5.4	7.4	13.0	22.2	27.5	32.8	46.4						
3112	5.7	7.7	13.1	23.7	31.7	38.0	51.2	84.0	130.1	162.6	193.2	223.4	227.0
3210	6.3	9.3	15.3	27.0	37.4	46.5	51.1						
3211	6.0	8.3	13.9	25.7	35.5	44.6	55.9						
3212	6.2	7.9	13.4	24.4	33.7	43.6	52.8						
3213	5.6	7.1	10.9	20.2	29.1	38.2	46.6	72.0	118.0	160.5	185.8	210.5	222.0
3310	6.1	8.7	14.3	26.0	36.7	43.0	58.0						
3311	6.3	8.7	15.3	26.3	36.3	47.1	60.7						
3312	6.3	8.4	14.7	26.4	35.0	44.5	57.0						
3314	5.5	8.6	15.1	26.2	34.8	43.8	53.8	90.8	136.9	175.6	200.5	219.3	234.0
3411	5.6	6.8	10.7	21.4	30.2	36.4	43.6						
3412	5.6	7.1	10.7	21.3	29.6	37.5	46.8						
3413	5.0	6.3	9.5	18.9	27.3	37.4	44.2						
3414	5.6	6.4	10.1	18.5	27.1	33.2	41.8	69.5	107.6	150.9	181.6	204.1	217.0
3503	6.1	7.6	12.3	22.6	30.4	39.0	51.3						
3510	6.3	9.1	14.4	24.3	34.3	42.3	52.7						
3511	6.3	8.1	13.3	24.7	33.6	41.7	53.5	83.0	129.5	171.0	198.6	225.2	237.0
3512	6.2	8.5	13.8	24.6	34.0	41.4	54.7	88.4	142.8	187.8	224.0	250.0	263.0
3708	5.7	8.4	15.7	29.7	39.9	51.6	57.0						
3709	5.5	7.5	14.3	25.9	34.4	46.5	54.8						
3710	5.9	8.6	15.5	27.2	36.2	48.4	57.5	90.9	135.1	176.4	207.4	234.7	255.0
3711	5.6	7.5	13.8	27.1	38.0	49.7	54.7	86.9	136.0	182.1	210.3	236.8	257.0

Last two digits of animal number denote pup number. Other digit(s) denote litter number.  
 Direct treatment of offspring: PND 11 to PND 21.

## APPENDIX 22 - continued

Bodyweight - individual values (g) for dosed female offspring

Group 3: malathion: 50 mg/kg/day

Animal number	PND												
	1	4	7	11	14	17	21	28	35	42	49	56	60
4807	6.7	9.2	14.9	25.8	33.9	41.5	52.6						
4808	6.2	9.3	15.0	26.6	34.6	43.1	55.8						
4809	6.4	9.9	15.5	27.6	35.1	41.8	54.9						
4810	6.4	8.9	14.1	24.7	32.7	40.8	52.1	86.2	130.1	165.2	190.3	211.4	219.0
4911	5.4	7.4	12.0	22.4	30.4	39.6	51.2						
4912	5.9	7.7	12.1	22.8	30.5	39.6	51.3						
4913	5.8	7.5	12.3	24.3	33.2	41.0	55.6						
4914	6.0	7.7	12.6	24.6	31.9	40.1	49.0	79.1	122.5	155.2	183.1	209.5	216.0
5010KIE	6.9	9.7	16.3	26.4									
5011	7.0	9.5	15.2	24.9	32.0	40.9	54.0						
5012	7.0	9.6	15.1	26.0	32.4	41.2	54.8						
5013	6.8	8.9	15.3	26.4	34.3	43.2	54.8	89.2	130.6	162.0	184.7	212.8	226.0
5106	6.4	8.2	14.1	25.7	37.1	41.8	52.5						
5107	7.1	9.2	16.6	28.4	33.5	46.2	57.5						
5108	6.4	8.3	14.7	26.2	34.5	43.8	53.4						
5109	6.3	7.4	12.5	23.7	31.1	39.6	49.4	81.8	130.8	168.1	199.7	229.4	237.0
5207	7.6	11.1	19.1	31.9	42.3	52.2	66.6						
5208	8.1	11.6	19.9	31.6	40.6	48.7	64.3						
5209	7.2	10.7	18.3	29.8	38.4	47.2	59.3						
5210	7.8	11.5	19.8	32.0	40.7	50.3	67.4	109.3	162.3	196.7	227.3	258.5	280.0
5306	5.5	8.4	14.0	24.1	32.2	39.9	47.4						
5307	6.2	9.4	15.3	26.4	33.7	42.2	53.0						
5308	6.1	8.8	14.2	24.2	32.9	40.6	50.2						
5309	5.9	8.7	15.1	25.1	32.9	42.1	49.5	80.1	123.8	159.5	183.7	206.6	221.0
5405	6.8	10.5	17.0	28.2	36.8	46.0	56.2						
5406	7.0	10.5	18.8	30.0	37.7	46.7	59.3						
5407	7.0	10.8	18.0	28.6	37.4	42.0	60.0	94.4	143.2	186.1	218.6	257.4	274.0
5408	7.8	11.1	18.4	29.5	39.1	46.5	58.9	91.1	139.7	179.8	203.7	224.2	237.0
5610	5.7	8.1	13.3	22.1	30.4	38.6	47.8						
5611	5.8	8.5	14.1	23.6	32.2	40.4	48.5						
5612	6.3	9.1	14.0	23.6	32.8	41.7	51.2	75.8	116.3	145.0	177.5	199.7	212.0
5613	6.2	9.0	15.2	23.9	32.6	40.7	53.7	84.0	127.4	166.7	198.1	224.9	238.0

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

Direct treatment of offspring: PND 11 to PND 21.

KIE Killed *in extremis* PND 14.

## APPENDIX 22 - continued

Bodyweight - individual values (g) for dosed female offspring

Group 4: malathion: 150 mg/kg/day

Animal number	PND												
	1	4	7	11	14	17	21	28	35	42	49	56	60
6713	6.4	7.9	13.3	25.1	31.0	39.2	50.2						
6714	6.1	7.1	12.6	23.6	31.8	41.4	52.0						
6715	6.4	5.5 <sup>x</sup>	15.2	25.5	34.0	43.2	53.5						
6716	5.3	5.8	9.9	18.7	27.0	35.5	44.1	69.6	111.8	149.7	179.0	202.8	214.0
6807	5.9	8.5	14.3	25.7	33.4	41.1	52.3						
6808	5.5	8.1	14.4	25.9	33.3	41.0	52.1						
6809	6.0	8.6	14.9	26.5	33.0	41.3	52.6						
6810	5.5	7.8	13.9	25.4	32.5	38.7	48.4	77.7	114.5	150.0	176.7	204.5	215.0
6904	7.3	7.2	10.5	17.7	23.2	27.7	34.5						
6905	7.8	9.7	14.4	23.6	29.9	36.1	47.8						
6906	6.5	8.3	13.0	21.2	26.7	32.3	41.7						
6907	6.8	9.5	14.6	23.9	30.9	37.1	49.8						
6908	8.0	10.0	15.7	25.4	32.3	39.5	51.7	81.9	130.3	176.8	213.3	248.6	268.0
7010	6.7	9.4	15.7	26.5	33.5	41.7	53.6						
7011	6.4	8.6	14.6	24.3	30.1	44.9	50.4						
7012	7.6	10.6	18.2	28.9	35.6	47.5	59.8						
7013	6.7	9.3	15.8	26.2	34.6	41.9	55.2	88.3	135.8	170.0	191.5	221.4	232.0
7207	7.0	10.7	16.8	28.2	36.2	41.4	59.5						
7208	6.9	9.9	16.2	25.5	32.5	44.1	51.1						
7210	6.5	10.0	18.2	27.0	34.6	43.9	55.0						
7211	6.8	10.6	18.3	29.2	36.2	44.6	58.8	94.6	134.8	174.3	198.6	221.1	231.0
7307	6.7	9.6	16.3	28.2	36.6	45.1	58.0						
7308	6.6	9.9	17.4	29.3	38.6	48.0	59.6						
7309	5.8	8.8	15.2	26.1	33.9	42.0	52.5						
7310	6.2	9.2	16.0	27.8	36.1	44.9	57.6	93.0	142.2	191.9	221.7	254.9	276.0
7409	6.9	9.5	15.3	24.4	30.2	38.6	49.1						
7410	6.3	9.1	14.5	24.4	29.9	38.9	46.9						
7411	5.4	8.3	12.8	21.7	27.2	34.4	43.3	78.1	118.5	148.3	182.5	205.4	217.0
7412	6.9	9.2	15.2	26.0	32.4	41.0	51.9	90.6	141.7	183.2	223.3	246.8	267.0
7511	5.7	8.9	15.5	26.6	35.3	45.2	57.6						
7512	6.0	9.4	15.9	28.4	36.6	45.9	58.2						
7513	6.2	9.8	16.7	28.0	36.4	45.5	56.2	89.8	130.8	162.9	187.9	212.9	223.0
7514	6.1	9.9	16.5	27.3	35.4	44.8	56.0	87.2	129.7	161.8	188.9	215.0	219.0

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

Direct treatment of offspring: PND 11 to PND 21.

<sup>x</sup> Suspected weighing error, excluded from group mean values.

APPENDIX 23

Bodyweight - individual values (g) for undosed male offspring

Group 1: control

Animal number	PND							
	1	4	7	11	14	21		
1801	6.3	8.3	15.1	26.0	34.0	49.6		
1802	6.2	8.7	14.4	25.1	33.0	50.8		
1803	5.6	8.2	14.1	25.3	32.9	50.8		
1804	6.3	8.8	15.0	26.2	35.3	54.9		
1901	6.3	9.2	15.7	25.2	32.8	48.3		
1902	6.9	10.6	17.2	26.2	33.6	55.4		
1903	6.9	10.2	16.0	25.4	33.5	51.8		
1904	6.9	9.9	16.4	25.8	32.8	54.4		

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



## APPENDIX 23 - continued

Bodyweight - individual values (g) for undosed male offspring

Group 2: malathion: 5 mg/kg/day

Animal number	PND						
	1	4	7	11	14	21	
3601	6.7	10.4	16.4	27.1	34.6	52.4	
3602	5.8	8.6	13.1	24.3	33.0	48.3	
3603	7.4	10.9	17.5	28.2	37.9	54.4	
3604	6.5	9.1	14.4	24.5	32.7	47.6	
3801	5.6	7.8	13.0	25.9	32.8	60.0	
3802	5.8	7.9	13.2	24.0	34.7	54.3	
3803	5.4	7.3	11.5	21.9	31.2	53.3	
3805	5.8	8.0	13.8	24.7	35.0	56.6	

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

## APPENDIX 23 - continued

Bodyweight - individual values (g) for undosed male offspring

Group 3: malathion: 50 mg/kg/day

Animal number	PND						
	1	4	7	11	14	21	
5501	6.8	10.5	17.1	28.7	36.7	56.8	
5502	6.7	11.0	18.2	29.4	38.1	59.8	
5503	6.9	10.7	16.7	28.1	36.5	55.4	
5701	5.9	9.0	15.3	25.9	34.9	57.5	
5702	5.9	9.5	16.4	26.7	35.0	55.2	
5703	5.9	9.7	16.1	26.6	33.7	53.1	
5704	6.4	9.8	17.2	27.7	36.1	58.0	

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

## APPENDIX 23 - continued

Bodyweight - individual values (g) for undosed male offspring

Group 4: malathion: 150 mg/kg/day

Animal number	PND							
	1	4	7	11	14	21		
7101	7.0	8.6	14.8	25.1	33.1	57.3		
7102	7.8	9.5	16.1	26.5	35.2	61.0		
7103	7.4	9.1	15.9	26.4	33.6	59.1		
7601	6.9	10.4	15.4	22.5	27.9	47.0		
7602	6.9	10.5	15.8	23.7	29.8	47.6		
7603	6.7	10.0	15.1	22.9	28.1	41.9		
7604	6.5	9.5	13.8	21.6	27.0	42.7		

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

## APPENDIX 24

Bodyweight - individual values (g) for undosed female offspring

Group 1: control

Animal number	PND						
	1	4	7	11	14	21	
1805	5.0	4.7	7.6	13.7	20.2	33.9	
1806	5.9	8.5	14.3	26.2	33.5	50.8	
1807	5.3	7.8	14.2	24.3	32.2	48.4	
1808	6.1	8.8	15.5	27.0	35.9	53.6	
1908	6.4	9.6	15.7	25.8	32.4	50.1	
1909	6.8	9.7	16.5	25.5	32.5	54.9	
1910	6.2	8.9	14.2	22.3	29.0	45.4	
1911	7.5	10.5	17.5	26.3	32.5	53.2	

Last two digits of animal number denote pup number. Other digits denote litter number.

## APPENDIX 24 - continued

Bodyweight - individual values (g) for undosed female offspring

Group 2: malathion: 5 mg/kg/day

Animal number	PND						
	1	4	7	11	14	21	
3606	6.7	10.4	16.7	28.0	36.8	56.7	
3607	7.4	12.3	19.7	31.1	39.8	60.0	
3608	6.4	9.0	14.9	25.3	35.5	53.8	
3609	7.1	10.8	16.8	28.0	37.5	54.7	
3806	4.6	6.6	11.2	21.6	29.8	51.4	
3807	5.1	6.8	10.9	21.6	30.8	52.2	
3808	5.9	8.5	14.5	25.7	35.2	58.9	
3809	5.9	8.3	14.6	25.2	33.7	53.9	

Last two digits of animal number denote pup number. Other digits denote litter number.

APPENDIX 24 - continued

Bodyweight - individual values (g) for undosed female offspring

Group 3: malathion: 50 mg/kg/day

Animal number	PND							
	1	4	7	11	14	21		
5504	6.9	10.9	17.8	28.4	34.8	56.8		
5505	6.7	10.1	15.9	26.2	34.2	53.3		
5506	6.8	11.3	17.7	27.3	35.4	55.1		
5507	5.9	9.6	16.7	27.6	34.1	54.1		
5508	6.4	10.0	17.8	28.3	35.7	53.9		
5706	5.8	9.5	16.1	26.4	34.6	54.7		
5707	5.9	9.1	15.9	25.3	34.0	51.7		
5708	5.9	9.5	16.8	27.3	35.3	53.3		
5709	6.0	9.0	16.0	25.5	34.3	54.1		

Last two digits of animal number denote pup number. Other digits denote litter number.

## APPENDIX 24 - continued

Bodyweight - individual values (g) for undosed female offspring

Group 4: malathion: 150 mg/kg/day

Animal number	PND							
	1	4	7	11	14	21		
7104	6.6	8.3	15.0	24.7	32.5	55.2		
7105	6.9	8.7	15.1	23.3	30.9	52.0		
7106	7.1	8.7	14.9	25.5	33.4	56.7		
7107	6.7	8.4	13.6	22.3	30.7	52.3		
7108	7.1	7.9	13.4	22.7	31.3	52.5		
7606	6.5	9.9	15.2	22.4	29.6	44.8		
7607	6.6	9.8	15.3	25.0	29.4	48.2		
7608	6.8	9.8	14.6	22.5	31.3	46.1		
7609	5.9	8.5	12.6	21.5	25.4	43.2		

Last two digits of animal number denote pup number. Other digits denote litter number.

## APPENDIX 25

Necropsy findings – individual observations for offspring on PND 21 (11 days of dosing)

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	1108	NAD
1201	NAD	1210	NAD
1301	NAD	1310	<b>Kidneys and ureter:</b> unilateral renal cavitation and unilateral hydroureter
1401	NAD	1409	NAD
1501	<b>Kidneys and ureter:</b> unilateral renal cavitation and unilateral hydroureter	1509	NAD
1601	NAD	1607	<b>Kidneys and ureter:</b> bilateral renal cavitation and bilateral hydroureter
1701	NAD	1709	NAD

Group 2: malathion: 5 mg/kg/day

Pups selected for blood and brain sampling

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
2901	NAD	2906	NAD
3001	NAD	3011	NAD
3101	NAD	3109	NAD
3201	NAD	3210	NAD
3301	NAD	3310	NAD
3401	NAD	3411	<b>Kidneys and ureter:</b> unilateral renal cavitation and bilateral hydroureter
3501	NAD	3510	NAD
3701	NAD	3708	NAD

NAD No abnormalities detected.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.



## APPENDIX 25 – continued

Necropsy findings – individual observations for offspring on PND 21 (11 days of dosing)

Group 3: malathion: 50 mg/kg/day

Pups selected for blood and brain sampling

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
4801	NAD	4807	NAD
4901	NAD	4911	NAD
5002	NAD	5011	NAD
5101	NAD	5106	NAD
5201	NAD	5207	NAD
5301	NAD	5306	NAD
5401	NAD	5405	NAD
5601	NAD	5610	NAD

Group 4: malathion: 150 mg/kg/day

Pups selected for blood and brain sampling

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
6701	NAD	6713	NAD
6801	NAD	6807	NAD
6901	NAD	6904	NAD
7001	NAD	7010	NAD
7201	NAD	7207	NAD
7301	NAD	7307	<b>Kidneys and ureter:</b> bilateral renal cavitation and bilateral hydroureter
7401	NAD	7409	<b>Kidneys:</b> bilateral renal cavitation
7501	NAD	7511	NAD

NAD No abnormalities detected.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

## APPENDIX 25 – continued

Necropsy findings – individual observations for offspring on PND 21 (11 days of dosing)

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	1109	NAD
1103	NAD	1110	NAD
1202	NAD	1211	NAD
1203	NAD	1212	NAD
1302	NAD	1311	NAD
1304	NAD	1312	NAD
1402	NAD	1410	NAD
1403	NAD	1411	NAD
1502	<b>Kidneys and ureter:</b> unilateral renal cavitation and unilateral hydroureter	1510	NAD
1503	NAD	1511	NAD
1602	NAD	1608	NAD
1702	NAD	1710	NAD
1801#	NAD	1805#	NAD
1802#	NAD	1806#	NAD
1803#	NAD	1807#	NAD
1804#	NAD	1808#	NAD
1901#	NAD	1908#	NAD
1902#	NAD	1909#	NAD
1903#	NAD	1910#	NAD
1904#	NAD	1911#	NAD

NAD No abnormalities detected.

# Pups not directly dosed.

## APPENDIX 25 – continued

Necropsy findings – individual observations for offspring on PND 21 (11 days of dosing)

Group 2: malathion: 5 mg/kg/day

Pups not selected for blood and brain sampling

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
2902	NAD	2907	NAD
2903	NAD	2908	NAD
3002	NAD	3012	NAD
3003	NAD	3013	NAD
3102	NAD	3110	NAD
3103	NAD	3111	NAD
3202	NAD	3211	NAD
3203	NAD	3212	NAD
3302	NAD	3311	NAD
3303	NAD	3312	NAD
3402	NAD	3412	NAD
3403	NAD	3413	NAD
3502	NAD	3510	NAD
3601#	NAD	3606#	<b>Diaphragm:</b> hernia with right liver lobe protruding through diaphragm <b>Liver:</b> agenesis of posterior right lateral lobe
3602#	NAD	3607#	NAD
3603#	<b>Liver:</b> raised area on mediam liver lobe adhering to diaphragm	3608#	Trauma to tip of tail
3604#	<b>Liver:</b> raised area on mediam liver lobe adhering to diaphragm; pale patches on anterior and posterior right lateral liver lobes <b>Diaphragm:</b> hernia with protrusion of anterior right lateral liver lobe and portion of duodenum through aperture of diaphragm	3609#	NAD
3702	NAD	3709	NAD
3801#	NAD	3806#	NAD
3802#	NAD	3807#	NAD
3803#	NAD	3808#	NAD
3805#	NAD	3809#	NAD

NAD No abnormalities detected.

# Pups not directly dosed.

## APPENDIX 25 – continued

Necropsy findings – individual observations for offspring on PND 21 (11 days of dosing)

Group 3: malathion: 50 mg/kg/day

Pups not selected for blood and brain sampling

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
4802	NAD	4808	NAD
4803	NAD	4809	NAD
4902	NAD	4912	NAD
4903	NAD	4913	NAD
5003	NAD	5012	NAD
5004	NAD	5107	NAD
5102	NAD	5108	NAD
5103	NAD	5208	NAD
5202	NAD	5209	NAD
5203	NAD	5307	NAD
5302	NAD	5308	NAD
5303	NAD	5406	NAD
5402	NAD	5504#	NAD
5501#	NAD	5505#	NAD
5502#	NAD	5506#	NAD
5503#	NAD	5507#	NAD
5602	NAD	5508#	NAD
5701#	NAD	5611	NAD
5702#	NAD	5706#	NAD
5703#	NAD	5707#	NAD
5704#	NAD	5708#	NAD
		5709#	NAD

NAD No abnormalities detected.

# Pups not directly dosed.

## APPENDIX 25 – continued

Necropsy findings – individual observations for offspring on PND 21 (11 days of dosing)

Group 4: malathion: 150 mg/kg/day

Pups not selected for blood and brain sampling

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
6702	NAD	6714	NAD
6703	NAD	6715	NAD
6802	NAD	6808	<b>Kidneys:</b> bilateral renal cavitation
6803	NAD	6809	<b>Kidneys:</b> unilateral renal cavitation
6902	NAD	6905	NAD
7002	NAD	6906	NAD
7003	NAD	6907	NAD
7101#	NAD	7011	NAD
7102#	NAD	7012	NAD
7103#	NAD	7104#	NAD
7202	NAD	7105#	NAD
7203	NAD	7106#	NAD
7302	NAD	7107#	NAD
7303	NAD	7108#	NAD
7402	NAD	7208	NAD
7502	NAD	7210	NAD
7601#	NAD	7308	NAD
7602#	NAD	7309	NAD
7603#	NAD	7410	NAD
7604#	NAD	7512	NAD
		7606#	NAD
		7607#	NAD
		7608#	NAD
		7609#	NAD

NAD No abnormalities detected.

# Pups not directly dosed.

## APPENDIX 26

Brain weights – individual values (g) for dosed male and female offspring on PND 11 (1 day of dosing)

## Group 1: control

Sex	Animal number	Brain weight	Sex	Animal number	Brain weight
M	7701	1.012	F	7708	0.951
	7801	0.945		7808	1.036
	7901	1.033		7906	0.945
	8001	0.915		8007	0.917
	8101	1.031		8109	1.044
	108201	1.025		108206	1.013
	8301	1.062		8309	0.950
	8401	1.043		8407	0.981

## Group 2: malathion: 5 mg/kg/day

Sex	Animal number	Brain weight	Sex	Animal number	Brain weight
M	7702	0.967	F	7709	0.962
	7802	0.975		7809	0.914
	7902	0.952		7907	0.948
	8002	0.932		8008	0.949
	8102	1.011		8110	0.998
	108202	0.990		108207	0.962
	8302	1.083		8310	1.062
	8402	1.034		8408	1.074

Last two digits of animal number denote pup number. Other digits denote litter number.

## APPENDIX 26- continued

Brain weights – individual values (g) for dosed male and female offspring on PND 11 (1 day of dosing)

Group 3: malathion: 50 mg/kg/day

Sex	Animal number	Brain weight	Sex	Animal number	Brain weight
M	7703	0.960	F	7710	0.856
	7803	1.051		7810	0.894
	7903	1.005		7908	1.062
	8003	0.995		8009	1.028
	8103	1.006		8111	1.015
	108203	1.038		108208	1.014
	8303	1.034		8311	1.110
	8403	1.063		8409	0.969

Group 4: malathion: 150 mg/kg/day

Sex	Animal number	Brain weight	Sex	Animal number	Brain weight
M	7704	0.941	F	7711	0.917
	7804	0.978		7811	0.900
	7904	1.067		7909	0.988
	8004	1.042		8010	0.946
	8104	1.073		8112	0.981
	108204	0.938		108209	0.961
	8304	1.069		8312	1.099
	8404	1.061		8410	0.975

Last two digits of animal number denote pup number. Other digits denote litter number.

## APPENDIX 26 - continued

Brain weights – individual values (g) for dosed male and female offspring on PND 11 (1 day of dosing)

Group 11: malathion: 450 mg/kg/day

Sex	Animal number	Brain weight	Sex	Animal number	Brain weight
M	7705	0.913	F	7712	0.856
	7805	0.995		7812	1.029
	7905	1.074		7910	1.020
	8005	0.992		8011	0.967
	8105	1.025		8113	1.021
	108205	1.009		108210	1.010
	8305	1.005		8313	1.137
	8405	0.967		8411	0.988

Last two digits of animal number denote pup number. Other digits denote litter number.



## APPENDIX 27

Brain weights – individual values (g) for dosed male and female offspring on PND 21 (11 days of dosing)

## Group 1: control

Sex	Animal number	Brain weight (g)	Sex	Animal number	Brain weight (g)
M	1001	1.432	F	1009	1.530
	1101	1.556		1108	1.513
	1201	1.537		1210	1.446
	1301	1.503		1310	1.510
	1401	1.371		1409	1.422
	1501	1.548		1509	1.446
	1601	1.504		1607	1.477
	1701	1.492		1709	1.422

## Group 2: malathion: 5 mg/kg/day

Sex	Animal number	Brain weight (g)	Sex	Animal number	Brain weight (g)
M	2901	1.522	F	2906	1.256
	3001	1.432		3011	1.471
	3101	1.405		3109	1.423
	3201	1.513		3210	1.483
	3301	1.513		3310	1.454
	3401	1.368		3411	1.411
	3501	1.376		3510	1.422
	3701	1.528		3708	1.509

Last two digits of animal number denote pup number. Other digits denote litter number.

## APPENDIX 27 - continued

Brain weights – individual values (g) for dosed male and female offspring on PND 21 (11 days of dosing)

Group 3: malathion: 50 mg/kg/day

Sex	Animal number	Brain weight (g)	Sex	Animal number	Brain weight (g)
M	4801	1.352	F	4807	1.373
	4901	1.509		4911	1.443
	5002	1.582		5011	1.519
	5101	1.542		5106	1.425
	5201	1.606		5207	1.387
	5301	1.529		5306	1.394
	5401	1.570		5405	1.452
	5601	1.471		5610	1.355

Group 4: malathion: 150 mg/kg/day

Sex	Animal number	Brain weight (g)	Sex	Animal number	Brain weight (g)
M	6701	1.434	F	6713	1.436
	6801	1.492		6807	1.424
	6901	1.485		6904	1.302
	7001	1.530		7010	1.495
	7201	1.415		7207	1.505
	7301	1.587		7307	1.433
	7401	1.439		7409	1.438
	7501	1.451		7511	1.390

Last two digits of animal number denote pup number. Other digits denote litter number.

## APPENDIX 28

Clinical signs - individual observations for young adult males and females #

Group	:	6	7	8	9
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

Group / sex	Animal number	day of kill	Signs observed on days	Post-dose salivation seen on days
6F	98	11	Brown staining on head (2-11) Brown staining on upper dorsal thorax (11)	
7F	111	11	Hairloss on dorsal body surface (9-11) Encrustations on dorsal body surface (9-11)	
	113	11	Brown staining on head (11)	
8M	244	11	-	10
	247	11	-	7
8F	119	1	Hairloss on dorsal body surface and head (1)	
9M	257	11	-	4-5, 7, 9-10
	258	11	-	5, 7, 9
	259	11	-	4-5, 7, 9
	260	11	-	5-7
	261	11	-	2-4, 8-9
	262	11	-	8-9
	263	11	-	3-4, 8-9
	264	11	-	4, 6, 8-9
9F	141	11	-	5-7, 9-10
	142	11	-	4-7
	143	11	-	7
	144	11	-	7, 10
	145	11	Brown staining on head (11)	3-4, 6, 8-9,
	146	11	-	5-6, 9,
	147	11	-	3-6, 8-9,
	148	11	Brown staining on head (10-11)	4, 9

# Only animals with signs are presented.

- No clinical signs observed.

## APPENDIX 29

Bodyweights – 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 #	244	249										
202 #	289	301										
203 #	250	256										
204 #	258	266										
205 #	300	313										
206 #	245	259										
207 #	270	282										
208 #	271	281										
209	273	280	288	297	307	318	325	335	341	353	363	370
210	236	241	251	260	268	277	282	292	295	302	310	318
211	294	298	310	320	331	334	336	342	348	359	359	368
212	261	270	280	289	297	305	314	321	332	339	340	357
213	288	303	307	321	327	330	341	348	355	365	374	282x
214	244	253	261	270	280	284	290	303	311	319	329	338
215	302	317	321	331	341	342	351	361	367	377	384	392
216	311	325	335	343	353	359	366	378	390	395	402	410

# Animals killed on day 1 of treatment.

x Suspected weighing error; value excluded from group mean calculation.

## APPENDIX 29 - continued

Bodyweights – individual values (g) for adult males

Group 7: malathion: 5 mg/kg/day

Animal number	day of treatment											
	-1	1	2	3	4	5	6	7	8	9	10	11
217 #	254	266										
218 #	277	287										
219 #	242	251										
220 #	223	231										
221 #	292	303										
222 #	291	301										
223 #	280	289										
224 #	267	281										
225	251	257	268	276	288	298	298	314	324	335	343	356
226	250	262	269	280	290	300	303	312	317	328	339	347
227	232	240	247	255	266	274	277	284	287	295	301	311
228	291	298	309	316	329	337	340	345	359	369	380	383
229	297	309	317	325	336	338	344	357	364	374	381	386
230	256	268	273	281	290	290	298	307	317	324	328	332
231	300	306	309	320	328	329	338	344	355	362	372	375
232	293	300	307	319	328	334	338	349	355	361	369	372

# Animals killed on day 1 of treatment.

## APPENDIX 29 - continued

Bodyweights – individual values (g) for adult males

Group 8: malathion: 50 mg/kg/day

Animal number	day of treatment											
	-1	1	2	3	4	5	6	7	8	9	10	11
233 #	223	233										
234 #	285	293										
235 #	265	277										
236 #	251	263										
237 #	287	300										
238 #	306	311										
239 #	234	243										
240 #	262	274										
241	256	263	273	282	290	300	304	310	323	333	341	347
242	248	260	266	272	282	293	299	304	313	317	325	333
243	273	283	291	302	313	320	326	333	346	357	363	373
244	273	280	294	300	314	327	330	339	353	360	374	380
245	293	299	310	316	323	324	330	301	353	357	366	374
246	246	255	262	270	279	286	292	301	311	315	324	332
247	281	286	297	308	313	316	324	331	340	345	354	357
248	311	321	329	341	347	354	358	364	368	376	385	392

# Animals killed on day 1 of treatment.

## APPENDIX 29 - continued

Bodyweights – individual values (g) for adult males

Group 9: malathion: 150 mg/kg/day

Animal number	day of treatment											
	-1	1	2	3	4	5	6	7	8	9	10	11
249 #	230	238										
250 #	286	295										
251 #	262	267										
252 #	265	272										
253 #	286	296										
254 #	279	291										
255 #	252	262										
256 #	241	253										
257	268	278	284	294	300	311	315	327	339	343	353	363
258	233	240	248	254	264	270	273	275	284	289	292	300
259	272	281	283	299	305	316	321	331	337	344	346	359
260	258	265	271	282	285	294	300	312	321	324	333	340
261	314	327	337	343	356	362	370	378	386	387	398	405
262	268	280	286	298	307	316	321	336	342	345	356	366
263	306	317	326	338	342	346	356	361	369	366	377	383
264	305	316	323	328	338	344	350	357	358	365	372	381

# Animals killed on day 1 of treatment.

## APPENDIX 29 - continued

Bodyweights – individual values (g) for adult males

## Group 10: control

Animal number	day of treatment	
	-1	1
281#	318	329
282#	291	304
283#	291	301
284#	297	313
285#	303	311
286#	295	309
287#	314	322
288#	308	322

# Animals killed on day 1 of treatment.

## Group 11: malathion: 450 mg/kg/day

Animal number	day of treatment	
	-1	1
289#	288	295
290#	292	304
291#	293	307
292#	314	334
293#	319	333
294#	294	300
295#	312	324
296#	301	312



## APPENDIX 30

Bodyweights – 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 #	177	186										
86 #	205	202										
87 #	215	218										
88 #	188	193										
89 #	194	197										
90 #	212	218										
91 #	197	204										
92 #	231	238										
93	202	202	210	216	222	221	228	235	238	235	240	248
94	166	167	167	173	177	179	176	189	192	191	191	200
95	201	205	204	209	217	218	220	224	233	233	233	241
96	212	210	209	218	218	217	215	226	234	231	227	239
97	207	214	217	216	219	221	228	223	231	238	236	232
98	225	230	238	235	242	246	244	248	256	266	266	263
99	228	239	245	249	245	251	258	263	264	270	276	282
100	194	194	191	199	204	202	209	215	223	222	222	229

# Animals killed on day 1 of treatment.

## APPENDIX 30 - continued

Bodyweights – individual values (g) for adult females

Group 7: malathion: 5 mg/kg/day

Animal number	day of treatment											
	-1	1	2	3	4	5	6	7	8	9	10	11
101 #	176	184										
102 #	180	189										
103 #	196	204										
104 #	194	200										
105 #	194	196										
106 #	197	198										
107 #	223	223										
108 #	209	214										
109	187	190	196	202	199	204	208	211	209	217	223	226
110	176	177	183	179	186	194	196	195	204	206	211	212
111	196	200	203	202	207	211	216	212	220	224	229	224
112	203	203	204	209	208	214	216	220	219	220	222	229
113	227	228	223	231	239	241	238	244	252	254	246	261
114	217	226	232	233	233	239	249	249	248	257	261	262
115	224	227	233	238	245	243	249	253	260	261	269	273
116	198	203	207	209	207	213	220	222	220	227	234	238

# Animals killed on day 1 of treatment.

## APPENDIX 30 - continued

Bodyweight – individual values (g) for adult females

Group 8: malathion: 50 mg/kg/day

Animal number	day of treatment											
	-1	1	2	3	4	5	6	7	8	9	10	11
117 #	181	186										
118 #	202	207										
119 #	210	213										
120 #	179	183										
121 #	197	200										
122 #	197	204										
123 #	187	194										
124 #	216	219										
125	194	202	204	209	205	216	221	223	223	227	240	239
126	205	209	213	212	219	226	230	229	233	239	243	243
127	192	198	202	204	201	213	218	220	220	229	240	241
128	189	180	185	192	193	188	196	202	201	200	212	211
129	185	190	194	192	199	202	204	201	210	216	218	216
130	203	207	208	213	219	222	220	227	234	238	234	244
131	189	192	198	202	203	206	211	213	217	224	226	228
132	209	215	219	214	225	229	232	236	243	250	249	248

# Animals killed on day 1 of treatment.

## APPENDIX 30 - continued

Bodyweights -- individual values (g) for adult females

Group 9: malathion: 150 mg/kg/day

Animal number	day of treatment											
	-1	1	2	3	4	5	6	7	8	9	10	11
133 #	171	176										
134 #	230	232										
135 #	196	200										
136 #	208	216										
137 #	188	195										
138 #	198	200										
139 #	206	214										
140 #	224	225										
141	195	197	195	201	210	211	211	209	222	226	225	235
142	161	168	172	176	173	181	186	180	186	193	195	199
143	198	198	204	208	213	208	218	218	222	223	231	238
144	197	200	192	201	203	205	203	211	216	216	213	220
145	202	206	198	207	212	215	210	213	218	221	222	229
146	222	232	233	231	240	244	247	249	254	258	263	261
147	200	203	202	213	214	219	216	224	228	229	232	239
148	181	182	186	192	193	196	202	208	207	204	216	221

# Animals killed on day 1 of treatment.

APPENDIX 30 - continued

Bodyweights – individual values (g) for adult females

Group 10: control

Animal number	day of treatment	
	-1	1
265#	175	183
266#	191	199
267#	190	194
268#	211	219
269#	207	217
270#	186	191
271#	193	199
272#	191	194

#      Animals killed on day 1 of treatment.

Group 11: malathion: 450 mg/kg/day

Animal number	day of treatment	
	-1	1
273#	179	189
274#	184	192
275#	195	200
276#	212	221
277#	184	191
278#	194	206
279#	186	188
280#	217	221

## APPENDIX 31

Necropsy findings – individual observations for young adult animals on day 11 of treatment

Group	:	6	7	8	9
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

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	Brown staining on head and upper dorsal thorax
	215	NAD	99	NAD
	216	NAD	100	NAD
7	225	NAD	109	NAD
	226	NAD	110	NAD
	227	NAD	111	Hairloss on dorsal surface and encrustation 7x3mm
	228	NAD	112	NAD
	229	NAD	113	Slight brown staining on head
	230	NAD	114	NAD
	231	NAD	115	NAD
	232	NAD	116	NAD

NAD No abnormalities detected.

## APPENDIX 31 - continued

Necropsy findings – individual observations for young adult animals on day 11 of treatment

Group	:	6	7	8	9
Compound	:	control	-----malathion-----		
Dosage (mg/kg/day)	:	0	5	50	150

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	<b>Kidneys and ureter:</b> bilateral renal cavitation and bilateral hydroureter	141	NAD
	258	NAD	142	NAD
	259	NAD	143	NAD
	260	NAD	144	NAD
	261	NAD	145	Slight brown staining on head
	262	NAD	146	NAD
	263	NAD	147	NAD
	264	NAD	148	Brown staining on head

NAD No abnormalities detected.

## APPENDIX 32

Brain weights – individual values (g) for young adult animals on day 1 of treatment

Group : 6 and 10 7 8 9 11  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150 450

Group	Sex	Animal number	Brain weight	Sex	Animal number	Brain weight
6	M	201	1.899	F	85	1.811
		202	1.928		86	1.996
		203	1.888		87	1.889
		204	1.879		88	1.804
		205	1.885		89	1.818
		206	1.846		90	1.858
		207	1.943		91	1.916
		208	1.836		92	1.913
7	M	217	1.902	F	101	1.681
		218	1.940		102	1.806
		219	1.843		103	1.808
		220	1.925		104	1.840
		221	2.067		105	1.844
		222	2.000		106	1.807
		223	1.912		107	1.915
		224	1.910		108	1.779
8	M	233	1.728	F	117	1.744
		234	2.005		118	1.770
		235	1.907		119	1.956
		236	1.988		120	1.958
		237	1.929		121	1.759
		238	1.944		122	1.794
		239	1.893		123	1.830
		240	1.794		124	1.850
9	M	249	1.771	F	133	1.677
		250	1.960		134	1.832
		251	1.939		135	1.798
		252	1.963		136	1.884
		253	2.000		137	1.768
		254	1.869		138	1.807
		255	1.847		139	1.882
		256	1.752		140	1.945
10	M	281	1.966	F	265	1.726
		282	1.875		266	1.702
		283	1.957		267	1.795
		284	1.886		268	1.776
		285	1.976		269	1.789
		286	1.930		270	1.835
		287	1.970		271	1.870
		288	1.969		272	1.819
11	M	289	1.932	F	273	1.846
		290	1.871		274	1.815
		291	2.012		275	1.779
		292	2.016		276	1.893
		293	1.922		277	1.835
		294	1.954		278	2.020
		295	1.926		279	1.836
		296	1.931		280	1.871



## APPENDIX 33

Brain weights - individual values (g) for young adult animals on day 11 of treatment

Group : 6 7 8 9  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group	Sex	Animal number	Brain weight	Sex	Animal number	Brain weight
6	M	209	1.908	F	93	1.926
		210	1.902		94	1.978
		211	2.053		95	1.802
		212	1.761		96	1.874
		213	2.023		97	1.760
		214	1.906		98	2.048
		215	1.924		99	1.896
		216	2.041		100	1.904
		225	1.926	F	109	1.819
		226	1.956		110	1.793
7	M	227	2.008		111	1.820
		228	1.917		112	1.902
		229	1.951		113	1.823
		230	1.879		114	1.922
		231	1.855		115	1.925
		232	1.849		116	1.981
		241	1.813	F	125	1.929
		242	2.014		126	1.864
		243	2.059		127	1.825
		244	1.979		128	1.829
8	M	245	1.978		129	1.787
		246	1.931		130	2.141
		247	1.935		131	1.910
		248	1.923		132	1.907
		257	1.974	F	141	1.850
		258	1.885		142	1.767
		259	2.067		143	1.979
		260	1.861		144	1.705
		261	2.026		145	1.919
		262	1.867		146	1.930
9	M	263	1.960		147	1.922
		264	1.806		148	1.884

## APPENDIX 34

Necropsy findings – individual observations for offspring on PND 60

Group 1: control

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
1004	NAD	1012	NAD
1104	NAD	1111	Brown staining on head and muzzle
1204	NAD	1213	NAD
1305	NAD	1314	<b>Kidneys:</b> unilateral renal cavitation
1404	<b>Testes:</b> right testis enlarged with pale tubules in adjacent fat pad	1412	NAD
1504	<b>Kidneys:</b> unilateral renal cavitation	1512	NAD
1603	NAD	1609	NAD
1604	NAD	1610	NAD
1703	NAD	1711	NAD
1704	NAD	1712	NAD

NAD No abnormalities detected.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

Direct treatment of offspring: PND 11 to PND 21.

## APPENDIX 34 – continued

Necropsy findings – individual observations for offspring on PND 60

Group 2: malathion: 5 mg/kg/day

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
2904	NAD	2909	NAD
3004	Brown staining on head	3014	NAD
3204	Brown staining on head	3112	Brown staining on muzzle
3304	NAD	3213	NAD
3404	NAD	3314	NAD
3504	NAD	3414	NAD
3505	NAD	3511	NAD
3703	NAD	3512	Brown staining on muzzle, head and forelimbs
3704	NAD	3710	NAD
		3711	Brown staining on head

NAD No abnormalities detected.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

Direct treatment of offspring: PND 11 to PND 21.

## APPENDIX 34 – continued

Necropsy findings – individual observations for offspring on PND 60

Group 3: malathion: 50 mg/kg/day

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
4804	NAD	4810	NAD
4904	NAD	4914	NAD
5005	NAD	5013	NAD
5104	NAD	5109	NAD
5204	NAD	5210	NAD
5304	NAD	5309	NAD
5403	NAD	5407	NAD
5404	NAD	5408	NAD
5603	NAD	5612	NAD
5604	NAD	5613	NAD

NAD No abnormalities detected.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

Direct treatment of offspring: PND 11 to PND 21.

## APPENDIX 34 – continued

Necropsy findings – individual observations for offspring on PND 60

Group 4: malathion: 150 mg/kg/day

Male offspring number	Necropsy observations	Female offspring number	Necropsy observations
6704	NAD	6716	Brown staining on head
6804	Hairloss on head	6810	Brown staining on head
6903	NAD	6908	NAD
7004	NAD	7013	NAD
7204	NAD	7211	Brown staining on head
7304	NAD	7310	NAD
7403	NAD	7411	Brown staining on head
7404	NAD	7412	NAD
7503	Brown staining on head	7513	NAD
7504	NAD	7514	Brown staining on head

NAD No abnormalities detected.

Last two digits of animal number denote pup number.

Other digit(s) denote litter number.

Direct treatment of offspring: PND 11 to PND 21.

## APPENDIX 35

Brain weights – individual values (g) for male and female offspring at PND 60

## Group 1: control

Sex	Animal number	Brain weight (g)	Sex	Animal number	Brain weight (g)
M	1004	1.935	F	1012	1.847
	1104	2.109		1111	1.968
	1204	2.043		1213	1.884
	1305	1.990		1314	1.745
	1404	2.018		1412	1.810
	1504	1.976		1512	1.878
	1604	2.165		1610	1.908
	1704	1.889		1712	1.857

## Group 2: malathion: 5 mg/kg/day

Sex	Animal number	Brain weight (g)	Sex	Animal number	Brain weight (g)
M	2904	1.943	F	2909	1.865
	3004	1.990		3014	1.939
	3204	2.092		3112	1.812
	3304	2.123		3213	1.864
	3404	1.997		3314	1.774
	3505	1.968		3414	1.830
	3703	1.872		3512	1.895
	3704	2.039		3711	1.838

Last two digits of animal number denote pup number. Other digits denote litter number.

Direct dosing of offspring: PND 11 to PND 21

## APPENDIX 35 - continued

Brain weights – individual values (g) for male and female offspring at PND 60

Group 3: malathion: 50 mg/kg/day

Sex	Animal number	Brain weight (g)	Sex	Animal number	Brain weight (g)
M	4804	1.926	F	4810	1.764
	4904	1.959		4914	1.844
	5005	2.052		5013	1.970
	5104	2.065		5109	1.746
	5304	1.988		5309	1.922
	5204	1.995		5210	1.983
	5404	1.963		5408	1.974
	5604	1.971		5613	1.737

Group 4: malathion: 150 mg/kg/day

Sex	Animal number	Brain weight (g)	Sex	Animal number	Brain weight (g)
M	6704	1.981	F	6716	1.790
	6804	1.921		6810	1.782
	6903	1.959		6908	1.801
	7004	2.042		7310	1.791
	7204	1.999		7013	1.927
	7304	1.903		7211	1.820
	7404	2.075		7412	1.861
	7504	1.917		7514	1.861

Last two digits of animal number denote pup number. Other digits denote litter number.  
 Direct dosing of offspring: PND 11 to PND 21

## APPENDIX 36

Plasma, erythrocyte and brain cholinesterase activity - individual values for dams on GD 20

Group : 1 2 3 4  
Compound : control  
Dosage (mg/kg/day) : 0 5 -----malathion----- 50 150

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1F	1	1205	1125	13400
	2	1222	1250	13850
	3	1459	1200	13000
	4	1294	975	13400
	5	1978	1375	12800
	6	1389	1350	13100
	7	1310	1225	12550
	8	1200	1375	13500
2F	20	1310	1275	11750
	21	1234	1150	12800
	22	1223	1225	13350
	23	1182	1325	13950
	24	1210	1200	13350
	25	1162	1200	12800
	26	944	1300	12700
	27	1411	1275	13400

CHE Cholinesterase.  
Dams treated from GD 6.



APPENDIX 36 - continued

Plasma, erythrocyte and brain cholinesterase activity - individual values for dams on GD 20

Group : 1 2 3 4  
Compound : control -----malathion-----  
Dosage (mg/kg/day) : 0 5 50 150

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
3F	39	1344	1050	12850
	40	1088	1000	13200
	41	1267	925	12650
	42	1540	1100	12650
	43	1405	925	14200
	44	1156	1000	13050
	45	1475	950	12800
4F	46	1099	1000	13400
	58	1441	575	12950
	59	1027	600	12500
	60	930	575	12800
	61	1120	650	12200
	62	1572	750	12550
	63	916	650	13000
	64	1347	525	12600
	65	1276	525	12550

CHE Cholinesterase.  
Dams treated from GD 6.

APPENDIX 37

Plasma, erythrocyte and brain cholinesterase activity - individual litter values for fetuses on GD 20

Group : 1 2 3 4  
Compound : control  
Dosage (mg/kg/day) : 0 5 -----malathion----- 150

Group	Litter number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1	1	317	825	1450
	2	263	850	1850
	3	287	875	1550
	4	292	925	1650
	5	303	1000	1650
	6	248	1025	1600
	7	269	850	1550
	8	299	1150	1550
2	20	313	875	1300
	21	277	900	1700
	22	268	975	1550
	23	257	775	1850
	24	243	975	1800
	25	244	875	1800
	26	264	850	1600
	27	254	950	1650

CHE Cholinesterase.  
Dams treated from GD 6.

## APPENDIX 37 - continued

Plasma, erythrocyte and brain cholinesterase activity - individual litter values for fetuses on GD 20

Group : 1 2 3 4  
 Compound : control  
 Dosage (mg/kg/day) : 0 5 50 150

-----malathion-----

Group	Litter number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
3	39	237	925	1650
	40	238	850	1650
	41	218	775	1550
	42	INS	700	1800
	43	242	850	1350
	44	248	875	1300
	45	329	925	1400
4	46	208	750	1450
	58	257	825	1700
	59	223	725	1550
	60	220	700	1750
	61	268	825	1850
	62	250	775	2050
	63	228	750	1550
	64	256	725	1450
	65	244	725	1200

CHE Cholinesterase.

INS Insufficient sample for testing.

Dams treated from GD 6.

## APPENDIX 38

Plasma, erythrocyte and brain cholinesterase activity – individual values for male and female offspring on PND 4

Group : 1 2 3 4  
 Compound : control  
 Dosage (mg/kg/day) : 0 5 -----malathion----- 150

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1M	1005	628	1025	2800
	1006	596	1175	2450
	1106	609	1175	3200
	1205	612	1550	2700
	1206	703	950	3100
	1306	726	1050	3150
	1307	653	950	3050
	1405	544	925	3250
	1406	533	975	3200
	1505	492	850	2850
	1506	654	1075	3000
	1605	581	1200	2750
	1606	628	1300	3000
	1705	561	1075	2800
	1706	622	1275	3100
	1905	629	1025	3350
	1906	636	1125	3550

CHE Cholinesterase.

Last two digits of animal number denote pup number. Other digit(s) denote litter number.  
 Dams treated from GD 6.

## APPENDIX 38 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for male and female offspring on PND 4

Group : 1 2 3 4  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
2M	2905	667	975	3350
	3005	501	1250	3150
	3007	492	950	3050
	3105	578	1250	2850
	3106	547	1225	3100
	3205	620	1400	3200
	3206	608	1125	3600
	3305	577	1200	3050
	3306	589	1150	3050
	3405	563	1275	3000
	3406	606	1275	2600
	3506	663	900	3150
	3507	625	1200	2900
	3605	759	1100	3000
	3705	548	1025	3050
	3706	566	850	3150

CHE Cholinesterase.

Last two digits of animal number denote pup number. Other digit(s) denote litter number.  
 Dams treated from GD 6.

## APPENDIX 38 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for male and female offspring on PND 4

Group : 1 2 3 4  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
3M	4805	543	1225	2400
	4806	589	1000	2900
	4905	630	1125	2750
	4906	640	1100	2350
	5006	638	1200	2750
	5007	642	1275	3450
	5105	596	950	3000
	5205	751	1300	3150
	5206	667	800	3350
	5305	579	1175	3050
	5605	644	925	2950
	5606	642	1075	3100
	5705	576	825	2700

CHE Cholinesterase.

Last two digits of animal number denote pup number. Other digit(s) denote litter number.  
 Dams treated from GD 6.

APPENDIX 38 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for male and female offspring on PND 4

Group : 1 2 3 4  
Compound : control -----malathion-----  
Dosage (mg/kg/day) : 0 5 50 150

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
4M	6705	538	1400	3050
	6706	633	N/S	2800
	6805	549	950	1900
	6806	548	N/S	2750
	7005	567	1050	3200
	7006	689	1150	3000
	7205	718	950	3050
	7206	646	950	2950
	7305	751	1375	1950
	7306	602	850	2800
	7405	645	850	3150
	7406	664	700	2950
	7505	549	1025	3350
	7506	559	1000	2850
	7605	643	975	3250

CHE Cholinesterase.  
Last two digits of animal number denote pup number. Other digit(s) denote litter number.  
Dams treated from GD 6.

## APPENDIX 38 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for male and female offspring on PND 4

Group : 1 2 3 4  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1F	1013	716	1125	2750
	1014	666	1175	3050
	1112	584	1000	2950
	1113	600	925	3200
	1214	668	1100	3300
	1215	699	1275	2950
	1315	628	1250	2800
	1316	697	825	3200
	1513	634	1050	3000
	1514	542	1650	2650
	1611	625	975	3300
	1612	596	975	2950
	1713	567	1050	3200
	1714	567	1350	2500
	1809	543	1375	2850
	1810	557	1225	3100
	1912	589	1175	2650
	1913	713	1150	3500

CHE Cholinesterase.

Last two digits of animal number denote pup number. Other digit(s) denote litter number.  
 Dams treated from GD 6.



## APPENDIX 38 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for male and female offspring on PND 4

Group : 1 2 3 4  
 Compound : control  
 Dosage (mg/kg/day) : 0 5 50 150  
 -----malathion-----

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
2F	2910	656	950	3100
	2911	499	1250	3000
	3113	616	1250	2650
	3114	616	1225	3250
	3214	639	925	2950
	3215	602	1200	3100
	3315	584	1100	2750
	3316	652	1500	3450
	3415	546	825	2700
	3513	610	875	2850
	3514	672	1450	2900
	3610	691	1150	2800
	3611	643	1225	2100
	3712	529	1075	3100
	3713	593	1175	3400
	3810	477	1000	2800
	3811	536	950	3100

CHE Cholinesterase.

Last two digits of animal number denote pup number. Other digit(s) denote litter number.  
 Dams treated from GD 6.

APPENDIX 38 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for male and female offspring on PND 4

Group : 1 2 3 4  
Compound : control -----malathion-----  
Dosage (mg/kg/day) : 0 5 50 150

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
3F	4811	645	1300	2000
	4812	633	1100	2350
	4915	568	1300	2250
	4916	629	1125	2700
	5014	600	1925	2850
	5015	652	1175	2350
	5110	587	1500	3100
	5111	641	800	2950
	5211	693	1200	3750
	5212	705	1075	3900
	5310	577	1350	2700
	5311	612	950	2800
	5409	589	1075	3950
	5410	552	1150	3600
	5509	512	1075	2750

CHE Cholinesterase.  
Last two digits of animal number denote pup number. Other digit(s) denote litter number.  
Dams treated from GD 6.

APPENDIX 38 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for male and female offspring on PND 4

Group : 1 2 3 4  
Compound : control  
Dosage (mg/kg/day) : 0 5 -----malathion----- 150

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
4F	6717	599	1025	2900
	6811	598	950	3200
	6812	624	1075	3150
	6909	586	1175	2850
	6910	546	1300	2500
	7014	664	1150	3600
	7015	571	1100	2900
	7109	671	975	3000
	7110	678	1050	3150
	7212	616	925	3200
	7213	624	950	3350
	7311	608	875	2600
	7312	675	1350	2200
	7413	671	925	2800
	7414	585	925	2950
	7515	552	1325	3300
	7610	578	1800	3200
	7611	662	975	2550

CHE Cholinesterase.  
Last two digits of animal number denote pup number. Other digit(s) denote litter number.  
Dams treated from GD 6.

## APPENDIX 39

Plasma, erythrocyte and brain cholinesterase activity – individual values for dosed male and female offspring on PND 11 (1 day of dosing)

Group : 1 2 3 4 11  
Compound : control  
Dosage (mg/kg/day) : 0 5 -----malathion----- 150 450

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1M	7701	768	1350	5800
	7801	813	1300	5900
	7901	873	1575	5800
	8001	695	1425	5350
	8101	813	2100	5650
	8301	693	1525	6100
	8401	659	1350	5600
	108201	734	1450	5850
2M	7702	704	1025	5600
	7802#	784	1300	5350
	7902	707	900	5800
	8002	641	1600	5950
	8102	737	1300	5800
	8302	637	1175	5400
	8402	677	1325	5850
	108202	748	1550	5750

CHE Cholinesterase.

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

# Samples for 7802 and 7805 believed to have been transposed. Values presented have been assigned to the animals/dosage levels where they are believed to have occurred.

## APPENDIX 39 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for dosed male and female offspring on PND 11 (1 day of dosing)

Group : 1 2 3 4 5  
 Compound : control  
 Dosage (mg/kg/day) : 0 50 150 450

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
3M	7703	600	975	5350
	7803	686	1050	5350
	7903	674	975	5650
	8003	552	1150	5300
	8103	598	1250	5700
	8303	607	1250	5550
	8403	606	1050	5400
4M	108203	592	1350	4800
	7704	533	650	3000
	7804	552	850	3850
	7904	583	725	4450
	8004	473	700	2900
	8104	399	600	2500
	8304	395	600	3000
	8404	500	725	3750
	108204	418	525	2500

CHE Cholinesterase.

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

APPENDIX 39 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for dosed male and female offspring on PND 11 (1 day of dosing)

Group : 1 2 3 4 11  
Compound : control -----malathion-----  
Dosage (mg/kg/day) : 0 5 50 150 450

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
11M	7705	347	300	350
	7805#	314	450	900
	7905	430	475	700
	8005	239	275	500
	8105	370	500	1400
	8305	301	425	1000
	8405	419	550	1650
	108205	346	450	850

CHE Cholinesterase.

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

# Samples for 7802 and 7805 believed to have been transposed. Values presented have been assigned to the animals/dosage levels where they are believed to have occurred.

## APPENDIX 39 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for dosed male and female offspring on PND 11 (1 day of dosing)

Group : 1 2 3 4 11  
 Compound : control -----malathion-----  
 Dosage (mg/kg/day) : 0 5 50 150 450

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1F	7708	830	1225	5500
	7808	698	1200	5550
	7906	876	1375	6250
	8007	692	1425	5800
	8109	758	1350	6100
	8309	711	1500	5550
	8407	656	1225	6000
2F	108206	674	1250	5850
	7709	746	950	5650
	7809	775	950	5850
	7907	771	1075	5800
	8008	690	1300	5300
	8110	728	1475	5700
	8310	636	1550	5500
	8408	704	1350	5500
	108207	687	1175	5500

CHE Cholinesterase.

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

## APPENDIX 39 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for dosed male and female offspring on PND 11 (1 day of dosing)

Group : 1 2 3 4 5  
 Compound : control  
 Dosage (mg/kg/day) : 0 5 50 150 450

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
3F	7710	603	825	4900
	7810	678	1225	5100
	7908	771	1050	5450
	8009	560	1100	5800
	8111	576	925	5490
	8311	600	1025	5850
4F	8409	518	900	3650
	108208	653	1075	5750
	7711	486	675	2500
	7811	514	600	2800
	7909	574	750	3900
	8010	438	700	2450
	8112	466	725	3050
	8312	463	725	3200
	8410	430	600	2650
	108209	476	725	3800

CHE Cholinesterase.

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



APPENDIX 39 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for dosed male and female offspring on PND 11 (1 day of dosing)

Group : 1 2 -----malathion----- 4 11  
Compound : control  
Dosage (mg/kg/day) : 0 5 50 150 450

Group	Animal	Plasm CHE U/L	RCHE DTNA U/L	Brain CHE U/Kg
11F	7712	223	275	450
	7812	451	625	1850
	7910	396	450	1000
	8011	383	475	650
	8113	348	700	1300
	8313	344	625	1750
	8411	374	550	950
	108210	307	450	700

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

## APPENDIX 40

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

Group : 6 and 10 : 8 : 9 : 11  
 Compound : control : -----malathion-----  
 Dosage (mg/kg/day) : 0 : 50 : 150 : 450

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
6M	201	345	1075	13250
	202	342	1000	12750
	203	370	925	13250
	204	328	1000	13350
	205	362	900	14750
	206	416	650	15250
	207	313	650	13800
	208	260	725	13300
7M	217	324	950	12450
	218	297	1050	12700
	219	392	1025	13750
	220	319	975	13000
	221	341	550	12650
	222	396	950	13350
	223	338	725	13050
	224	317	900	12950

CHE Cholinesterase.

## APPENDIX 40 - continued

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

Group : 6 and 10 7 8 9 11  
 Compound : control  
 Dosage (mg/kg/day) : 0 5 -----malathion----- 150 450

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
8M	233	358	850	13450
	234	409	950	13600
	235	498	1100	13850
	236	343	1050	12700
	237	308	925	13200
	238	346	925	13500
	239	329	950	11650
	240	284	1050	12700
9M	249	342	875	13450
	250	320	850	13900
	251	511	900	13700
	252	278	875	12750
	253	352	825	12450
	254	344	725	12000
	255	274	925	11550
	256	274	850	12150

CHE Cholinesterase.

## APPENDIX 40 - continued

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

Group : 6 and 10 7 8 9 11  
 Compound : control  
 Dosage (mg/kg/day) : 0 5 -----malathion----- 150 450

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
10M	281	323	1100	13900
	282	350	1075	13750
	283	339	1100	13000
	284	331	1200	13450
	285	367	1175	14050
	286	312	1025	13050
	287	519	1225	13850
	288	287	975	13450
11M	289	278	825	14000
	290	266	775	13500
	291	303	925	12800
	292	218	975	13000
	293	329	750	12500
	294	264	775	13050
	295	247	875	13100
	296	239	750	13100

CHE Cholinesterase.

## APPENDIX 40 - continued

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

Group : 6 and 10 7 8 9 11  
 Compound : control  
 Dosage (mg/kg/day) : 0 5 50 150 450

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
6F	85	567	875	13300
	86	580	975	13350
	87	955	1000	13550
	88	978	950	12800
	89	492	975	12600
	90	915	975	12950
	91	1046	1025	12300
	92	814	825	12350
7F	101	711	950	13950
	102	738	1150	13250
	103	875	950	13300
	104	614	875	13000
	105	498	1075	13400
	106	744	1050	12600
	107	839	1150	12750
	108	714	900	13450

CHE Cholinesterase.

APPENDIX 40 - continued

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

Group : 6 and 10 7 8 9 11  
Compound : control  
Dosage (mg/kg/day) : 0 5 -----malathion----- 150 450

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
8F	117	752	900	13000
	118	633	1000	13700
	119	1078	1000	13150
	120	806	925	12300
	121	553	1100	13000
	122	956	975	13300
	123	1004	1025	12200
	124	794	750	13650
9F	133	494	825	13300
	134	594	925	13450
	135	555	875	13150
	136	907	975	13700
	137	567	975	13150
	138	1034	800	12950
	139	841	875	13000
	140	822	875	13250

CHE Cholinesterase.

## APPENDIX 40 - continued

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

Group : 6 and 10 7 8 9 11  
 Compound : control  
 Dosage (mg/kg/day) : 0 5 50 150 450

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
10F	265	804	1050	14150
	266	531	975	13900
	267	598	1175	13850
	268	620	1075	13700
	269	601	1000	13400
	270	611	1125	13250
	271	547	1050	12550
	272	679	1100	13300
11F	273	573	825	13000
	274	707	950	12900
	275	479	900	13250
	276	593	800	13500
	277	588	1075	12550
	278	578	775	11700
	279	437	900	13800
	280	506	850	13100

CHE Cholinesterase.

## APPENDIX 4I

Plasma, erythrocyte and brain cholinesterase activity – individual values for dosed male and female offspring on PND 21 (11 days of dosing)

Group : 1 2 3 4  
Compound : control  
Dosage (mg/kg/day) : 0 5 50 150  
-----malathion-----

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1M	1001	336	2075	10700
	1101	331	1575	10500
	1201	411	1550	10800
	1301	372	1475	10050
	1401	562	1525	10300
	1501	404	1975	10800
	1601	347	2200	10200
	1701	379	2550	10650
	2901	288	1800	10200
	3001	458	1550	9750
2M	3101	352	1925	10700
	3201	316	1875	10450
	3301	288	1475	10250
	3401	313	1375	10300
	3501	453	1200	10500
	3701	260	1250	10750

CHE Cholinesterase.

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



APPENDIX 41 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for dosed male and female offspring on PND 21 (11 days of dosing)

Group : 1 2 3 4  
Compound : control -----malathion-----  
Dosage (mg/kg/day) : 0 5 50 150

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
3M	4801	270	1200	11100
	4901	314	1025	10200
	5002	280	1125	10900
	5101	360	1100	9850
	5201	344	1050	10500
	5301	270	875	10200
	5401	373	1625	11150
4M	5601	350	1150	10000
	6701	295	575	7200
	6801	359	600	9050
	6901	320	500	9000
	7001	334	950	9500
	7201	245	425	9050
	7301	216	725	8850
	7401	296	350	8350
	7501	325	850	9800

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

## APPENDIX 41 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for dosed male and female offspring on PND 21 (11 days of dosing)

Group : 1 2 3 4  
 Compound : control  
 Dosage (mg/kg/day) : 0 5 50 150

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1F	1009	378	1450	10450
	1108	303	2275	10600
	1210	445	2000	10200
	1310	436	1700	10150
	1409	462	1600	10050
	1509	313	1675	10150
	1607	335	2650	10750
	1709	322	1800	10500
2F	2906	452	1750	9650
	3011	338	1925	10050
	3109	329	1325	10100
	3210	289	1550	10400
	3310	300	1200	10350
	3411	296	1375	10250
	3503	380	2600	11000
	3708	316	1125	10200

CHE Cholinesterase.

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

## APPENDIX 41 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for dosed male and female offspring on PND 21 (11 days of dosing)

Group : 1 2 3 4  
Compound : control -----malathion-----  
Dosage (mg/kg/day) : 0 5 50 150

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
3F	4807	245	1175	10550
	4911	435	1250	10100
	5011	233	1425	10800
	5106	276	1375	10050
	5207	276	1475	10550
	5306	378	1150	10400
	5405	311	1125	11150
4F	5610	281	1025	9950
	6713	244	575	7600
	6807	223	825	8700
	6904	284	275	7250
	7010	333	800	9300
	7207	220	600	9650
	7307	227	725	9050
	7409	230	450	8000
	7511	268	525	9650

CHE Cholinesterase.

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

## APPENDIX 42

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

Group : 6 7 8 9  
Compound : control  
Dosage (mg/kg/day) : 0 5 50 150  
-----malathion-----

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
6M	209	365	1175	13000
	210	317	1075	13950
	211	303	1100	12550
	212	315	1100	12400
	213	336	1050	13150
	214	327	1050	13650
	215	336	1100	14000
	216	363	1025	13050
7M	225	367	1125	13050
	226	299	1050	13750
	227	322	975	13850
	228	312	975	13300
	229	322	1000	12350
	230	282	1100	13400
	231	317	1000	14000
	232	356	1125	12600

CHE Cholinesterase.

APPENDIX 42 - continued

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

Group : 6 7 8 9  
Compound : control  
Dosage (mg/kg/day) : 0 5 -----malathion----- 50 150

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
8M	241	285	775	13800
	242	303	925	13900
	243	315	825	13600
	244	304	775	13650
	245	242	825	13150
	246	310	900	13650
	247	292	1075	13500
	248	325	850	12700
9M	257	372	625	12350
	258	256	525	14200
	259	296	725	12900
	260	243	600	13100
	261	223	550	12350
	262	326	575	12750
	263	297	725	12550
	264	302	600	14050

CHE Cholinesterase.

APPENDIX 42 - continued

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

Group : 6 7 8 9  
Compound : control -----malathion-----  
Dosage (mg/kg/day) : 0 5 50 150

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
6F	93	1415	1150	18100
	94	994	1200	12150
	95	750	1050	13100
	96	944	1225	13850
	97	893	1025	13700
	98	999	1075	12500
	99	823	950	12950
	100	1402	1075	13500
	109	919	1100	13500
	110	890	1100	13450
7F	111	818	1025	13050
	112	910	1175	13750
	113	1473	850	14050
	114	1050	1200	13400
	115	607	1225	13350
	116	1155	875	13150

CHE Cholinesterase.

## APPENDIX 42 - continued

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

Group : 6 7 8 9  
 Compound : control  
 Dosage (mg/kg/day) : 0 5 50 150

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
8F	125	1017	800	13900
	126	712	875	14100
	127	615	900	13900
	128	985	875	14250
	129	787	825	12900
	130	1294	850	13350
	131	584	950	13300
	132	974	950	13900
9F	141	770	600	13350
	142	1100	725	12750
	143	940	625	12100
	144	644	575	13400
	145	878	475	13250
	146	1087	575	13150
	147	710	450	12900
	148	1014	500	13350

CHE Cholinesterase.

## APPENDIX 43

Plasma, erythrocyte and brain cholinesterase activity – individual values for male and female offspring on PND 60

Group : 1 2 3 4  
 Compound : control  
 Dosage (mg/kg/day) : 0 5 -----malathion----- 150

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1M	1004	453	1000	13550
	1104	374	800	13650
	1204	375	850	13650
	1305	377	675	12550
	1404	339	950	13750
	1504	374	975	12900
	1604	284	1000	12450
	1704	325	975	13350
2M	2904	358	800	13500
	3004	319	875	13350
	3204	360	1050	13400
	3304	289	950	12550
	3404	387	925	13150
	3505	367	825	13350
	3703	449	1075	14650
	3704	490	1050	12200

CHE Cholinesterase.

Last two digits of animal number denote pup number. Other digit(s) denote litter number.  
 Direct dosing of offspring: PND 11 to PND 21.



## APPENDIX 43 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for male and female offspring on PND 60

Group : 1 2 3 4  
 Compound : control  
 Dosage (mg/kg/day) : 0 5 50 150

-----malathion-----

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
3M	4804	258	975	14400
	4904	357	1025	12850
	5005	377	1200	13100
	5104	340	975	12350
	5204	397	850	13150
	5304	361	900	13150
	5404	335	1075	12900
4M	5604	325	975	13100
	6704	218	925	12100
	6804	348	950	14450
	6903	340	1725	13200
	7004	446	1025	13000
	7204	260	925	13350
	7304	337	850	11500
	7404	252	1075	11450
	7504	333	925	13550

CHE Cholinesterase.

Last two digits of animal number denote pup number. Other digit(s) denote litter number.  
 Direct dosing of offspring: PND 11 to PND 21.

## APPENDIX 43 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for male and female offspring on PND 60

Group : 1 2 3 4  
 Compound : control  
 Dosage (mg/kg/day) : 0 5 50 150  
 -----malathion-----

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
1F	1012	1197	900	13350
	1111	1159	800	13500
	1213	1237	875	13850
	1314	701	975	12950
	1412	793	1200	13500
	1512	1638	1100	13900
	1610	1065	900	13700
	1712	929	975	13350
2F	2909	869	1000	13550
	3014	1166	900	13100
	3112	795	1000	13600
	3213	728	1075	12900
	3314	768	1000	13950
	3414	714	950	10950
	3512	1105	925	13500
	3711	1006	1050	14950

CHE Cholinesterase.

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

Direct dosing of offspring: PND 11 to PND 21.

## APPENDIX 43 - continued

Plasma, erythrocyte and brain cholinesterase activity – individual values for male and female offspring on PND 60

Group : 1 2 3 4  
 Compound : control  
 Dosage (mg/kg/day) : 0 5 50 150  
 -----malathion-----

Group	Animal number	Plasma CHE U/L	Erythrocyte CHE U/L	Brain CHE U/Kg
3F	4810	860	925	14500
	4914	680	925	13700
	5013	816	1000	12850
	5109	1029	1025	13150
	5210	1225	875	13050
	5309	842	1100	13350
	5408	1004	950	13650
	5613	848	850	13200
4F	6716	981	1100	13500
	6810	937	1250	13500
	6908	814	975	13250
	7013	855	1000	13450
	7211	822	975	13550
	7310	648	950	12550
	7412	839	975	13750
	7514	844	725	13100

CHE Cholinesterase.

Last two digits of animal number denote pup number. Other digit(s) denote litter number.  
 Direct dosing of offspring: PND 11 to PND 21.

ADDENDUM 1

Certificate of Analysis for diet



# CONTROL DATA

VRF1CP2.5 lot 00712

Date of Manufacture 2000/07/12 Sell by date 2000/11/12  
 Use by date 2001/07/12

Reg numbers	151 to 250
Quantity manufactured	(tonnes) 25
Variation from theoretical weight	Conform

## SIEVE ANALYSIS (mm)

Diameter 0.00 - 0.10	0.1
Diameter 0.10 - 0.25	16.1
Diameter 0.25 - 0.50	42.8
Diameter 0.50 - 1.00	38.5
Diameter 1.00 - 2.00	4.5
Diameter 2.00 - 3.15	1.0
Diameter > 3.15	0.0

## NUTRITIVE QUALITY

Incorporation of macro-mineral mix	(Na)	Positive
Incorporation of micro-mineral premix	(Mn and Cu)	Positive
Incorporation of vitamin premix	(Vit. A and Z)	Positive
Moisture	(%)	12.3
Crude protein	(%)	18.7
Crude oil	(%)	4.9
Nitrogen free extract	(%)	55.2
of which starch	(%)	38.9
of which total sugars	(%)	3.9
Crude fibre	(%)	3.3
Hemicellulose	(%)	3.3
True cellulose	(%)	
Lignine	(%)	
Total minerals	(%)	5.4
Calcium	(mg / Kg)	8 700
Phosphorus	(mg / Kg)	5 800
Sodium	(mg / Kg)	3 000
Potassium	(mg / Kg)	7 200
Manganese	(mg / Kg)	64
Copper	(mg / Kg)	19
Vitamin A	(UI / Kg)	48 300
Vitamin C	(mg / Kg)	2 500
Vitamin D3	(UI / Kg)	110
Vitamin E	(mg / Kg)	

## CONTAMINANTS

BACTERIOLOGY			MYCOTOXINS (ug / Kg)	
Viable organisms	(/g)	< 100	Aflatoxin	< 1
Moulds and yeasts	(/g)	< 10	Mycotoxin global risk	Negative
Total coliforms	(/g)	0		
Faecal coliforms	(/g)	0		
Aerobes S.R.	(/g)	< 10		
Salmonella	(/25g)	0		

2000/77/12

NITROGEN DERIVATIVE

DERIVATIVE		
MO2	(mg / kg)	0.5
MO3	(mg / kg)	12.0
NDA	(µg / kg)	( $z < 500$ )
NDBA	(µg / kg)	0.8
NDEA	(µg / kg)	< 0.2
NDBA	(µg / kg)	< 0.3
NETF	(µg / kg)	< 0.3
NFYF	(µg / kg)	< 0.3
NMOR	(µg / kg)	< 0.5
		< 0.6

(Total < 200)

Heptachlor	< 1
Heptachlor epoxide	< 1 ( $\leq 10$ )
Endrin	< 1 ( $\leq 10$ )
O-P'DDD	< 1
P-P'DDD	< 5
O-P'DDE	< 5
P-P'DDE	< 1
O-P'DDT	< 1 ( $\leq 50$ )
P-P'DDT	< 5

(Total < 7 000)

Iodofenphos	< 25	( $\leq 5$ 000)
Malathion	24	( $\leq 5$ 000)
Methamidophos	< 15	( $\leq 5$ 000)
Methidathion	< 25	( $\leq 5$ 000)
Mevinphos	< 1b	( $\leq 5$ 000)
Monocrotophos	< 90	( $\leq 5$ 000)
Naled	< 15	( $\leq 5$ 000)
Oxydemeton methyl	< 400	( $\leq 5$ 000)
Parathion ethyl	< 20	( $\leq 5$ 000)
Parathion methyl	< 20	( $\leq 5$ 000)
Phosalene	< 50	( $\leq 5$ 000)
Phosmet	< 50	( $\leq 5$ 000)
Phosphamidon	< 25	( $\leq 5$ 000)
Profenofos	< 50	( $\leq 5$ 000)
Prothoate	< 20	( $\leq 5$ 000)
Pyridaphenthion	< 15	( $\leq 5$ 000)
Pyrimiphos ethyl	< 20	( $\leq 5$ 000)
Pyrimiphos methyl	44	( $\leq 2$ 500)
Sulfotep	< 20	( $\leq 5$ 000)
Tanephos	< 15	( $\leq 5$ 000)
Tetrachlorvinphos	< 30	( $\leq 5$ 000)
Thiomethon	< 40	( $\leq 5$ 000)
Triazophos	< 30	( $\leq 5$ 000)
Trichlorfon	< 10	( $\leq 5$ 000)
Trichlorfonate	< 25	( $\leq 5$ 000)

ND.

## 1574

2000/09/13

Le Responsable AO



# CONTROL DATA

VRP1C lot 01102

Date of Manufacture 2000/11/02      Sell by date 2001/03/02  
 Use by date 2001/11/02

Bag numbers	1 to 500
Quantity manufactured	10 (tonnes)
Variation from theoretical weight	Conforms

## PHYSICAL QUALITY OF THE PELLETS

Diameter	(mm)	12.95 ± 0.23	(12.0 to 13.4)
Resistance to crushing	(Kgf / cm <sup>2</sup> )	16.7 ± 1.8	(13 to 20)
Resistance to abrasion	(%)	99.0	(> 97)
Specific mass	(g / l)	622.0	
Average pellet weight	(g)	3.005 ± 0.229	
Average pellet length	(mm)	20.70 ± 2.50	(15.0 to 24.0)
Length / Diameter	(%)	0.6	(< 2)
Number of pellets burnt	( / Kg)	9	(< 10)

## NUTRITIVE QUALITY

Incorporation of macro-mineral mix	(Na)	Positive	
Incorporation of micro-mineral premix	(Mn and Cu)	Positive	
Incorporation of vitamin premix	(Vit. A and E)	Positive	
Moisture	(%)	12.5	(9 to 14)
Crude protein	(%)	18.7	(17.4 to 20.4)
Crude oil	(%)	4.8	(3.8 to 6.2)
Nitrogen free extract	(%)	54.4	(48.0 to 60.0)
of which starch	(%)	35.8	
" which total sugars	(%)	4.2	
Crude fibre	(%)	3.6	(2.8 to 5.2)
Hemicellulose	(%)		
True cellulose	(%)		
Lignine	(%)		
Total minerals	(%)	6.0	(4.5 to 7.0)
Calcium	(mg / Kg)	9 400	(8 000 to 12 000)
Phosphorus	(mg / Kg)	6 700	(4 000 to 8 300)
Sodium	(mg / Kg)	3 600	(2 500 to 3 700)
Potassium	(mg / Kg)	8 900	(5 700 to 9 700)
Manganese	(mg / Kg)	83	(20 to 100)
Copper	(mg / Kg)	26	(13 to 23)
Vitamin A	(UI / Kg)	47 880	(20 000 to 55 000)
Vitamin C	(mg / Kg)		
Vitamin B3	(UI / Kg)	1 900	(≤ 3 000)
Vitamin E	(mg / Kg)	100	

## CONTAMINANTS

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

VRFIC lot 01102

2000/11/02

HEAVY METALS			NITROGEN DERIVATIVES		
Lead - Pb	(µg / Kg)	20 (< 1 500)	NO <sub>2</sub>	(mg / Kg)	0.5 (< 500)
Mercury - Hg	(µg / Kg)	10 (< 100)	NO <sub>3</sub>	(mg / Kg)	7.9 (< 10)
Arsenic - Ar	(µg / Kg)	10 (< 1 000)	NDMA	(µg / Kg)	< 0.2 (< 10)
Cadmium - Cd	(µg / Kg)	117 (< 250)	NDEA	(µg / Kg)	< 0.3 (< 10)
Selenium	(µg / Kg)	420 (< 600)	NDPA	(µg / Kg)	< 0.3 (< 10)
			NDEA	(µg / Kg)	< 0.3 (< 10)
			NFIP	(µg / Kg)	< 0.3 (< 10)
			NFPA	(µg / Kg)	< 0.5 (< 10)
			NMOR	(µg / Kg)	< 0.6 (< 10)
			(Total < 200)		
			Heptachlor		< 1 (< 10)
			Heptachlor Epoxide		< 1 (< 10)
			Endrin		< 1 (< 10)
			o,p'-DDD		< 5 (< 50)
			p,p'-DDD		< 5
			o,p'-DDE		< 1
			p,p'-DDE		< 1
			o,p'-DDT		< 5
			p,p'-DDT		< 5
			(Total < 7 000)		
			Indofenphos		< 25 (< 5 000)
			Malathion		27 (< 5 000)
			Methamidophos		< 15 (< 5 000)
			Methidathion		< 25 (< 5 000)
			Merlinphos		< 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		< 13 (< 2 500)
			Quatrop		< 20 (< 5 000)
			Tenophos		< 15 (< 5 000)
			Tetrahydrovinphos		< 30 (< 5 000)
			Thiomethon		< 40 (< 5 000)
			Triazophos		< 30 (< 5 000)
			Trichlorfon		< 10 (< 5 000)
			Trichloronate		< 25 (< 5 000)
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)					
Acophate		< 500 (< 5 000)			
Azinphos ethyl		< 50 (< 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		18 (< 1 500)			
Chlorthiofos		< 15 (< 5 000)			
Diazinon		< 15 (< 5 000)			
Dichlorfenthion		< 19 (< 5 000)			
Dichlorvos		120 (< 5 000)			
Diethion		< 10 (< 5 000)			
Dimafos		< 20 (< 5 000)			
Dimethoate		< 30 (< 1 000)			
Dioxathion		< 15 (< 5 000)			
Disulfoton		< 30 (< 5 000)			
Ethoprophos		< 20 (< 5 000)			
Fenchlorphos		< 20 (< 5 000)			
Fenitrothion		< 15 (< 5 000)			
Fenthion		< 30 (< 5 000)			
Fonofos		< 20 (< 5 000)			
Formothion		< 20 (< 5 000)			
Heptenophos		< 30 (< 5 000)			
SYNTHETIC PYRETHROIDES (µg / Kg)					
ND			ND		

## NOTES

Result of copper is over OAS average but stays very inferior to toxic level.

Laboratoire Contrôle AQ  
Le Responsable

2000/11/22

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	No. of samples planned per annum	No. of samples taken in year	PCV	No. Of samples in contravening PCV	% of samples in contravening PCV	Concentration or value (all samples)	Min.	Mean	Max
CONDUCTIVITY	US/cm @20	12	7	>1500	0	0.000	787.000	821.286	855.000	
ODOUR (QUAL)		12	7		0	0.000	0.000	0.000	0.000	
TASTE (QUAL)		12	7		0	0.000	0.000	0.000	0.000	
ALUMINIUM	Al ug/l	4	2	>200	0	0.000	< 14.600	< 14.600	< 14.600	
COLOUR	hazen	4	2	>20	0	0.000	1.100	1.415	1.730	
IRON	Fe ug/l	4	2	>200	0	0.000	11.000	15.000	19.000	
MANGANESE	Mn ug/l	4	2	>50	0	0.000	< 1.200	< 1.250	1.300	
AMMONIUM	NH4 mg/l	4	2	>0.5	0	0.000	< 0.025	< 0.025	< 0.025	
NITRITE	NO2 mg/l	4	2	>0.1	0	0.000	< 0.009	< 0.009	< 0.009	
NITRATE	NO3 mg/l	4	2	>50	0	0.000	1.600	1.650	1.700	
ODOUR (QUANT)	DN	4	2	>3	0	0.000	0.000	0.000	0.000	
HYDROGEN ION (pH)	pH units	4	2	9.5	0	0.000	7.370	7.375	7.380	
TASTE (QUANT)	DN	4	2	>3	0	0.000	0.000	0.000	0.000	
TEMP	deg C	4	2	>25	0	0.000	7.500	9.350	11.200	
TURBIDITY	FTU	4	2	>4	0	0.000	0.110	0.205	0.300	
2,4-DB	ug/l	4	2	>0.1	0	0.000	< 0.009	< 0.009	< 0.009	
236-TBA	ug/l	4	2	>0.1	0	0.000	< 0.012	< 0.012	< 0.012	
2,4,5-T	ug/l	4	2	>0.1	0	0.000	< 0.007	< 0.007	< 0.007	
2,4-D	ug/l	4	2	>0.1	0	0.000	< 0.010	< 0.010	< 0.010	
BENTAZONE	ug/l	4	2	>0.1	0	0.000	< 0.011	< 0.011	< 0.011	
BROMOXNYL	ug/l	4	2	>0.1	0	0.000	< 0.010	< 0.010	< 0.010	
CLOPYRALID	ug/l	4	2	>0.1	0	0.000	< 0.009	< 0.009	< 0.009	
DICAMBA	ug/l	4	2	>0.1	0	0.000	< 0.012	< 0.012	< 0.012	
DICHLOROPROP	ug/l	4	2	>0.1	0	0.000	< 0.008	< 0.008	< 0.008	
IOXNYL	ug/l	4	2	>0.1	0	0.000	< 0.009	< 0.009	< 0.009	
MCPA	ug/l	4	2	>0.1	0	0.000	< 0.008	< 0.008	< 0.008	
MCPB	ug/l	4	2	>0.1	0	0.000	< 0.008	< 0.008	< 0.008	
MCCOPROP (MCPp)	ug/l	4	2	>0.1	0	0.000	< 0.013	< 0.013	< 0.013	
COPPER	Cu ug/l	4	2	>0.1	0	0.000	< 0.009	< 0.009	< 0.009	
CHLOROTHALONIL	ug/l	4	2	>3000	0	0.000	33.000	39.500	46.000	
CHLORTHAL	ug/l	4	2	>0.1	0	0.000	< 0.010	< 0.010	< 0.010	
HEXACHLOROBENZENE	ug/l	4	2	>0.1	0	0.000	< 0.013	< 0.013	< 0.013	
HEPTACHLOR	ug/l	4	2	>0.1	0	0.000	< 0.010	< 0.010	< 0.010	
TECHNAZENE	ug/l	4	2	>0.1	0	0.000	< 0.017	< 0.017	< 0.017	
TRIFLURALIN	ug/l	4	2	>0.1	0	0.000	< 0.012	< 0.012	< 0.012	
INDENO	ug/l	4	2	>0.2	0	0.000	< 0.018	< 0.018	< 0.018	
BENZO-11,12-FLUORANTHENE	ug/l	4	2	>0.2	0	0.000	< 0.003	< 0.003	< 0.003	
BENZO-3,4'-FLUORANTHENE	ug/l	4	2	>0.2	0	0.000	0.001	< 0.001	< 0.001	
BENZO-3,4-PYRENE	ug/l	4	2	>10	0	0.000	< 0.003	< 0.003	< 0.003	
BENZO-GHI-PERYLENE	ug/l	4	2	>0.2	0	0.000	< 0.600	< 0.600	< 0.600	

FLUORANTHENE	ug/l	5	4	2	>0.2	0	0.000	0.003	0.004	0.004
PAH (TOTAL)	ug/l	S	4	2	>0.2	0	0.000	0.003	0.004	0.004
LEAD	Pb ug/l	S	4	2	>50	0	0.000	< 3.100	< 3.100	< 3.100
TETRACHLOROETHENE	ug/l	S	4	2	>10	0	0.000	< 0.180	< 0.270	< 0.360
TETRACHLOROMETHANE	ug/l	S	4	2	>3	0	0.000	< 0.050	< 0.090	< 0.130
TRICHLOROETHENE	ug/l	S	4	2	>30	0	0.000	< 0.250	< 0.400	< 0.550
TRICHLOROMETHANE	ug/l	S	4	2	>100	0	0.000	< 0.520	< 0.815	< 1.110
BROMODICHLOROMETHANE	ug/l	S	4	2	>100	0	0.000	4.520	4.530	4.540
BROMOFORM	ug/l	S	4	2	>100	0	0.000	14.500	14.905	15.310
CHLOROFORM	ug/l	S	4	2	>100	0	0.000	< 1.180	< 1.440	1.700
DIBROMOCHLOROMETHANE	ug/l	S	4	2	>100	0	0.000	12.400	12.730	13.060
TOTAL HALOFORMS	ug/l	S	4	2	>100	0	0.000	31.420	33.015	34.610
TOTAL PESTICIDE	ug/l	S	4	2	>0.5	0	0.000	0.000	0.000	0.000
ATRAZINE	ug/l	S	4	2	>0.1	0	0.000	< 0.016	< 0.016	< 0.016
PIRIMICARB	ug/l	S	4	2	>0.1	0	0.000	< 0.012	< 0.012	< 0.012
PROMETRYNE	ug/l	S	4	2	>0.1	0	0.000	< 0.017	< 0.017	< 0.017
PROPAZINE	ug/l	S	4	2	>0.1	0	0.000	< 0.010	< 0.010	< 0.010
SIMAZINE	ug/l	S	4	2	>0.1	0	0.000	< 0.012	< 0.012	< 0.012
TERBUTRYN	ug/l	S	4	2	>0.1	0	0.000	< 0.030	< 0.030	< 0.030
TERBUTHYLAZINE	ug/l	S	4	2	>0.1	0	0.000	< 0.015	< 0.015	< 0.015
TRIFLAZINE	ug/l	S	4	2	>0.1	0	0.000	< 0.019	< 0.019	< 0.019
CHLOROTOLURON	ug/l	S	4	2	>0.1	0	0.000	< 0.005	< 0.005	< 0.005
DIURON	ug/l	S	4	2	>0.1	0	0.000	< 0.006	< 0.006	< 0.006
ISOPROTURON	ug/l	S	4	2	>0.1	0	0.000	< 0.010	< 0.010	< 0.010
LINURON	ug/l	S	4	2	>0.1	0	0.000	< 0.003	< 0.003	< 0.003
METHIBENZURON (MET)	ug/l	S	4	2	>0.1	0	0.000	< 0.005	< 0.005	< 0.005
MONURON	ug/l	S	4	2	>0.1	0	0.000	< 0.009	< 0.009	< 0.009
TERBUTHIURON	ug/l	S	4	2	>5000	0	0.000	10.000	167.000	324.000
ZINC	Zn ug/l	S	4	2	>10	0	0.000	< 0.860	< 0.860	< 0.860
SILVER	Ag ug/l	S	1	1	>30	0	0.000	381.000	381.000	381.000
ALKALINITY	HCO3 mg/l	S	1	1	>50	0	0.000	< 0.400	< 0.400	< 0.400
ARSENIC	As ug/l	S	1	1	>2000	0	0.000	50.000	50.000	50.000
BORON	B ug/l	S	1	1	>1000	0	0.000	43.000	43.000	43.000
BARIUM	Ba ug/l	S	1	1	>250	0	0.000	138.000	138.000	138.000
CALCIUM	Ca mg/l	S	1	1	>5	0	0.000	< 0.340	< 0.340	< 0.340
CADMIUM	Cd ug/l	S	1	1	>400	0	0.000	57.000	57.000	57.000
CHLORIDE	Cl mg/l	S	1	1	>50	0	0.000	< 5.000	< 5.000	< 5.000
CYANIDE	CN ug/l	S	1	1	>50	0	0.000	< 1.600	< 1.600	< 1.600
CHROMIUM	Cr ug/l	S	1	1	>1500	0	0.000	292.000	292.000	292.000
FLOORIDE	F ug/l	S	1	1	>1	0	0.000	< 0.004	< 0.004	< 0

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 malathion



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

Phone (+45) 96 90 96 90  
Fax (+45) 96 90 96 91  
www.cheminova.com  
CVR-No. DK 1276 00 43

## BATCH ANALYTICAL CERTIFICATE

ARTICLE IDENTIFICATION				
Article Name: <b>Fyfanon Technical</b>		Reg. Dept. Code:		
Manufacturer: <b>Cheminova A/S</b>		Batch No.: <b>9010501</b>		
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 <input type="checkbox"/> ; Analytical <input type="checkbox"/> ; Liquid <input checked="" type="checkbox"/> ; Solid <input type="checkbox"/> ; Colour: <b>Pale yellowish</b>				
<u>Recommended Storage Conditions</u>				
Ambient temperature in the dark _____		<u>Expiry Date:</u>		
In refrigerator ..... <input checked="" type="checkbox"/> _____		The article is stable at least <u>1</u> year from date		
In deep freezer ..... _____		of analysis/last date of reanalysis when stored at:		
Additional Comments:		recommended conditions.		
ACTIVE INGREDIENT IDENTIFICATION				
Common Name/ISO-Name: <b>Malathion</b>		CAS-Name: <b>Butanedioic acid, ((dimethoxyphosphinothioyl)thio)-, diethyl ester</b>		
CAS No.:	<b>121-75-5</b>	Structural Formula:		
Empirical Formula:	<b>C<sub>10</sub>H<sub>19</sub>O<sub>6</sub>PS<sub>2</sub></b>			
Molecular Weight:	<b>330.4</b>			
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.: <b>96.0% w/w</b>				
Analytical Method: <b>VAM 001-01.</b>				
Analytical Report (incl. amendments): <b>TEM 010-02</b>				
Date of analysis/ reanalysis (yyymmdd)	<b>990223</b>	<b>000105</b>	<b>001206</b>	
-for article stored at -	<b>Cheminova A/S. Regist. storage, DK</b>	<b>Cheminova A/S</b>	<b>Cheminova A/S. Regist. storage, DK</b>	
GLP - COMPLIANCE				
The identification and determination of purity/content of active ingredient were performed at Cheminova 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, study plans, test articles, reference samples, and report are retained in the GLP archives of Cheminova A/S, Denmark.				
Date: <u>December 12, 2000</u>		Signature: <u>Barbara Hinz</u> Barbara Hinz		

ADDENDUM 4

Formulation Chemistry report

**MALATHION**  
**FORMULATION CHEMISTRY**

**Authors:**

M A Collard  
A D Clemson



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## INTRODUCTION

This report details the analytical procedure used and the results obtained for:

The determination of concentrations of malathion in formulations prepared for dosing to all groups on the first day of treatment and for the last day of treatment for one group.

## EXPERIMENTAL PROCEDURE

Malathion (Batch No. 9010501) was dissolved in corn oil for oral administration to the study animals. The homogeneity and re-suspendibility of malathion in corn oil had been assessed analytically in trial formulations, at concentrations of 1 and 250 mg/ml, prepared for the associated study (HLS Report No. CHV066/013331). The concentrations of malathion in corn oil were determined in all formulations prepared for dosing to all groups on the first day of treatment and for the last day of treatment for one group for the current study.

### ACHIEVED CONCENTRATIONS IN DOSE FORMULATIONS

Samples (1 ml) were taken for analysis from all dosage concentrations prepared for dosing to all groups on the first day of treatment and for the last day of treatment for one group.

### PROCEDURAL RECOVERIES

At each analytical occasion, procedural recoveries were prepared to cover the range of inclusion levels being examined and were analysed concurrently with dose formulations. Procedural recovery analysis was performed as a quality control measure and was used to accept or reject analysed batches of samples in accordance with the relevant Huntingdon Life Sciences Standard Operating Procedure. Analytical results were corrected for the appropriate recovery values.

## RESULTS

The mean concentrations of malathion in dose formulations analysed and the mean found concentrations as a percentage of nominal values, are summarised in Table 1. Individual analytical results are detailed in Table 2. Procedural recovery values are presented in Table 3.

The analytical method used is presented in Appendix 1.

Typical chromatograms of study samples are presented in Figures 1A - D.

## DISCUSSION AND CONCLUSION

The mean concentrations of malathion in formulations, prepared for dosing for the first and last days of treatment, ranged from 91.0 to 109 % of nominal concentrations and were considered satisfactory.

TABLE 1

Mean achieved concentrations of malathion in dose formulations

Occasion of dosing	Group	Intended concentration (mg/ml)	Found concentration (mg/ml)	Achieved concentration as % of intended
First day	control	0	ND	-
	2	1	1.01	101
	3	10	9.29	92.9
	4	30	27.3	91.0
	11	90	86.9	96.6
Last day	11	90	97.7	109

ND = Not detected

TABLE 2

## Achieved concentrations of malathion in dose formulations

Occasion of dosing	Group	Intended Concentration (mg/ml)	Found concentration (mg/ml)		Mean
First day	control	0	ND	ND	ND
	2	1	1.01	1.01	1.01
	3	10	9.29	9.28	9.29
	4	30	28.6	25.4	
			27.3	27.9	27.3
	11	90	82.0	91.7	86.9
Last day	11	90	99.1	96.3	97.7

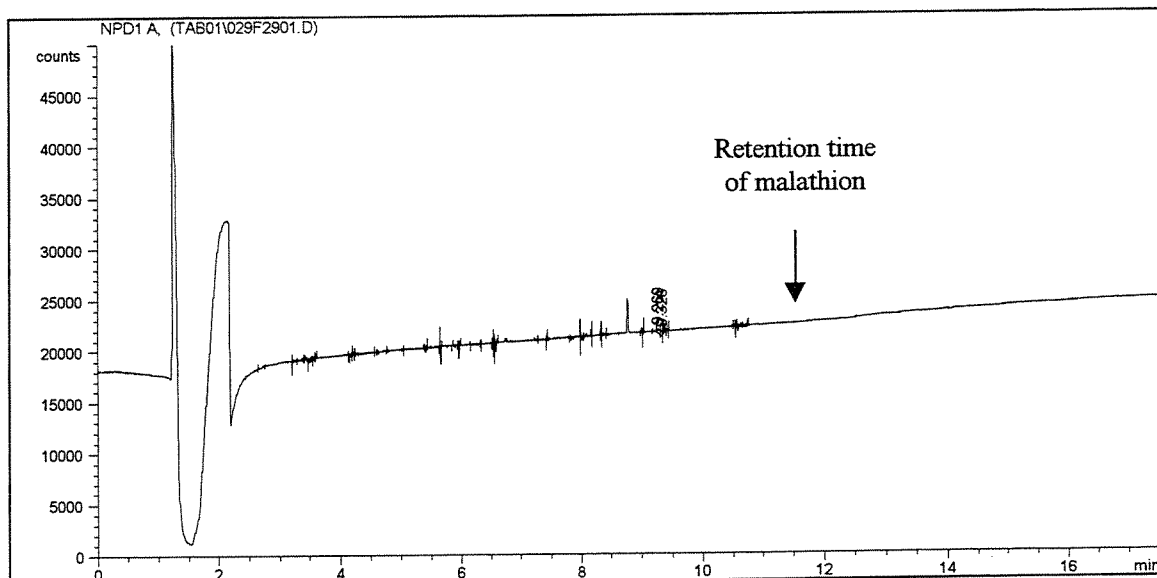
ND = Not detected

**TABLE 3****Procedural recovery data for malathion in corn oil formulation**

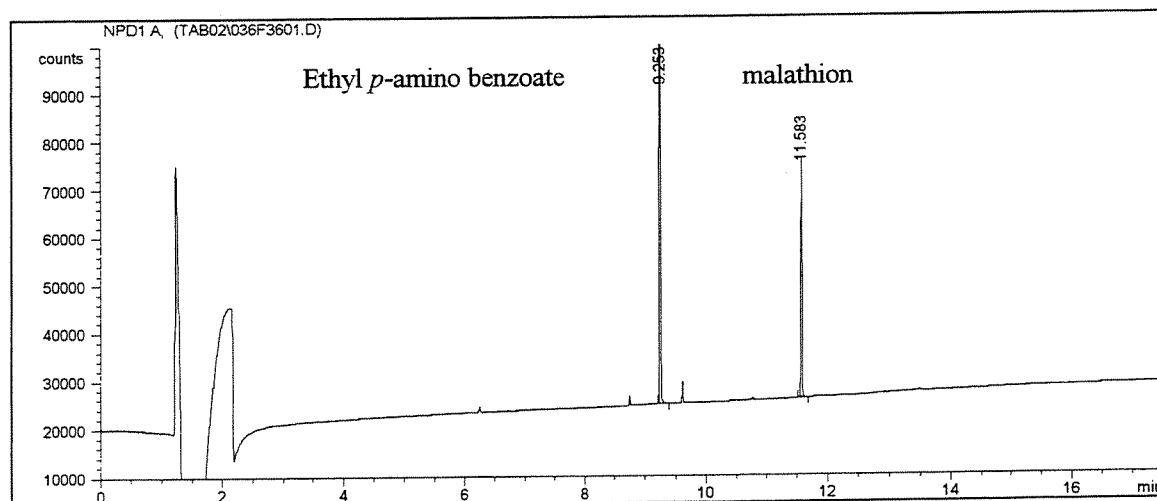
Occasion	Recovery (%) at nominal fortification (mg/ml)			
	1	10	30	90
1	90.6	102.6	105.4	-
2	-	-	-	99.0, 101.6

## FIGURES

**Figure 1A**  
Typical sample chromatogram - Group 1 control



**Figure 1B**  
Typical sample chromatogram - Group 2 1 mg/ml





## FIGURES (continued)

Figure 1C

Typical sample chromatogram - Group 4 30 mg/ml

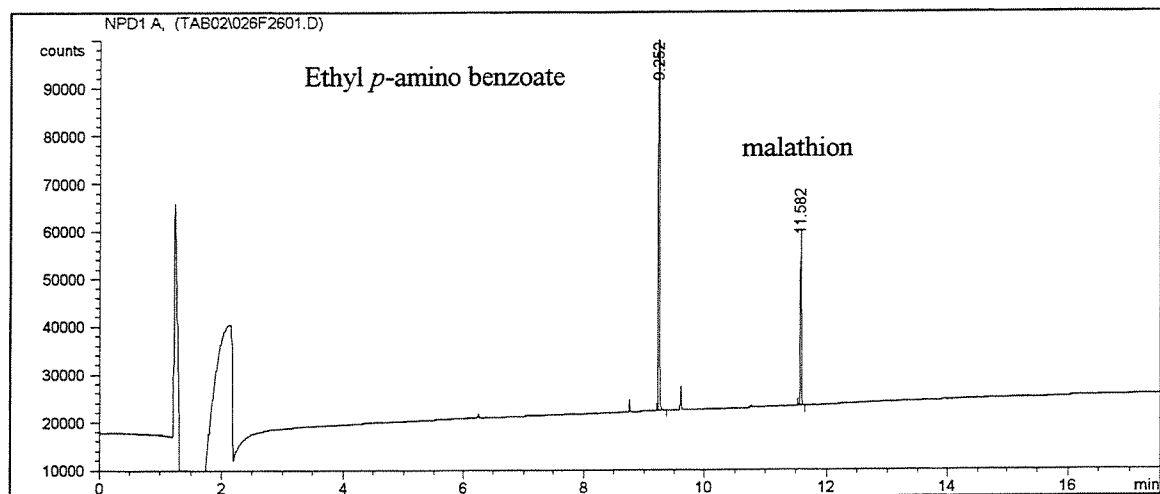
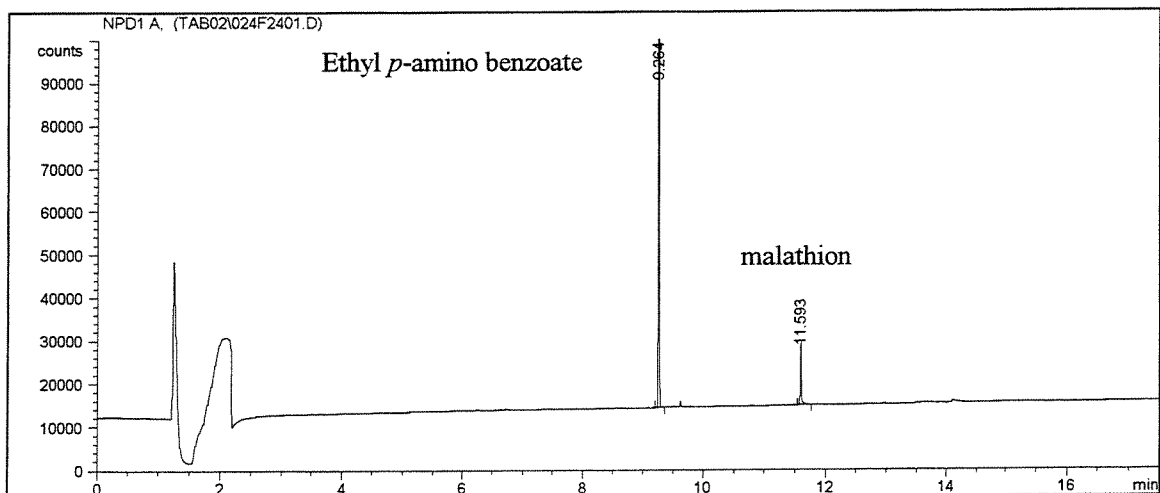


Figure 1D

Typical sample chromatogram - Group 11 90 mg/ml



**APPENDIX 1A****Analysis of malathion in corn oil formulations**

This method details the procedure used for the Gas Chromatography (GC) assay of malathion in corn oil formulations at concentrations in the range 1 mg/ml - 250 mg/ml.

**1. TEST SUBSTANCE**

Name:	Malathion
Supplier:	Sponsor
Appearance:	Clear, colourless liquid
Batch :	
Analytical:	Not supplied
Technical:	Same batch as used in the formulation
Storage:	
Test substance:	Refrigerator (+4°C)
Test formulations:	Freezer (-20°C)
Sample extracts:	Refrigerator (+4°C)
Hazard classification:	Huntingdon Life Sciences CLASS 2

**2. APPARATUS**

GC system:	As detailed in the chromatographic section.
Balances:	Analytical capable of weighing to 0.01 mg.
General laboratory apparatus.	

### 3. REAGENTS

Control vehicle:	Corn oil
Acetone:	Rathburn glass distilled grade
Ethyl <i>p</i> -amino benzoate:	Sigma reagent

### 4. SAMPLE PROCESS

The formulations were analysed as soon as possible after preparation. If it was not possible to analyse the samples on the day of receipt, they were stored in a freezer at  $-20^{\circ}\text{C}$ .

#### 4.1 Extraction

Each sample was quantitatively transferred to the volumetric flask, dissolved in a suitable volume of acetone with the aid of swirling and diluted (Table 1, Dilution A) to volume using acetone.

#### 4.2 Dilution

The extract was further diluted with acetone (Table 1, Dilution B) to a known volume incorporating internal standard in the final dilution (except for controls) to provide a solution containing the test substance in the range 200 - 800 ng/ml. The amount of internal standard added was adjusted such that the nominal concentration was 10  $\mu\text{g/ml}$ .

Table 1: Summary of sample preparation

Nominal  (mg/ml)	Sample volume  (ml)	Dilution				Dilution (ml/dose)	
		A*	B*				
0	1	25	1 ml	→	50 ml	1250	
1	1	25	1 ml	→	50 ml <sup>a</sup>	1250	
10	1	200	1 ml	→	100 ml <sup>b</sup>	20000	
30	1	200	1 ml	→	50 ml	Then, 2 ml → 10 ml <sup>c</sup>	50000
90	1	200	1 ml	→	50 ml	Then, 1 ml → 20 ml <sup>d</sup>	200000

\* acetone

<sup>a</sup> 1 ml 500  $\mu\text{g/ml}$  1° internal standard

<sup>b</sup> 2 ml 500  $\mu\text{g/ml}$  1° internal standard

<sup>c</sup> 2 ml 50  $\mu\text{g/ml}$  2° internal standard

<sup>d</sup> 4 ml 50  $\mu\text{g/ml}$  2° internal standard

## 5. STANDARD PREPARATION

### 5.1 Calibration

The test substance (about 50 mg) was weighed (to an accuracy of 0.01 mg) into a volumetric flask (50 ml). It was dissolved in acetone and diluted to volume (50 ml) using acetone to provide primary standard A (approximately 1000 µg/ml). Primary standard A (2.5 ml) was diluted to 50 ml with acetone to provide secondary standard A (approximately 50 µg/ml). Secondary standard A (2 ml) was diluted to 50 ml with acetone to provide tertiary standard A (approximately 2 µg/ml). A second set of standards was prepared as above to provide primary, secondary and tertiary standards B.

Ethyl *p*-amino benzoate (about 25 mg) was weighed (to an accuracy of 0.01 mg) into a volumetric flask (50 ml). It was dissolved in acetone and diluted to volume (50 ml) using acetone to provide primary internal standard (approximately 500 µg/ml). The primary internal standard (10 ml) was diluted to 100 ml in acetone to provide secondary internal standard (approximately 50 µg/ml).

The tertiary standards were sequentially diluted using acetone, to provide calibration standards for the nominal concentration range 200 ng/ml – 1000 ng/ml containing internal standard at a nominal concentration of 10 µg/ml as detailed in Table 2.

**Table 2: Summary of standard preparation**

Standard reference	Nominal concentration	Standard preparation			
1° standard A,	1000 µg/ml	Test substance,	approx 50 mg	→	50 ml <sup>#</sup>
1° standard B,	1000 µg/ml	Test substance,	approx 50 mg	→	50 ml <sup>#</sup>
2° standard A,	50 µg/ml	1° standard A,	2.5 ml	→	50 ml <sup>#</sup>
2° standard B,	50 µg/ml	1° standard B,	2.5 ml	→	50 ml <sup>#</sup>
3° standard A,	2 µg/ml	2° standard A,	2 ml	→	50 ml <sup>#</sup>
3° standard B,	2 µg/ml	2° standard B,	2 ml	→	50 ml <sup>#</sup>
Standard 1A,	1000 ng/ml	Dilute 3° standard A,	5 ml*	→	10 ml <sup>#</sup>
Standard 1B,	1000 ng/ml	Dilute 3° standard B,	5 ml*	→	10 ml <sup>#</sup>
Standard 2,	800 ng/ml	Dilute 3° standard A,	4 ml*	→	10 ml <sup>#</sup>
Standard 3,	600 ng/ml	Dilute 3° standard A,	3 ml*	→	10 ml <sup>#</sup>
Standard 4,	400 ng/ml	Dilute 3° standard A,	2 ml*	→	10 ml <sup>#</sup>
Standard 5,	200 ng/ml	Dilute 3° standard A,	1 ml*	→	10 ml <sup>#</sup>

Diluent: <sup>#</sup> acetone

\* 2 ml of 50 µg/ml 2° internal standard was also added to give a final nominal internal standard concentration of 10 µg/ml.

Standards 1A and 1B were injected, in duplicate, to assess standard reproducibility and each analytical run calibrated by injecting, at the beginning of the run, the five point calibration prepared from standard A and a reagent blank. Standard 3 was injected, as a running standard, after a maximum of every four sample injections.

## 5.2 Procedural Recoveries

Procedural recovery analysis was performed with the analysis of study samples as a quality control measure and used to accept or reject analysed batches/runs of study samples in accordance with the relevant Huntingdon Life Sciences Standard Operating Procedure.

Control corn oil (1 ml) was fortified by adding the test substance as detailed in Table 3, either as a solution in acetone (inclusion levels <20 mg/ml) or as the neat liquid test substance (inclusion levels  $\geq 20$  mg/ml). The prepared procedural recoveries were analysed in accordance with the sample extraction procedure.

At each analytical occasion, procedural recoveries were prepared at each inclusion level being examined and analysed concurrently with test formulations.

**Table 3: Summary of procedural recovery fortification**

Nominal inclusion (mg/ml)	Fortification	
1	1 ml primary standard A	+ 1 ml control
10	10 ml primary standard A	+ 1 ml control
30	30 mg test substance	+ 1 ml control
90	90 mg test substance	+ 1 ml control

## 6. TYPICAL CHROMATOGRAPHIC CONDITIONS

### 6.1 Instrumentation

GC system: HP 5890 with NPD and HP ChemStation  
 Analytical column: HP-5 MS (30 m × 0.25 mm id) film thickness 0.25 µm

### 6.2 Operating conditions

Column temperature: Initial: 50°C, held for 1 min  
 Rate: 20°C/min  
 Final: 280°C, held for 5 min

Carrier gas: Helium: 2.0 ml/min at 50°C

Injector temperature: 220°C

Injector volume: 1 µl

Injection mode: Splitless

Septum purge: 3.0 ml/min

Split vent purge: 100 ml/min

Detector: Nitrogen Phosphorus (NPD)

Temperature: 275°C  
 Gas flows: Hydrogen: 3.3 ml/min  
 Air: 114 ml/min  
 Auxiliary: 21 ml/min (helium)

Retention times:  
 malathion: Approx 11.6 min  
 Ethyl *p*-amino benzoate: Approx 9.3 min

Slight variations of the gas flow rates may have been carried out on occasion in order to provide a suitable retention time for the test substance and to optimise resolution. Standard 1A was injected in triplicate at the start of the run, the areas of the second and third injections had usually to be within 5% for the analysis to be accepted.

## 7. CALCULATION

From the calculated peak area ratio response of the test compound and internal standard in each calibration standard chromatogram, calibration curves were constructed by linear regression of standard response versus standard concentration to demonstrate linearity. The concentration of the test compound in each sample and procedural recovery was calculated, from the area ratio response observed at the characteristic retention times for the test compound and internal standard, by reference to the bracketing calibration standards. The calculations were based upon the following equation.

$$\text{Analysed concentration, mg/g} = Y \times \frac{C_c}{R_c} \times P \times \frac{V}{W} \times 10^{-3}$$

Procedural recovery values were determined using the following equation:

$$\text{Procedural recovery (\%)} = \frac{\text{Analysed concentration, mg/ml}}{\text{Fortified concentration, mg/ml}} \times 100$$

Concentration of test samples were corrected for density and procedural recovery values using the following equation:

$$\text{Corrected concentration, mg/ml} = \text{Analysed concentration, mg/g} \times D \times \frac{100}{R}$$

Where

- Y = Calculated peak area ratio response for the test substance and internal standard in test chromatogram
- C<sub>c</sub> = Concentration (µg/ml) of the single point bracketing calibration standard
- R<sub>c</sub> = Mean peak area ratio response of bracketing calibration standards
- V = Dilution volume of sample (ml)
- W = Weight of sample (g)
- D = Density (g/ml), a value of 1 was used if all formulation densities were within the range 0.99 - 1.01 g/ml
- R = Procedural recovery value at analysis (%)
- P = Purity factor for bracketing calibration standard (= % purity / 100), if applicable

ADDENDUM 5

Data for Group 5 untreated dams and litters providing offspring for PND 11 investigations



## ADDENDUM 5 - continued

Bodyweight – individual values (g) after mating

Animal number	GD						
	0	3	6	10	14	17	20
77	255	273	291	327	354	396	445
78	245	275	291	327	353	401	443
79	236	273	283	306	334	378	426
80	234	260	268	286	307	342	384
81	240	269	288	317	344	388	438
83	229	252	258	291	317	360	402
84	214	244	253	281	303	345	381

Bodyweight – individual values (g) during lactation

Animal number	PND			
	1	4	7	11@
77	346	360	368	370
78	333	356	361	351
79	322	340	356	355
80	295	306	321	332
81	341	361	373	373
1082	‡	374	395	380
83	309	323	331	327
84	293	298	310	297

@ Bodyweight recorded at necropsy

Gestation length – individual values

Female number	Gestation length (days)
77	22
78	22
79	22
80	22
81	22
83	22.5
84	22

‡ Dam number 1082 was a replacement for number 82; no data was recorded for Dam number 1082 prior to PND 4

## ADDENDUM 5 – continued

## Litter size up to PND 11– individual values

Animal number	Implants	Total litter size PND 1	Live litter size on				
			Before cull		After cull		
			PND 1	PND 4	PND 4	PND 7	PND 11
77	16	15	15	15	10	10	10
78	18	17	16	16	10	10	10
79	17	15	15	15	10	10	10
80	15	14	14	14	10	10	10
81	17	17	17	17	10	10	10
1082	15	‡	‡	12	10	10	10
83	16	15	15	15	10	10	10
84	14	13	13	13	10	10	10

## Offspring survival indices up to PND 11– individual values

Animal number	Post implantation survival index (%)	Live birth index (%)	Viability index (%)	Lactation index (%)	
				PND 7	PND 11
77	94	100	100	100	100
78	94	94	100	100	100
79	88	100	100	100	100
80	93	100	100	100	100
81	100	100	100	100	100
1082	‡	‡	‡	100	100
83	94	100	100	100	100
84	93	100	100	100	100

## Sex ratio up to PND 11 – individual litter values

Animal number	Total on			Live (before cull)									Live (after cull)								
	PND 1			PND 1			PND 4			PND 4			PND 7			PND 11					
	M	F	%M	M	F	%M	M	F	%M	M	F	%M	M	F	%M	M	F	%M	M	F	%M
77	7	8	46.7	7	8	46.7	7	8	46.7	5	5	50.0	5	5	50.0	5	5	50.0	5	5	50.0
78	8	9	47.1	7	9	43.8	7	9	43.8	5	5	50.0	5	5	50.0	5	5	50.0	5	5	50.0
79	5	10	33.3	5	10	33.3	5	10	33.3	5	5	50.0	5	5	50.0	5	5	50.0	5	5	50.0
80	6	8	42.9	6	8	42.9	6	8	42.9	5	5	50.0	5	5	50.0	5	5	50.0	5	5	50.0
81	8	9	47.1	8	9	47.1	8	9	47.1	5	5	50.0	5	5	50.0	5	5	50.0	5	5	50.0
1082	‡	‡	‡	‡	‡	‡	7	5	58.3	5	5	50.0	5	5	50.0	5	5	50.0	5	5	50.0
83	8	7	53.3	8	7	53.3	8	7	53.3	5	5	50.0	5	5	50.0	5	5	50.0	5	5	50.0
84	6	7	46.2	6	7	46.2	6	7	46.2	5	5	50.0	5	5	50.0	5	5	50.0	5	5	50.0

‡ Dam number 1082 was a replacement for number 82; no data was recorded for Dam number 1082 prior to PND 4

## ADDENDUM 5 – continued

## Bodyweight – individual litter values (g) for male offspring

Animal number	Before Cull				After Cull					
	PND 1		PND 4		PND 4		PND 7		PND 11	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
77	6.1	0.4	8.2	0.6	8.1	0.6	14.0	1.1	22.6	1.8
78	6.5	0.2	9.6	0.2	9.6	0.3	15.5	0.4	24.1	0.7
79	6.7	0.2	9.1	0.6	9.1	0.6	15.0	1.1	25.0	1.5
80	6.0	0.8	8.8	1.2	8.6	1.2	13.4	2.0	23.0	3.1
81	6.5	0.4	9.6	0.8	9.5	1.0	15.4	1.3	25.6	1.4
1082	‡	‡	‡	‡	10.0	0.7	15.6	1.1	26.0	1.9
83	6.9	0.8	9.8	1.0	9.4	1.0	15.9	1.6	26.9	2.1
84	6.4	0.1	9.9	0.3	9.9	0.3	17.1	0.5	26.9	1.3

## Bodyweight – individual litter values (g) for female offspring

Animal number	Before Cull				After Cull					
	PND 1		PND 4		PND 4		PND 7		PND 11	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
77	5.7	0.3	7.6	0.8	7.4	0.8	12.6	1.4	21.2	1.8
78	6.0	0.3	8.6	0.7	8.7	0.9	14.0	1.3	21.4	1.4
79	6.1	0.2	9.1	0.4	9.0	0.4	14.9	0.9	24.3	1.1
80	5.7	0.2	8.4	0.5	8.3	0.6	13.4	1.5	22.0	2.2
81	6.1	0.2	9.1	0.6	9.1	0.7	15.4	1.2	25.5	1.9
1082	‡	‡	‡	‡	9.6	0.6	15.1	1.2	25.4	1.6
83	6.5	0.7	9.0	1.0	9.3	0.9	16.6	1.1	27.6	1.5
84	6.0	0.4	9.2	0.5	9.2	0.6	15.4	1.1	25.0	1.9

‡ Dam number 1082 was a replacement for number 82; no bodyweight data was recorded for Dam number 1082 prior to cull on PND 4

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Life Sciences**

**PROTOCOL**  
**MALATHION**  
**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

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*Huntingdon Life Sciences Ltd, registered in England: 1815730*

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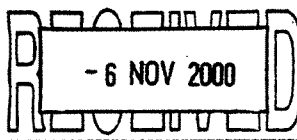
**CONTACT DETAILS**

Study Monitoring : M. Jensen  
Cheminova A/S  
Lemvig  
DENMARK

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**PROTOCOL APPROVAL**

**MALATHION**

**EFFECTS ON CHOLINESTERASE**

**IN THE CD RAT (ADULT AND JUVENILE) BY ORAL**

**GAVAGE ADMINISTRATION**

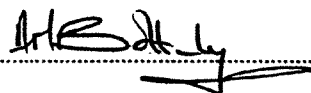
  
.....

S.M. Fulcher, B.A., F.I.A.T.  
Study Director,  
Huntingdon Life Sciences Ltd.

27 October 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.

  
.....

A.M. Bottomley, B.Sc., Dip.R.C.Path(Tox).  
Management,  
Huntingdon Life Sciences Ltd.

27 October 2000

Date

  
.....

M. Jensen  
Sponsor,  
Cherninova A/S

3/11-2000

Date

*Please sign both copies of this page, retain one for your records and return one to the Study Director  
at Huntingdon Life Sciences.*

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Life Sciences**

**MALATHION**  
**EFFECTS ON CHOLINESTERASE**  
**IN THE CD RAT (ADULT AND JUVENILE) BY ORAL**  
**GAVAGE ADMINISTRATION**

Enquiry Number: 21594C

Number of pages for internal distribution: 20

This working document is approved for circulation and use:

Stiller  
Study Director

27 October 2008  
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 : Huntingdon Research Centre, Huntingdon, Cambridgeshire, PE28 4HS.

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# Huntingdon Life Sciences

## 1. INTRODUCTION

### Management of study

Study Director : S.M. Fulcher

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.

: D.P. Myers

### 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 Malathion 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.

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### 2.2. Scheduled time plan

(to be up-dated as required in an amendment to protocol)

Sample of Malathion arrived	:	23 May 2000	
Pairing to commence	:	2 November 2000	
Draft report to be issued	:	May 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:

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---

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# Huntingdon Life Sciences

## 3.1. Test substance

Sponsor's identification	:	Malathion
Storage conditions	:	At 4°C, (Analytical standard 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	:	Malathion; low mg/ml.
Test group	:	Malathion; intermediate mg/ml.
Test group	:	Malathion; 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	:	Corn oil.
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.

Four samples (nominally 1 ml accurately weighed) from all groups; 2 assays from each group. The remainder will be retained frozen (nominally -20°C) as contingency for analysis if any result requires confirmation.

## 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.

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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 (F<sub>0</sub> generation)

Male/female ratio : 1:1 with stock males.  
Daily checks for evidence of mating : Ejected copulation plugs in cage tray and vaginal smear.  
Day 0 of gestation : When positive evidence of mating detected.

### 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.

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## 4.1.5. Identification

Numbering	:	Unique for each animal within study - from Day 28 of age.
Method	:	Tail tattoo (F <sub>0</sub> generation and, selected F <sub>1</sub> generation at 4 weeks of age). Toe marking (F <sub>1</sub> generation) on Day 1 of age within each litter. Tail marking with pen (F <sub>1</sub> ) 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.
------------------------------	---	-----------------

## 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.

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## 4.2.2. Animal accommodation

Study period	Number of animals/cage		Cage material	Cage flooring
	Male	Female		
<b>Generation F<sub>0</sub></b>				
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
<b>Generation F<sub>1</sub></b>				
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.

## 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.



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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.

### 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.

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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 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 1-4) : Once daily at approximately the same time each day, from Day 6 after mating until 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.

Suspensions are stirred using a magnetic stirrer before and throughout the dosing procedure.

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## 4.3.2. Clinical observations (F<sub>0</sub> 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 F <sub>1</sub> 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.

## 4.3.3. Mortality

Debilitated animals	:	Observed carefully.
Premature sacrifice	:	Animals may be killed on humane grounds or if considered <i>in extremis</i> .
Animals found dead, killed <i>in extremis</i> 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.

## 4.3.4. Bodyweight

F <sub>0</sub> females (Groups 1-5)	:	After mating: Days 0, 3, 6, 10, 14, 17 and 20, then daily until parturition. During lactation: Days 1, 4, 7, 11, 14, 17 and 21.
F <sub>1</sub> offspring	:	Days 1, 4, 7, 11, 14, 17, 21 and 28 of age, then weekly until termination

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(Groups 1-5) and on Day 60 of age (where appropriate)\*.

Adult males and females (Groups 6-9) : On the day before treatment commences and then daily to termination.

\* Dosed offspring will be weighed daily during their dosing period to allow daily adjustment of the dose volume given based on bodyweight.

### 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 ≥ 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 <sub>0</sub> females failing to produce viable litter	:	Day 25 after mating.
F <sub>0</sub> females with litters	:	After recording of bodyweight on Day 21 of lactation or on day of litter loss.
Unallocated F <sub>1</sub> 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. Decapitation for offspring selected for assessment of blood and brain cholinesterase activity.
- F<sub>1</sub> animals at Day 11 of age : Decapitation.
- Unallocated F<sub>1</sub> 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.

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## 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.



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## 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 F<sub>1</sub> 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.

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## 8.2. Archiving

All raw data, samples and specimens arising from the performance of this study will remain the property of the Sponsor.

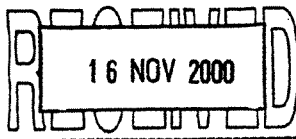
Types of sample and specimen which are unsuitable, by reason of instability, for long term retention and archiving may be disposed of after the periods stated in Huntingdon Life Sciences Standard Operating Procedures.

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.

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**MALATHION**  
**EFFECTS ON CHOLINESTERASE**  
**IN THE CD RAT (ADULT AND JUVENILE) BY ORAL**  
**GAVAGE ADMINISTRATION**

**Total number of pages: 10**

**Number of pages for internal distribution: 10**

**Study Director : S.M. Fulcher, B.A., F.I.A.T.**

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: *S. Fulcher* Date: 9 November 2000  
(Study Director)

**For the Sponsor**

Approved by: *Nick K. Gurney* Date: 15 Nov. 2000

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**MALATHION**  
**EFFECTS ON CHOLINESTERASE**  
**IN THE CD RAT (ADULT AND JUVENILE) BY ORAL**  
**GAVAGE ADMINISTRATION**

Reason for amendment :

- : Section 2.1: Addition of dosages and additional dosage groups
- : Section 3.2: Addition of concentration for dose formulations and additional dosage groups.
- : Section 4.1.1: Addition of details for Group 10 and 11. Clarification that the sibling relationship of animals ordered for 6-9 is known.
- : Section 4.1.4: Correction to allocation methodology to ensure litter mates are spread throughout groups. Addition of allocation methodology for Groups 10-11.
- : Section 4.3.1: Addition of frequency of dosing for Groups 10-11.
- : Section 4.3.4: Addition of weighing schedule for Groups 10-11. Clarification of weighing schedule for offspring (Groups 1-5).
- : Section 4.3.6: Increase in size of Group 5 litters to allow investigation of additional dosage.
- : Section 4.3.7: Addition of Groups 10-11
- : Section 5.1: Addition of Groups 10-11. Addition of details for Group 5 dams.

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#### Amendments

## 2. STUDY SCHEDULE AND STRUCTURE

### 2.1 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	<u>Malathion</u>	<u>5</u>	19F	20-28 A 29-38 B
3	<u>Malathion</u>	<u>50</u>	19F	39-47 A 48-57 B
4	<u>Malathion</u>	<u>150</u>	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	<u>Malathion</u>	<u>5</u>	16F 16M	101-108 C, 109-116 D 217-224 C, 225-232 D
8	<u>Malathion</u>	<u>50</u>	16F 16M	117-124 C, 125-132 D 233-240 C, 241-248 D
9	<u>Malathion</u>	<u>150</u>	16F 16M	133-140 C, 141-148 D 249-256 C, 257-264 D
10	<u>Control</u>	=	8F 8M	265-272 281-288
11	<u>Malathion</u>	<u>450</u>	8F 8M	273-280 289-296

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.

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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 5 mg/kg/day  
Y 50 mg/kg/day  
Z 150 mg/kg/day  
450 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.

### 3. TEST SUBSTANCE AND FORMULATION

#### 3.2 Formulation

##### Treatment

Control ( <u>Groups 1, 6 and 10</u> )	:	Vehicle.
<del>Test group</del> <u>Groups 2 + 7</u>	:	Malathion; <del>low</del> 1.0mg/ml.
<del>Test group</del> Groups 3 + 8	:	Malathion; <del>intermediate</del> 10mg/ml.
<del>Test group</del> Groups 4 + 9	:	Malathion; <del>high</del> 30mg/ml.
<u>Group 11</u>	:	<u>Malathion 90mg/ml</u>
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	:	Corn oil.
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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#### 4. ANIMAL MANAGEMENT

##### 4.1 Animals - supply, acclimatisation and allocation

##### 4.1.1 Animals

Species	:	Rat.
Strain	:	Ctrl: CD*BR.
Age ordered	:	Groups 1 to 5: Females 9-10 weeks of age. Groups 6 to 9: Males and females ( <u>litter mates identified</u> ) 5-6 weeks of age. <u>Groups 10 to 11</u> <u>Males and females 5-6 weeks of age</u>
Weight range ordered	:	Groups 1 to 5: Females 200-220 g. Groups 6 to 9: Males 120-175 g, females 100-150 g. <u>Group 10 to 11</u> <u>Males 120-175 g, females 100-150 g</u>
Supplier	:	Males and females: Charles River (UK). Males for mating of Groups 1-5: Stock animals from same source, retained at Huntingdon Life Sciences.

##### 4.1.4. 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.

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### 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.~~  
By sex. After exclusion of animals showing signs of ill-health allocated so as to ensure not more than 1 offspring from each litter is present in each group. Animals at the extremes of the bodyweight range or litters showing large variation in individual weights will not be selected if alternatives are available. Record of littermate relationships will be retained.
- Cage distribution : Arrangement designed to minimise environmental variables.

### Males and Females (Groups 10-11):

- 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.

## 4.3 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.



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- Individual dose volume - offspring : Calculated from the most recently recorded 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 males and females (Groups 10-11) : On one occasion.
- Frequency - adult females (Group 1-4) : Once daily at approximately the same time each day, from Day 6 after mating until 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.

Suspensions are stirred using a magnetic stirrer before and throughout the dosing procedure.

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#### 4.3.4 Bodyweight

F<sub>0</sub> 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.

F<sub>1</sub> offspring : Days 1, 4, 7, ~~11, 14, 17, 21~~ 11-21 and 28 of age, then weekly until  
(Groups 1-5) termination and on Day 60 of age (where appropriate)\*.

Adult males and females : On the day before treatment commences and then daily to termination.  
(Groups 6-9)

Adult males and females : On the day before treatment and the day of treatment.  
(Groups 10-11)

\* Dosed offspring ~~will be~~ are weighed daily during their dosing period to allow daily adjustment of the dose volume given based on bodyweight. Weighing of any non-dosed offspring prior to weaning will only be conducted on Days 1, 4, 7, 11, 14, 17 and 21 of age.

#### 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 – Group 1-4  
Litters culled to 10 (5M + 5F where possible) on Day 4 of age – Group 5

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 6-9-11	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 ≥ 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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Life Sciences**

All samples will be examined for the following characteristics:

Using lithium heparin as anticoagulant

Plasma pseudo cholinesterase  
Erythrocyte acetyl cholinesterase.

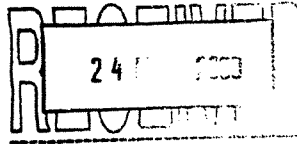
For assessment of brain acetyl cholinesterase activity: brains will be taken (after decapitation for Day 4 and Day 11 pups) and weighed at necropsy and brain cholinesterase activity will be measured. For the Day 20 fetuses, the brains for all fetuses in the litter will be pooled for analysis.

## 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 <sub>0</sub> females failing to produce viable litter	:	Day 25 after mating.
F <sub>0</sub> females with litters	:	After recording of bodyweight on Day 21 of lactation or on day of litter loss.
Unallocated F <sub>1</sub> offspring	:	After Day 21 or Day 60 of age (Groups 1-4).
Selected offspring (Group 5)	:	Day 11 of age.
<u>Adult males and females (Groups 10-11)</u>	:	<u>On Day 1 of treatment after dosing.</u>
<u>Adult females (Group 5)</u>	:	<u>On Day 11 of lactation.</u>

Study Number : CHV067  
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**MALATHION**  
**EFFECTS ON CHOLINESTERASE**  
**IN THE CD RAT (ADULT AND JUVENILE) BY ORAL**  
**GAVAGE ADMINISTRATION**

Total number of pages: 2

Number of pages for internal distribution: 2

Study Director : S.M. Fulcher, B.A., F.I.A.T.

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: Stuher Date: 20 November 2000  
(Study Director)

**For the Sponsor**

Approved by: Nelle K. Lunn Date: 23. nov. 2000

Study Number : CHV067  
 Protocol Amendment Number : 2

**Huntingdon  
Life Sciences**

**MALATHION**  
**EFFECTS ON CHOLINESTERASE**  
**IN THE CD RAT (ADULT AND JUVENILE) BY ORAL**  
**GAVAGE ADMINISTRATION**

Reason for amendment : Section 4.1.6: Precautionary retention of 4 mated females as possible replacements for Group 5 dams/litters.

**Amendments**

**4. ANIMAL MANAGEMENT**

**4.1 Animals - supply, acclimatisation and allocation**

**4.1.6. Precommencement animal replacement**

Spare animals will be ordered to replace any individuals rejected before the start of treatment.

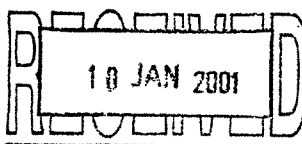
Replacement before treatment : Ill-health.  
 Bodyweight range extremes.  
 Inadequate mating performance. #

Replacement during treatment : None scheduled.

# : 4 spare time-mated females will be retained until Group 5 litters have been assessed for adequate size and sex ratio. Group 5 females failing to litter or with inadequate numbers of male and females offspring will be replaced using these animals.

Study Number : CHV067  
Protocol Amendment Number : 3

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**MALATHION**  
**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** : S.M. Fulcher, B.A., F.I.A.T.

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: S. Fulcher Date: 1 December 2000  
(Study Director)

**For the Sponsor**

Approved by: Nelle K. Lumsden Date: 8/1 - 2001

Study Number : CHV067  
Protocol Amendment Number : 3

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**Life Sciences**

**MALATHION**  
**EFFECTS ON CHOLINESTERASE**  
**IN THE CD RAT (ADULT AND JUVENILE) BY ORAL**  
**GAVAGE ADMINISTRATION**

Reason for amendment : Section 2.1: Replacement of Litter 82 – proportion of  
offspring insufficient to meet the requirements of Group 5  
: Section 3.3: Modification to schedule of sampling  
formulations (to accommodate 450 mg/kg/day)



Study Number : CHV067  
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#### Amendments

## 2. STUDY SCHEDULE AND STRUCTURE

### 2.1 Duration of treatment

Group	Treatment	Level (mg/kg/day)	Number of animal/sex	Animal numbers
1	Control	-	19F	1-9 A 10-19 B
2	Malathion	5	19F	20-28 A 29-38 B
3	Malathion	50	19F	39-47 A 48-57 B
4	Malathion	150	19F	58-66 A 67-76 B
5	Untreated	-	8F	<del>77-84 77-81, 83-84</del> <del>1082@</del>
6	Control	-	16F 16M	85-92 C, 93-100 D 201-208 C, 209-216 D
7	Malathion	5	16F 16M	101-108 C, 109-116 D 217-224 C, 225-232 D
8	Malathion	50	16F 16M	117-124 C, 125-132 D 233-240 C, 241-248 D
9	Malathion	150	16F 16M	133-140 C, 141-148 D 249-256 C, 257-264 D
10	Control	-	<del>8F</del> <del>8M</del>	265-272 281-288
11	Malathion	450	8F 8M	273-280 289-296

@ Litter 82 replaced with spare litter as it was unable to fulfil neonate requirements for Group 5. Spare replacement litter given study number 1082.

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.

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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.

### 3. TEST SUBSTANCE AND FORMULATION

#### 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.

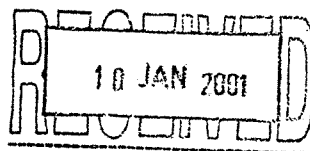
#### Achieved concentration

Sampling and determination : On two occasions. Formulations prepared for use during the first week of treatment and again during ~~the first week of lactation.~~ last week of treatment at each dosage.  
 Other sampling regimens may be specified by the Sponsor.

Four samples (nominally 1 ml accurately weighed) from all groups; 2 assays from each group. The remainder will be retained frozen (nominally -20°C) as contingency for analysis if any result requires confirmation.

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**MALATHION**  
**EFFECTS ON CHOLINESTERASE**  
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**GAVAGE ADMINISTRATION**

**Total number of pages: 3**

**Number of pages for internal distribution: 3**

**Study Director : S.M. Fulcher, B.A., F.I.A.T.**

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**FOURTH AMENDMENT APPROVAL**

**For Huntingdon Life Sciences Ltd**

Authorised by: SKL Date: 15 December 2000  
(Study Director)

**For the Sponsor**

Approved by: Nelle K. Lumb Date: 8/1-2001

Study Number : CHV067  
Protocol Amendment Number : 4

**Huntingdon**  
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**MALATHION**  
**EFFECTS ON CHOLINESTERASE**  
**IN THE CD RAT (ADULT AND JUVENILE) BY ORAL**  
**GAVAGE ADMINISTRATION**

Reason for amendment : Section 4.3.4:Clarification of bodyweight schedule.

Study Number : CHV067  
 Protocol Amendment Number : 4

**Huntingdon  
 Life Sciences**

#### Amendments

#### 4. ANIMAL MANAGEMENT

##### 4.3. Animals – procedures

##### 4.3.4 Bodyweight

F<sub>0</sub> 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.  
 (Groups 1-4)

Group 5 : After mating: Days 0, 3, 6, 10, 14, 17, and 20, then daily until parturition  
During lactation: Days 1, 4, 7 and 11.

F<sub>1</sub> offspring : Days 1, 4, 7, 11-21 and 28 of age, then weekly until termination and on  
 (Groups 1-5) Day 60 of age (where appropriate)\*.  
 (Groups 1-4)

Group 5 : Days 1, 4, 7 and 11 of age.

Adult males and females : On the day before treatment commences and then daily to termination.  
 (Groups 6-9)

Adult males and females : On the day before treatment and the day of treatment.  
 (Groups 10-11)

\* Dosed offspring are weighed daily during their dosing period to allow daily adjustment of the dose volume given based on bodyweight. Weighing of any non-dosed offspring prior to weaning will only be conducted on Days 1, 4, 7, 11, 14, 17 and 21 of age.



**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**

**TEST TYPE**

**Huntingdon Life Sciences  
Eye Research Centre  
Eye  
Suffolk  
IP23 7PX**

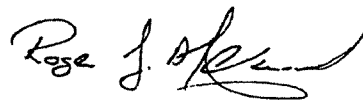
**Analytical Chemistry  
Clinical Chemistry  
Ecosystems  
Environmental Fate  
Environmental Toxicity  
Mutagenicity  
Phys/Chem Testing  
Toxicology**

**DATE OF INSPECTION**

**29<sup>th</sup> 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.

  
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**

**TEST TYPE**

**Huntingdon Life Sciences  
Huntingdon Research Centre  
Wooley Road  
Alconbury  
Huntingdon  
Cambs.  
PE28 4HS**

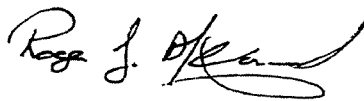
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3/4/01

Dr. Roger G. Alexander  
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