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elf atochem

ELF ATOCHEM NORTH AMERICA, INC.
900 First Avenue, P.O. Box 1536
King of Prussia, PA 19406-0018

Tel: 215-337-6500

8EHQ-92-12586

8892001070

INIT

October 20, 1992

CERTIFIED MAIL

RETURN RECEIPT REQUESTED

Document Processing Center (TS-790)
Office of Toxic Substances
U.S. Environmental Protection Agency
401 M St., S.W.
Washington, D.C. 20460

Attn: Section 8(e) Coordinator (CAP Agreement)

RE: Report Submitted Pursuant to the TSCA Section 8(e) Compliance Audit Program

CAP Identification Number: 8ECAP-0026

Dear Sir/Madam:

Elf Atochem North America Inc. (Elf Atochem) is submitting the attached studies to the Environmental Protection Agency (EPA) pursuant to Toxic Substances Control Act (TSCA) Section 8(e). These studies do not involve effects in humans.

These studies are responsive to EPA/FIFRA registration requirements. The data has been developed by an industrial consortium of which Elf Atochem is a member. The consortium contract provides for each member to maintain the confidentiality of reports consistent with Sections 3 and 10 of FIFRA regulations in order to preclude their use by non-members for registration purposes without data compensation. We have enclosed a copy of these study reports, but consider the study reports confidential and subject to the Data Compensation conditions pursuant to FIFA Sections 3 and 10. The studies will be submitted to FIFRA by the consortium.

The enclosed studies provide information on Ziram Technical (zinc dimethyldithiocarbamate). Its exact chemical name is zinc, bis(dimethylcarbamodithiato-S,S')- and its CAS number is 137-30-4.

2/9/95

The title of the enclosed study reports are The Acute Toxicity of Ziram Technical to Bluegill Sunfish, The Acute Toxicity of Ziram Technical to Daphnia Magna, A Study of the Effect of Ziram on Pregnancy of the Rat, Ziram Preliminary Dietary Toxicity Study in Beagle Dogs, 13-Week Dietary Toxicity Study in Beagle Dogs, and Ziram: 52-Week Dietary Toxicity Study in Beagle Dogs (ZIR/10-G). The following is a summary of the adverse effects observed in these studies.

In the rat teratology study, signs of maternal toxicity were noted in the 16 and 64 mg/kg treatment groups. Signs of developmental toxicity (significant reductions in mean fetal and litter weight) were also noted at 64 mg/kg. Results from this study suggest that while Ziram Technical induces developmental effects in rats, it does so only at dose levels that also produce significant maternal toxicity.

Under dynamic exposure conditions, the 96-hour median lethal (LC_{50}) concentration of Ziram Technical to bluegill sunfish was 0.0097 mg/l and, under static exposure conditions, the 48-hour LC_{50} to Daphnia magna was 0.048 mg/l.

In the repeat dosing studies (4- or 13-weeks), dogs fed the test material exhibited body weight and hematological parameter changes, liver toxicity and, in one animal only in each study, convulsive episodes. Lower doses given to dogs for a longer period (1-year), produced similar effects to those reported in the 4- or 13-week studies.

Atochem previously submitted a TSCA Section 8(e) notice on zinc dimethyldithiocarbamate and on a formulation (Ziram W76) containing zinc dimethyldithiocarbamate as active ingredient. The submissions were made July 9, 1992 and June 26, 1992, respectively; we have not been notified by EPA of the EPA Document Control Number for this substance.

Further questions regarding this submission may be directed to me at 215 337-6892.

Sincerely,

C.H. Farr, PhD, DABT
Manager, Product Safety
and Toxicology

Enclosures

A STUDY OF THE EFFECT OF ZIRAM ON PREGNANCY OF THE RAT

Study completed: 4 July 1989

Regulation: EPA FIFRA 83-3,

ADDRESS:

Dr. M. Giurgea,
Ziram Task Force,
U.C.B. S.A.,
Chemical Sector,
Avenue Louise 326 Box 7,
B-1050 Brussels,
BELGIUM.

Mr. R. Husson,
Ziram Task Force,
U.C.B. S.A.,
Chemical Sector,
Avenue Louise 326 Box 7,
B-1050 Brussels,
BELGIUM.

Mr. P. Larson,
Ziram Task Force,
C/o U.C.B. Chemicals Corporation,
5505A Robin Hood Road,
Norfolk,
Virginia 23513,
U.S.A.

AUTHORS:

Janet A. Smith,
Andrew M. Bryson,
David M. John,
Alan Anderson.

Huntingdon Research Centre Ltd.,
P.O. Box 2,
Huntingdon,
Cambridgeshire,
PE18 6ES,
ENGLAND.

EPA MRID
41908701

SPONSOR

Ziram Task Force
Consortium No. 62405
c/o UCB Chemicals Corporation
5505-A Robin Hood Road
Norfolk, Virginia 23513 U.S.A.

STUDY TITLE

A Study of the Effect of Ziram on Pregnancy of the Rat

DATA REQUIREMENTS

EPA FIFRA 83-3

AUTHORS

Janet A. Smith
Andrew M. Bryson
David M. John
Alan Anderson

PERFORMING LAB

Huntingdon Research Centre Ltd.
P. O. Box 2
Huntingdon, Cambridgeshire
PE18 6ES ENGLAND

HRC REPORT NO.

ZIR 15/24/891371

FINAL REPORT DATE

11 June 1990

STATEMENT OF NO CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10(d)(1)(A), (B), or (C).

COMPANY: Ziram Task Force
c/o UCB Chemicals Corporation

COMPANY AGENT: Paul R. Larson

DATE: October 12, 1990

TITLE: Manager, Agricultural Products

SIGNATURE: Paul R. Larson

These data are the property of the Ziram Task Force (Consortium No. 62405) and, as such, are considered to be confidential for all purposes other than compliance with FIFRA 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any other statute or in any other country.

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

HRC Report No. ZIR 15/24/891371

To the best of our knowledge and belief the study described in this report was conducted in compliance with the following Good Laboratory Practice Standards:

United States Environmental Protection Agency, (FIFRA), Title 40 Code of Federal Regulations Part 160, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

Organisation for Economic Co-operation and Development, ISBN 92-64-12367-9, Paris 1982.

Japan Ministry of Agriculture, Forestry and Fisheries, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984.

Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health & Social Security 1986 and subsequent revision, Department of Health, 1989.

JA Smith

Janet A. Smith, B.Sc. (Hons.), M.Sc.,
Study Director
Huntingdon Research Centre Ltd.

11 September 1990
Date

Keith F. Rivett, B.Sc., C.Biol., M.I.Biol.,
For Laboratory Management

19.09.1990

M. GIURGEA M.D.
SPONSOR'S MONITORING
SCIENTIST

We the undersigned, hereby declare that the work was performed under our supervision according to the procedures herein described, and that this report provides a correct and faithful record of the results obtained.

J. A. Smith

Janet A. Smith, B.Sc., M.Sc.,
Study Director,
Department of Reproductive Toxicology

A. Bryson

Andrew M. Bryson, B.Sc.(Hons.),
Study Supervisor,
Department of Reproductive Toxicology

D M John

David M. John, B.Sc.,
Teratologist,
Department of Pathology

Alan Anderson, B.Sc., C.Chem., F.R.S.C.,
Head of Drug Analysis,
Department of Analytical Chemistry

DEPARTMENT OF QUALITY ASSURANCE
REPORT AUDIT STATEMENT

HRC REPORT No. ZIR 15/24/891371

This report has been audited by HRC Quality Assurance Department. It is considered to be an accurate description of the procedures and practices employed during the course of the study and an accurate presentation of the findings.

Peter H.C.V. Richold, B.Sc.,
Systems Compliance Auditor
Huntingdon Research Centre Ltd.

15-1-90

DEPARTMENT OF QUALITY ASSURANCE

DATES OF STUDY INSPECTIONS

HRC REPORT No. ZIR 15/24/891371

Inspections were made by the Quality Assurance Department of the various phases of the study described in this Report. The dates on which inspections were made and the dates on which the findings were reported to the Study Director and to HRC Management are given below:

Phase of Study	Date of Inspection*	Date of Reporting
Protocol Review	-	15. 6.89
Pre-experimental Period	-	-
Experimental Period	21. 6.89	26. 6.89
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"		
Final Report Audit	-	6.12.89

15-1-90

Peter H.C.V. Richold, B.Sc.,
Systems Compliance Auditor,
Department of Quality Assurance,
Huntingdon Research Centre Ltd.

***Week beginning. This has been adopted to avoid the presentation of excessive dates in instances of an inspection being conducted over several days.**

Dates on which any 'process-based' inspections were made while the study was in progress are not included in the above listing.

PART I

A study of the effect of Ziram on pregnancy of the rat.

PART II

A preliminary study of the effect of Ziram on pregnancy of the rat.

PART III

The analysis of Ziram in 1% methylcellulose.

PART IV

The analysis of Ziram (Data supplied by Sponsor)

PART I

A STUDY OF THE EFFECT OF ZIRAM ON PREGNANCY OF THE RAT

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1. In this assessment of the effect of the fungicide, Ziram Technical, on pregnancy and embryofoetal development of the rat, daily dosages of 0(Control), 1, 4, 16 and 64 mg/kg/day were administered by oral gavage to females from Day 6 through to Day 15 of pregnancy inclusive. On Day 20 of pregnancy, the females were killed and subjected to post mortem examination. Litter parameters were determined and half of the foetuses subsequently examined for visceral, and half for skeletal abnormalities.

2. Dosages were based on results of a preliminary study which is presented in Part II to this report. This was also conducted on pregnant animals but the foetuses were not examined in detail.

3. Treatment at 64 and 16 mg/kg/day was associated with the following responses in the adult:

(a) Post-dosing salivation in occasional animals.
(b) Increased water consumption and decreased food consumption during the dosing period.
(c) Dosage-related bodyweight loss to Day 8 followed by retarded weight gain throughout the dosing period.

Treatment at 64 mg/kg/day was also associated with the following effect on litter parameters:

(a) Significantly lower mean foetal and litter weight.

Treatment at 4 mg/kg/day was associated with:

(a) Marginally increased water consumption during the dosing period.

4. Treatment at 1 mg/kg/day did not produce any overt maternal response. Mean litter parameters as assessed by pre- and post implantation losses, litter size and litter and mean foetal weight were unaffected by treatment at 16, 4 and 1 mg/kg/day.

5. Embryofoetal development as assessed by the incidence of malformation skeletal and visceral anomalies were unaffected by treatment. At 64 mg/kg/day the incidence of unossified sternebrae was marginally higher than the control, this was probably related to the lower foetal weight.

Within the context of this study oral administration of Ziram to rats during Days 6 to 15 of pregnancy produced maternal toxicity at 64 and 16 mg/kg/day in terms of increased water consumption, decreased food consumption and retarded mean bodyweight gain, at 4 mg/kg/day marginally increased water consumption only was observed. Treatment of females at 1 mg/kg/day was without any adverse maternal effect.

Treatment at 64 mg/kg/day was associated with reduced litter and mean foetal weight. Mean litter parameters were unaffected by treatment at 16, 4 and 1 mg/kg/day.

No differences in embryofoetal development or morphology were seen at dosages that did not also produce evidence of a maternal response.

The results of this study show the no toxic effect level to be in the region of 4 mg/kg/day.

This report describes the experiment performed to assess the effect of the fungicide, Ziram Technical, on pregnancy and embryofoetal development in the rat when administered by oral gavage once per day from Day 6 to Day 15 of pregnancy inclusive. Oral administration was requested by the Sponsor as this is a possible route of human exposure.

The dosages employed were based on the results obtained in a preliminary study on the pregnant rat, results of which are presented in Part II of this report.

Dosages of 80, 20 and 5 mg/kg/day were employed in the preliminary study, as the degree of maternal response at 80 mg/kg/day was considered too marked for use in the main embryotoxicity study 64 mg/kg/day was chosen as the highest dosage and a low dosage of 1 mg/kg/day was selected as some maternal response was apparent at 5 mg/kg/day in the preliminary study, 16 and 4 mg/kg/day were chosen as the intermediate dosages.

Ziram Technical, Batch No. 8331 AA was received in these laboratories on 12 January 1989. It was a white powder with a melting point of 240°C and stated purity of 98.9%. It was stored at room temperature in the dark. It was known by the Sponsor that the material was stable for the duration of the study.

Stability of the test article in suspension was investigated prior to the start of dosing and achieved concentrations of the test article in liquid formulations were analysed on the second day of formulation. The results of which are presented in Part III of this report.

KEY STUDY DATES

Date of approval by HRC Management	:	13 June 1989
Date of approval by Study Director	:	13 June 1989
Date of Sponsor approval	:	20 June 1989
Date of animal arrival	:	15 June 1989
Date of first dose	:	19 June 1989
Date of last dose	:	29 June 1989
Date of final sacrifice	:	4 July 1989

Parent animals

One hundred and twenty-five sexually mature (8-10 weeks old), Specific Pathogen Free female rats (Crl: CD® (SD) BR VAF/Plus strain) which were time-mated to males of the same strain, were ordered from Charles River Portage, Michigan, USA. The first batch (A) consisted of 78 animals followed by a second batch (B) of 52 animals mated one day later. The day of mating, as judged by the appearance of sperm in the vaginal smear or by the presence of a vaginal plug, was considered as Day 0 of pregnancy. Six additional animals were ordered for health check purposes.

On arrival all animals were examined for abnormalities and for signs of overt ill health. Six animals designated as health check animals were numbered alphabetically and killed within 24 hours after arrival at HRC and subjected to routine macroscopic examination. Any abnormalities seen were processed immediately and examined microscopically. Lungs, liver, kidneys, spleen and heart were preserved in fixative, but not processed further. Results are reported in Addendum 1.

The remaining animals were weighed on arrival. Allocation to treatment groups occurred on Day 2 of pregnancy when animals were weighed and those in the weight range 161 g to 218 g assigned to four groups by computerised stratified randomisation to give approximately equal initial group mean bodyweights within each batch. Following allocation, the animals were earmarked to give individual identification. Prior to the commencement of treatment, all animals were inspected by a Veterinary Officer.

The animals delivered that were excess to requirement were not dosed prior to sacrifice.

General animal management and accommodation

Animal room controls for temperature and relative humidity were set to maintain 21°C and 55% respectively, recorded range was 21 ± 2°C and 56 ± 4% respectively. Lighting was controlled to give 12 hours light (8 am to 8 pm) and 12 hours dark per 24 hours.

The animals were housed five to a cage in suspended galvanised metal cages (Bowman® equipped with solid sides and back, wire mesh front, floor and top. The cages constituting each treatment group were dispersed within the batteries so that possible environmental influences arising from their spatial distribution was equilibrated as far as possible for all treatments.

Throughout the study each cage was identified by a label coloured according to the group and recording the study schedule number, animal numbers, details of treatment and the name of the Study Supervisor.

All animals were given free access to Labsure Laboratory Animal Diet No. 1 and to tap water.

There was no information available to the Study Director to indicate that any non-nutrient substance likely to influence the effect of the test compound could reasonably be expected to be present in the diet or water. (Quality Assurance Aspects for Food and Water are presented in Procedure Addenda 1 and 2). These analytical data are lodged in HRC Archives.

Experimental procedures

The experimental design was as follows:

Group/ colour code	Treatment Ziram (mg/kg/ day)#	Concentration of suspension (% w/v)#	Vehicle	Dose volume (ml/ 100 g)	No. of rats ♀ A	B	Animal numbers A	B
1: White	Control	-	1% MC	0.1	15	10	1- 15	16- 25
2: Yellow	1	0.1	1% MC	0.1	15	10	26- 40	41- 50
3: Blue	4	0.4	1% MC	0.1	15	10	51- 65	66- 75
4: Green	16	1.6	1% MC	0.1	15	10	76- 90	91-100
5: Red	64	6.4	1% MC	0.1	15	10	101-115	116-125

Material as supplied
MC Methylcellulose

Method and frequency of dose preparation

Suspensions of the test article were prepared on a daily basis. On each occasion of formulation the highest required concentration was prepared by suspending a weighed quantity of the test article in the vehicle. The low concentrations were prepared by serial dilution. Immediately prior to and during dosing suspensions were placed on a magnetic stirrer.

Samples were taken for analysis at HRC on the second day of dosing. Results are presented in Part III.

Treatment

Treatment by oral gavage commenced on Day 6 of pregnancy and continued daily up to and including Day 15 of pregnancy.

Dosage volumes were calculated for individual animals on Day 6 of pregnancy and adjusted according to bodyweight on Days 8, 10, 12 and 14.

Observations

The following observations were made during the study:

1. Parent animals

(a) Signs

All animals were regularly handled and observed daily for obvious changes or signs of reaction to treatment.

(b) Water consumption

Water consumption was measured daily from Day 2 to Day 20 of pregnancy inclusive. For clarity of presentation data was presented in one, two or three day blocks.

(c) Food consumption

Food consumption was measured from weighday to weighday.

(d) Bodyweights

All animals were weighed initially (=Day 1 of pregnancy for Batch B and Day 2 for Batch A and Batch B) and on Days 3, 6, 8, 10, 12, 14, 16, 18 and 20. Day 1 weights not reported as only recorded for Batch B animals.

2. Litter data and foetal examinations

On Day 20 of pregnancy the animals were killed by CO_2 asphyxiation, dissected and examined for congenital abnormalities and macroscopic pathological changes in maternal organs. Abnormal tissues were preserved at the discretion of the post mortem pathologist. The ovaries and uteri were examined immediately to determine:

(a) number of corpora lutea
(b) number and distribution of live young
(c) number and distribution of embryofoetal deaths
(d) individual foetal weight from which the litter weight is calculated
(e) foetal abnormalities

Embryofoetal deaths were classified as:

Early: only placenta visible at termination.

Late: both placental and embryonic remnants visible at termination.

Uteri or individual uterine horns without visible implantations were immersed in a 10% solution of ammonium sulphide to reveal evidence of embryonic death at very early stages of implantation.[a]

Live young were examined externally and weighed. Half the foetuses in each litter were preserved in Bouin's solution for subsequent free-hand sectioning to discover visceral abnormalities (Wilson technique[b]); the remainder were fixed in 74 OP industrial methylated spirit for subsequent macroscopic examination, evisceration, clearing and alizarin staining (modified Dawson technique[c]) for skeletal examination. Young showing suspected abnormalities were processed by the more appropriate technique for clarification of initial observations.

All foetuses were sexed by gonadal inspection following preservation.

Foetuses were uniquely identified to allow correlation of initial with subsequent findings.

Structural changes are presented as:

Malformations: rare and/or probably lethal, e.g. exencephaly, anury.

Anomalies: minor differences from 'normal' that are detected relatively frequently either by free-hand sectioning, e.g. increased renal pelvic dilatation, or at skeletal examination, e.g. bipartite centrum.

Variants: alternative structures occurring regularly in the control population are classified as variants e.g. unossified sternebra(e).

Assessment of results

Individual litter values

In assessing litter parameters, pre-implantation loss was calculated as a percentage from the formula:

$$\frac{\text{(No. of corpora lutea - no. of implantations)}}{\text{No. of corpora lutea}} \times 100$$

Post implantation loss was similarly calculated from the formula:

$$\frac{\text{(No. of implantations - no. of live young)}}{\text{No. of implantations}} \times 100$$

Litter weight and mean foetal weight were calculated from individual foetal weight.

For each group

All values expressed as a percentage or ratio were first calculated within each litter and the group values calculated as a mean of the individual litter percentages.

As there were no instances of total resorption, the mean values were calculated to include animals with live young at termination only. Mean values for water and food consumption include all animals because of gang-housing.

Statistical analysis

Statistical analyses were performed on litter data. Significance tests were two-tailed.

Litter data

The basic sample unit was the litter, and due to the preponderance of non-normal distributions, non-parametric analyses proved the most consistent.

Mean values of litter size, pre- and post implantation loss, litter weight, mean pup weight, sex ratio and the incidence of anomalous offspring were analysed by the Kruskal-Wallis test[1]. Intergroup comparisons were made by the non-parametric equivalent of the 't' test together with the Jonckheere test for an ordered series of treatments[1].

Where 75% of the values for a given variable consisted of one value, a Fisher's exact test[2] was used.

Water, food and bodyweight data

Analysis of variance[3], followed by Williams' test[4] were used for assessing intergroup differences in absolute values for mean water and food consumption and bodyweight during gestation.

Location of study records

All specimens, raw data and other documents generated at HRC during the course of this study are lodged in the Huntingdon Research Centre Ltd., Archives, Huntingdon, England.

All study related specimens and raw data will be kept for ten years after the issue of the final report and then discarded. The Sponsor will be notified of this date and given the option of receiving this material into their own archives.

Any such material arising from investigations made by the Sponsor or other laboratory, the findings of which are included in the final report, will be retained either by the organisation conducting these investigations or by HRC.

A hard copy of the final bound report will be kept in the Huntingdon Research Centre, Archives.

Good laboratory practice

The study was conducted in compliance with the principles of Good Laboratory Practice as set forth in:

The United Kingdom Compliance Programme, Department of Health and Social Security, 1986

Organisation for Economic Co-operation and Development, ISBN 92-64-12367-9, Paris 1982.

The study also complied with:

United States Environmental Protection Agency, Title 40 Code of Federal Regulations Part 160, Federal Register, 29 November 1983.

Japan Ministry of Agriculture, Forestry and Fisheries, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984.

Quality Assurance Department

The Quality Assurance Department conducted inspections of the various phases of the study and of certain repetitive operations, at the intervals required by the FDA Good Laboratory Practice Regulations. The dates on which the findings of these inspections were reported to the Study Director and to HRC Management are specified in this report.

The final report has been reviewed by HRC's Quality Assurance Department, comparing individual findings against raw data and comparing the statements and results presented in the report with individual data presented in the Appendices of the report.

References

a Salewski, E. 1964 — Farbemethode zum makroskopischen Nachweis von Implantationsstellen am Uterus der Ratte. Naunyn-Schmiedebergs Arch. exp. Pathol. Pharmakol. 247: 367.

b Wilson, J.G. 1965 — Methods for administering agents and detecting malformations in experimental animals - In Teratology : Principles and Techniques. J.G. Wilson and J. Warkany Eds. University of Chicago Press, p. 262-277.

c Dawson, A.B. 1926 — Note on the staining of the skeleton of cleared skeletal specimens with alizarin red S. Stain Techn. 1: 123-4.

Statistical references

1. Hollander, M. & Wolfe, D.A. 1973 — Non-parametric statistical methods. Publ. J. Wiley & Sons, NY.

   Kruskal-Wallis & Jonckheere tests: page 114-132.

   Wilcoxon test: page 68-75.

2. Fisher, R.A. 1950 — Fisher's exact test for 2 x 2 contingency table: 'Statistical Methods for Research Workers' para. 21.02 Oliver & Boyd, Edinburgh.

3. Snedecor, G.W. & Cochran, W.G. 1967 — 'Statistical Methods' 6th ed. The Iowa State University Press.

4. Williams, D.A. 1971/2 — Williams' test for comparing the effect of increasing doses of a substance with a zero dose.
   Biometrics, 27: 103 - 171.
   Biometrics, 28: 519.

Composition and quality assurance aspects of rodent diet

Labsure LAD was a closed formula diet suitable for normal health, growth and reproduction of laboratory rats and mice. The standards of production adopted by the manufacturers have been approved by the HRC Quality Assurance Department.

Analyses were made of all batches of diet for most nutrients and for specified substances and micro-organisms likely to be present in feed ingredients or the finished diet and which, if in excess of specified amounts, might have had an undesirable effect on the test system. Although occasional slight deviation was permitted, batches of diet conformed with the acceptable standards agreed by the Study Director and Department of Quality Assurance HRC as detailed below.

(A) Nutrients

		Target level	Maximum Tolerance (%)
Crude fat	%	3.7	±15
Crude protein	%	21.5	±10
Crude fibre	%	2.0	±40
Ash	%	5.5	±15
Moisture	%	9.5	10.5
Calcium	%	1.0	±20
Phosphorus	%	0.9	±20
Sodium	%	0.3	+100-50
Chlorine	%	0.5	+100-50
Potassium	%	0.8	+100-50
Magnesium	%	0.15	±50
Manganese	mg/kg	70	±50
Iron	mg/kg	220	±50
Copper	mg/kg	15	±50
Zinc	mg/kg	60	±50
Vitamin A	i.u./kg	12000	+50-20
Alpha tocopherols	mg/kg	35	+150-20

(B) Contaminants

	Maximum allowable concentration (mg/kg except where stated)
Lead	2.5
Cadmium	0.5
Arsenic	1.5
Mercury	0.1
Selenium	0.6
Fluorine	40
Nitrates (as sodium nitrate)	200
Nitrites (as sodium nitrite)	10
PCBs	0.05
Total DDT	0.15
Dieldrin	0.05
Lindane	0.15
Heptachlor	0.05
Malathion	5.0
Total aflatoxins (B_1, B_2, G_1, G_2)	5.0 µg/kg

(C) Microbiological content

Maximum count/g diet (at time of manufacture)

	LAD 1
Total viable organisms	10,000
Mesophilic spores	30,000
Presumptive *E. coli*	0
E. coli Type 1	0
Salmonella spp.	0
Fungal units	1,000
Antibiotic activity	0

Quality assurance aspects of water

Results of the routine physical and chemical examination of drinking water at source (Grafham Final Water) as conducted usually weekly by the supplier, Anglian Water Authority, were made available to HRC as quarterly summaries. Additionally, levels of specified substances known to be present from time to time in local water and which, if in excess of the maxima recommended (for humans) might have had undesirable effects on the test system, were determined in HRC tap water at approximately 6-monthly intervals.

Quarterly summary analyses of source water normally included levels of nitrites, nitrates, Ca, Mg, Na, K, P, Cl, Si, Fe.

Six-monthly analyses of HRC tap water currently include levels of As, Se, Ba, Ag, Sb, organophosphorus, organochlorine and other pesticides, haloforms, chlorophenols, polychlorinated biphenyls and polycyclic aromatic hydrocarbons.

1. Analysis of dosing suspensions (Part III)

On the second day of preparation samples of the dosing suspensions were taken and analysed for achieved concentrations. Results showed that achieved concentration was within 9% of nominal. Full results are presented in Part III.

2. Parent animals

Dosages: Ziram, 0(Control), 1, 4, 16 and 64 mg/kg/day.

(a) Signs (Table 1, Appendix 1).

Treatment at 64 mg/kg/day was associated with slight post-dosing salivation in 8/25 animals on one to four occasions (mainly during the last few days of dosing), and hair loss in 6/25 animals. At 16 mg/kg/day, 2/25 animals showed slight post-dosing salivation. There were no obvious treatment-related signs at 4 or 1 mg/kg/day.

There were no mortalities.

(b) Water consumption (Figure 1, Table 2, Apppendix 2)

Treatment at 64 and 16 mg/kg/day was associated with a dosage-related increased water consumption throughout the dosing period; differences from controls attained statistical significance ($P<0.01$); during the post-dosing period (Days 16 to 19) mean consumption was similar to controls. At 4 mg/kg/day mean water consumption during the dosing period was marginally higher than control, but differences were not statistically significant ($P>0.05$).

(c) Food consumption (Figure 2, Table 3, Appendix 3)

Treatment at 64 and 16 mg/kg/day was associated with a dosage-related decrease in mean food consumption throughout the dosing period; differences from controls attained statistical significance ($P<0.01$); on cessation of treatment appetite returned and was similar to controls. Treatment at 4 and 1 mg/kg/day did not noticeably affect mean food consumption.

(d) Bodyweight change (Figure 3, Tables 4a and 4b, Appendix 4)

Following initiation of treatment on Day 6 of pregnancy there was an initial slight bodyweight loss to Day 8 in 13/23 and 20/24 dams at 64 and 16 mg/kg/day, respectively, against a combined incidence of 0/69 in Groups 1 to 3 inclusive; differences from controls attained statistical significance throughout ($P<0.01$ or $P<0.05$). At 64 mg/kg/day weight gain was slightly retarded to Day 16 and parity with controls was not regained by termination, at 16 mg/kg/day weight gain to Day 16 paralleled controls. Bodyweight gain at 4 and 1 mg/kg/day was essentially similar to controls.

(e) Pregnancy rate and pre-implantation loss (Tables 1 and 5, Appendix 5)

Pregnancy rate was high, ranging from 88% in Group 3 to 96% in Groups 2 and 5. There were no differences in mean pre-implantation loss. The implantation rate was comparable in all groups at the start of treatment.

(f) Terminal autopsy (Appendix 1)

Other than confirmation of hair loss in occasional animals at 64 mg/kg/day there were no obvious treatment-related macroscopic changes detected at terminal autopsy.

2. Litter data

(a) Total resorption

There were no instances of total resorption. The following assessment is therefore based on dams with viable young.

(b) Litter size, post implantation loss and sex ratio (Tables 5 and 6, Appendices 5 and 6)

Slight intergroup variation in both post implantation loss and litter size was not considered treatment-related.

Sex ratio, as expressed by the percentage of males per litter, was essentially similar among both control and treated animals.

(c) Litter and mean foetal weight (Table 5, Appendix 5)

Mean litter size at 64 mg/kg/day was identical to the control value but mean foetal weight was significantly lower than the control value (P<0.001) and this was reflected in the low mean litter weight.

At 16, 4 and 1 mg/kg/day mean foetal weight and litter weight showed no consistent dosage-related pattern.

(d) Malformations and anomalies (Table 7, 8a, 8b, 8c, Appendices 7 and 8)

The incidence of malformed foetuses was 4, 2, 1, 1 and 4 in control, 1, 4, 16 and 64 mg/kg/day the type of malformations observed were as follows:

Description	No. foetuses in Group				
	1	2	3	4	5
Microphthalmia		1			1
Microphthalmia and cardiac malformation					1
Cranial and cardiac malformation, situs inversus and anal atresia		1			
Cardiac malformation	1			1	1
Cardiac malformation and situs inversus	1				
Diaphragmatic hernia	1		1		
Umbilical hernia					1
Situs inversus	1				

The overall incidence of affected foetuses at 64 mg/kg/day was identical to the control, however, 2/4 affected foetuses had microphthalmia, as the incidence is low these are considered to be coincidental.

The incidence of foetuses with visceral anomalies was marginally higher at 16 and 64 mg/kg/day compared with controls, this increase was not dosage-related but 5/20 and 5/16 at 16 and 64 mg/kg/day respectively showed thinning of the diaphragm. Although a high proportion of foetuses at the top two dosages showed the same visceral anomaly this is thought unlikely to be treatment-related as no foetuses at these dosages showed diaphragmatic hernia.

The incidence of foetuses with skeletal anomalies was similar in both control and treated groups.

(e) Skeletal variants (Table 9, Appendix 9)

The marginally higher incidence of foetuses at 64 mg/kg/day with unossified sternebrae was probably related to the lower foetal weight; differences in percentage incidence were not statistically significant (P>0.05). Treatment at 1, 4 and 16 mg/kg/day was not considered to adversely affect the incidence of skeletal variants, differences from controls were not statistically significant (P>0.05).

FIGURE 1

Water consumption - group mean values

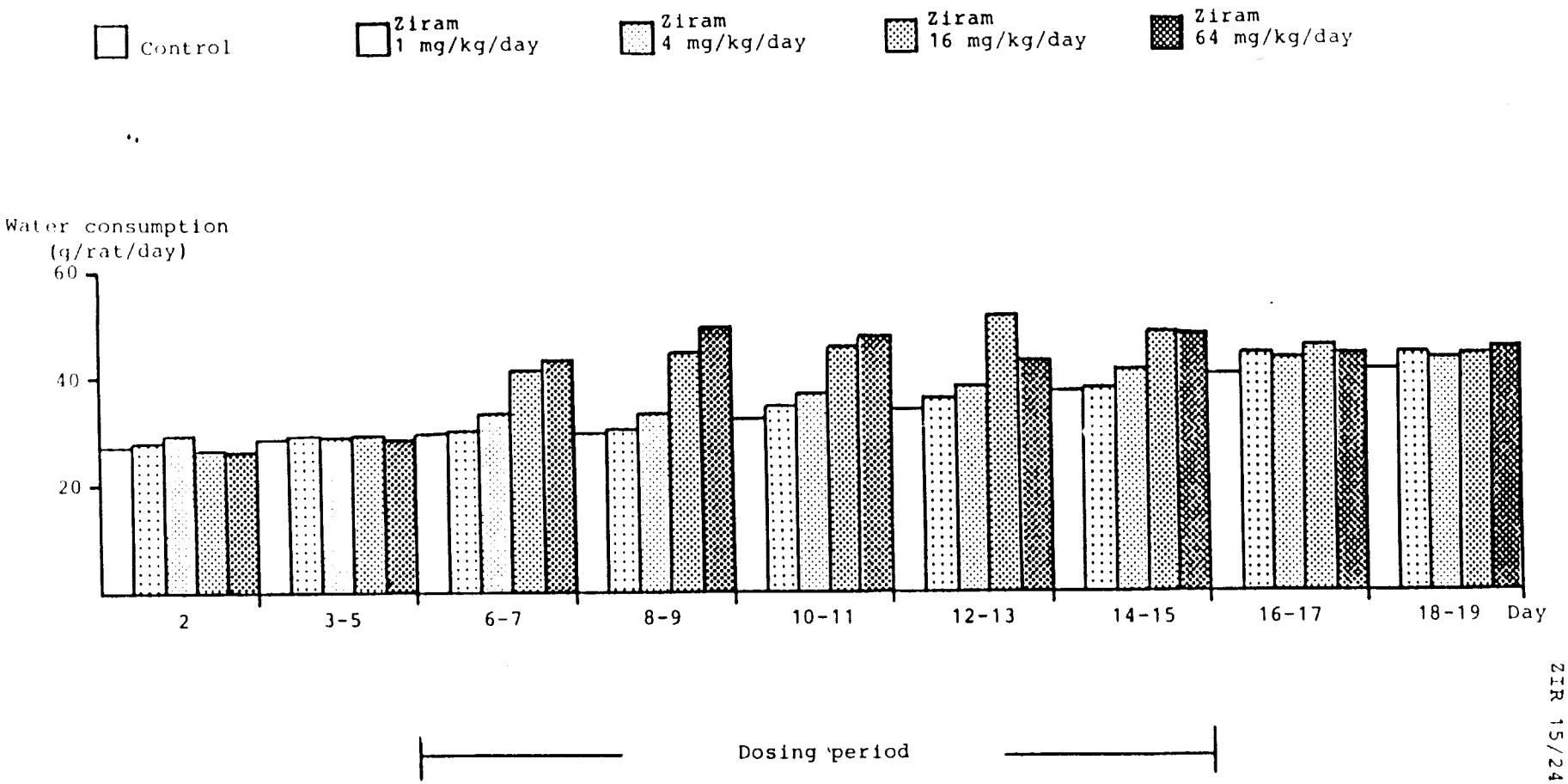

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FIGURE 2

Food consumption - group mean values

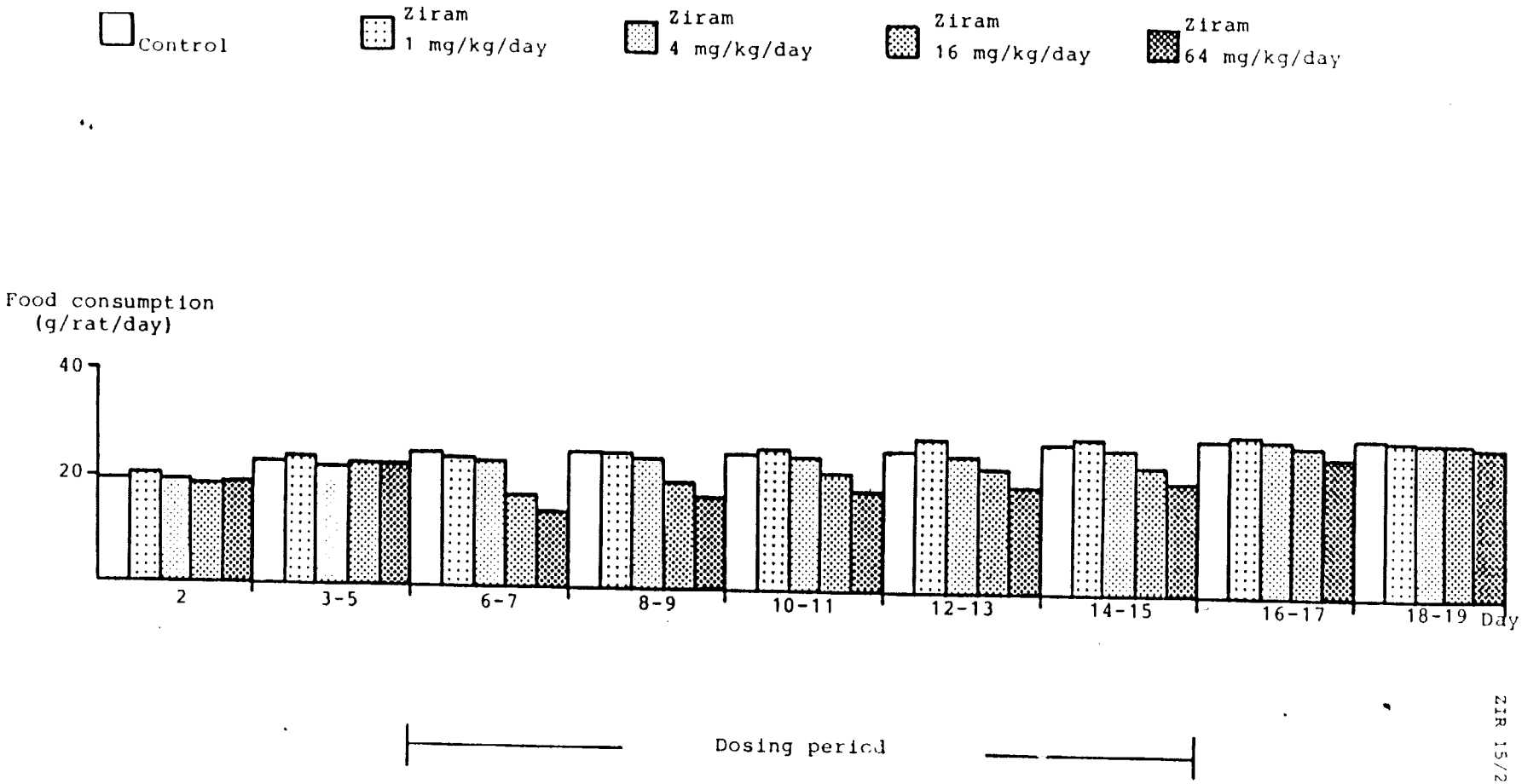

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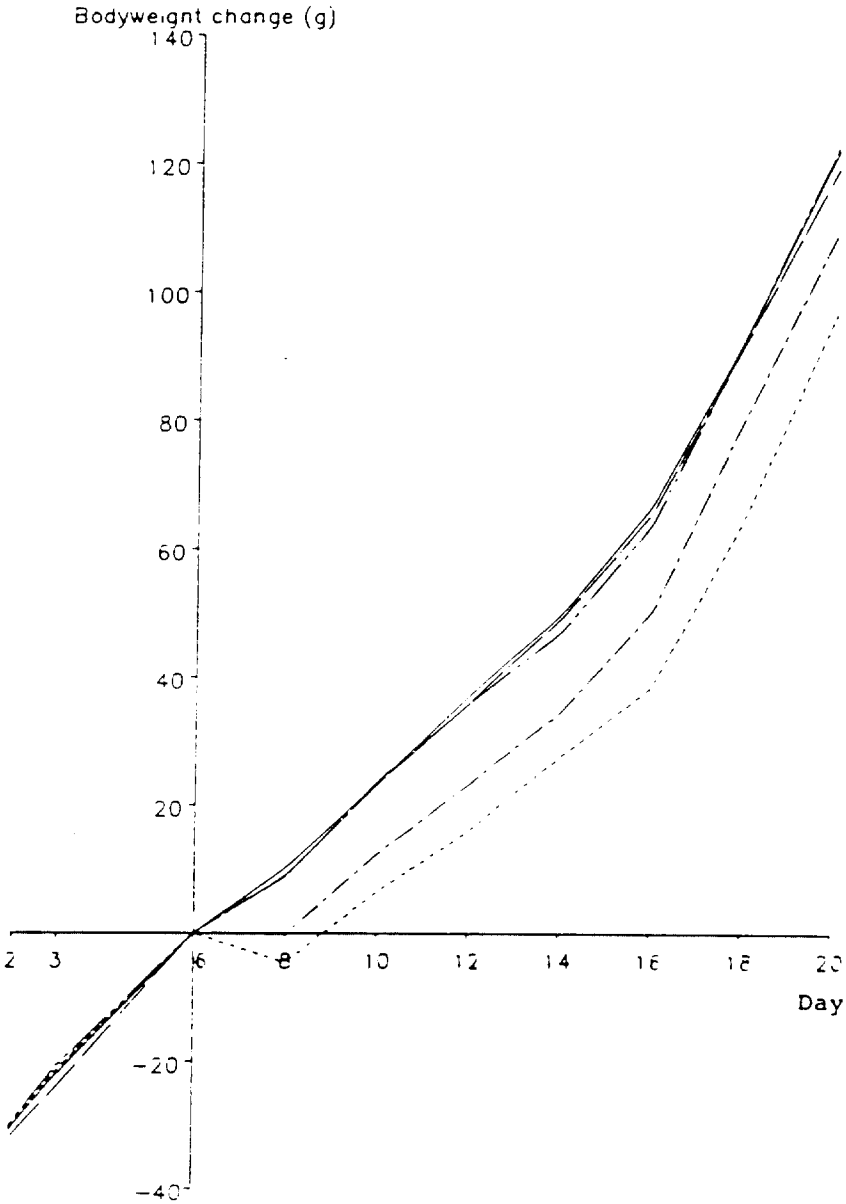
FIGURE 3

Bodyweight change of dams with live young - group mean values



Animals dosed from Day 6 to Day 15 of pregnancy inclusive

TABLE 1

Summary of adult performance

Group:	1	2	3	4	5
Compound:	Control		Ziram		
Dose (mg/kg/day):	-	1	4	16	64

Category	No. of animals in Group				
	1	2	3	4	5
Mated	25	25	25	25	25
Non-pregnant	2	1	3	2	1
With live young	23	24	22	23	24
Pregnancy rate (%)	92	96	88	92	96

TABLE 2

Water consumption - group mean values

Group:	1	2	3	4	5
Compound:	Control		Ziram		
Dose (mg/kg/day):	-	1	4	16	64

Group	No. of‡ animals	Mean water consumption (g/rat/day) during Days								
		2	3 - 5	6 - 7	8 - 9	10 - 11	12 - 13	14 - 15	16 - 17	18 - 19
1	25	27.2	28.5	29.8	29.9	32.0	33.9	37.1	40.1	40.5
2	25	28.0	29.3	30.1	30.5	34.4	36.0	38.4	44.0	43.8
3	25	29.7	28.7	33.6	33.2	36.9	38.0	41.1	43.2	43.1
4	25	26.7	29.5	41.2**	44.7**	45.2**	51.2**	48.1**	45.2*	43.9
5	25	26.0	28.4	43.5**	49.2**	47.7**	43.2**	47.6**	43.8*	44.7

Analysis of variance followed by Williams' test
Differences from controls statistically significant:
* P<0.05
** P<0.01

Other differences not statistically significant P>0.05

‡ Housed five per cage

TABLE 3

Food consumption - group mean values

Group:	1	2	3	4	5
Compound:	Control		Ziram		
Dose (mg/kg/day):	-	1	4	16	64

Group	No. of‡ animals	Mean food consumption (g/rat/day) during Days								
		2	3 - 5	6 - 7	8 - 9	10 - 11	12 - 13	14 - 15	16 - 17	18 - 19
1	25	19.6	23.1	24.8	25.2	25.7	26.4	27.7	29.3	29.6
2	25	20.6	23.6	24.2	24.9	26.2	28.7	28.5	30.2	29.3
3	25	19.2	22.4	23.5	24.0	25.1	25.3	26.5	29.2	28.6
4	25	18.8	22.8	17.4**	20.0**	22.0**	22.9**	24.0**	28.3	28.6
5	25	19.0	22.9	14.1**	17.3**	18.8**	19.6**	20.7**	26.2**	28.1

Analysis of variance followed by Williams' test
Differences from controls statistically significant:
** P<0.01

Other differences not statistically significant P>0.05

‡ Housed five per cage

TABLE 4a

Bodyweights of dams with live young - group mean values

Group:	1	2	3	4	5
Compound:	Control		Ziram		
Dose (mg/kg/day):	-	1	4	16	64

Group	No. of animals	Bodyweight (g) at Day gestation									
		2	3	6	8	10	12	14	16	18	20
1	23	188.4	196.9	219.3	229.5	242.7	256.3	269.0	286.2	312.4	341.7
2	24	188.8	196.4	220.8	229.8	244.1	256.8	270.0	286.6	313.2	340.4
3	22	189.8	199.0	220.0	228.9	243.9	255.8	267.0	284.0	313.1	343.1
4	23	188.1	196.7	219.1	219.0	231.5	242.3	253.6	269.3	299.4	328.7
5	24	188.1	196.5	218.3	213.8	224.9	234.2	245.8	256.8	282.9	315.3
Values adjusted for bodyweight at Day 6 as covariate											
1					229.7	243.0	256.6	269.2	286.5	312.7	342.1
2					228.6	242.8	255.3	268.5	285.0	311.5	338.5
3					228.4	243.3	255.2	266.5	283.4	312.4	342.3
4					** 219.4	** 231.9	** 242.8	** 254.0	** 269.9	** 300.0	* 329.3
5					** 214.9	** 226.1	** 235.5	** 247.1	** 258.2	** 284.4	** 317.0

Analysis of covariance followed by Williams' test
Differences from controls statistically significant:
* P<0.05
** P<0.01

Other differences not statistically significant P>0.05

TABLE 4b

Bodyweights of dams with live young - group mean change values from first day of dosing

Group:	1	2	3	4	5
Compound:	Control		Ziram		
Dose (mg/kg/day):	-	1	4	16	64

Group	No. of animals	Bodyweight change (g) at Day gestation									
		2	3	6	8	10	12	14	16	18	20
1	23	-30.8	-22.4	0.0	10.2	23.5	37.0	49.7	66.9	93.1	122.4
2	24	-32.1	-24.4	0.0	9.0	23.3	35.9	49.1	65.8	92.4	119.6
3	22	-30.2	-21.1	0.0	8.8	23.8	35.8	47.0	64.0	93.0	123.0
4	23	-31.0	-22.3	0.0	-0.1	12.4	23.2	34.5	50.3	80.3	109.6
5	24	-30.3	-21.8	0.0	-4.5	6.5	15.9	27.5	38.4	64.6	97.0

TABLE 5

Litter data - group values

Group:	1	2	3	4	5
Compound:	Control			Ziram	
Dose (mg/kg/day):	-	1	4	16	64

Group	No. of anims.	Live young	Embryonic deaths Early	Late	Total	Implants	Corpora lutea	Pre-implant loss %	Post implant loss %	Litter wt.(g)	Mean foetal wt.(g)
1	23	11.3	0.4	0.1	0.5	11.9	13.2	9.6	4.7	37.84	3.35
2	24	10.6	0.7	0.1	0.8	11.4	12.9	11.5	7.6	35.02	3.29
3	22	11.5	0.4	0.0	0.4	11.9	13.1	9.0	3.4	37.63	3.28
4	23	11.6	0.6	0.0	0.6	12.2	13.1	6.5	5.1	36.90	3.20
5	24	11.3	0.4	0.1	0.5	11.8	12.4	4.9	3.7	(*) 33.78	*** 2.98
Kruskal-Wallis 'H' statistic		NS	NS	F	NS	NS	NS	NS	NS	NS	P<0.001
Jonckheere 'J' statistic		NS	NS	NS	NS	NS	NS	NS	NS	P<0.05	P<0.001

Kruskal-Wallis 'H' statistic not significant (P>0.05)
Intergroup differences statistically significant: * P<0.05
*** P<0.001
() Not supported by a significant 'H' statistic: P>0.05
F Analysed by Fisher's Exact test due to frequency of tied values

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TABLE 6

Sex ratios - group mean values

Group:	1	2	3	4	5
Compound:	Control		Ziram		
Dose (mg/kg/day):	-	1	4	16	64

Group	No. of litters	No. of foetuses	♂	♀	Total	% ♂ / litter
1	23	261	5.3	6.0	11.3	48.1
2	24	255	6.2	4.4	10.6	57.6(*)
3	22	253	6.0	5.5	11.5	50.1
4	23	266	6.0	5.6	11.6	51.7
5	24	272	5.6	5.7	11.3	50.0

Jonckheere 'J' statistic not significant: P>0.05
Intergroup differences from control group significant at Kruskal-Wallis test: * P<0.05

() Not supported by significant 'H' statistic (P>0.05)

TABLE 7

Malformations and anomalies - group values

Group:	1	2	3	4	5
Compound:	Control		Ziram		
Dose (mg/kg/day):	-	1	4	16	64

Group	Number of foetuses								
	Malformations			Anomalies+					
				Visceral (Wilson technique)			Skeletal		
	Examined	Total no.	Mean %	Examined	Total no.	Mean %	Examined	Total no.	Mean %
1	261	4	1.6	126	9	8.3	131	29	23.8
2	255	2	0.7	125	6	4.9	128	26	19.5
3	253	1	0.4	123	9	6.9	129	31	22.5
4	266	1	0.4	133	20	14.9	132	30	22.1
5	272	4	1.4	134	16	12.2	134	30	21.3

+ Malformed foetuses are excluded

TABLE 8a

Skeletal anomalies - individual incidence

Group:	1	2	3	4	5
Compound:	Control	Ziram			
Dose (mg/kg/day):	-	1	4	16	64

	Foetuses					Litters				
Group	1	2	3	4	5	1	2	3	4	5
Number examined } excludes malformed foetuses	131	128	129	132	134	23	26	22	23	24
Number affected }	29	24	31	30	30	17	12	14	16	16
Description	Incidence Ø									
Reduced ossification of one or more cranial centre	4	4	4	6	3	4	3	4	5	1
Reduced ossification of cervical vertebral arches			1		1			1		1
Reduced ossification of thoracic/lumbar vertebral arches					1					1
Reduced ossification of sacrocaudal vertebral arches	18	14	15	16	10	14	9	10	8	8
Reduced ossification of one or more centres pelvic girdle	9	4	2	4	4	9	3	2	3	4
Reduced ossification of digital centres	1			3	3	1			2	3
Cranial										
Fused/part fused bilateral jugal to squamosal	1					1				
Cervical										
Cervical ribs	1		2			1		2		
Misshapen cervical vertebral arch					1					1
Thoracic										
Irregular ossification of vertebral centra	2	3	6		8	2	2	6		6
Minimal distortions affecting rib(s)	2					2				
Shortened/absent 13th rib(s)	9	6	7	5	11	7	6	5	5	7
Partially fused ribs	1					1				
Connected/misshapen sternebrae				2					2	
Asymmetric costal cartilage elements				2					2	
Lumbar										
Lumbar rib(s)			2	2				2	2	
One less pre-sacral vertebra	1					1				
Two less pre-sacral vertebra				1					1	
Sacrocaudal										
Asymmetric pelvic girdle			1					1		

Ø Individual foetuses may be included in more than one category

TABLE 8b

Visceral anomalies - individual incidence

Group:	1	2	3	4	5
Compound:	Control		Ziram		
Dose (mg/kg/day):	-	1	4	16	64

	Foetuses					Litters				
Group	1	2	3	4	5	1	2	3	4	5
Number examined } excludes malformed foetuses	126	125	123	133	134	23	24	22	20	24
Number affected }	9	6	9	20	16	8	5	7	16	11
Description	Incidence∅									
Subcutaneous haemorrhages cranium					1					1
Subcutaneous oedema	1					1				
Cranial										
Haemorrhages affecting brain/spine and surrounding tissues	2		1	3	5	2		1	3	4
Haemorrhages affecting eyes and surrounding tissues				4					4	
Increased dilatation of lateral ventricles				1					1	
Small eye					1					1
Thoracic										
Anomalous cervicothoracic arteries	1		1	2		1		1	2	
Anomalous cervicothoracic veins			1					1		
Abdominal										
Intra-abdominal haemorrhage		1					1			
Thinning of diaphragm/protrusion of liver			1	5	5			1	4	5
Increased dilatation renal pelvis	2	5	3	5	3	2	4	3	5	3
Displaced testis(es)	2	[1]		1[1]	[1]	2	[1]		1[1]	[1]
Abnormal lobation of liver	1[2]		2	[1]	1	1[2]		2	[1]	1

∅ Individual foetuses may be included in more than one category
[] Figures in brackets observed at processing for skeletal examination

TABLE 8c

Skeletal and visceral malformations - individual incidence

Group:	1	2	3	4	5
Compound:	Control		Ziram		
Dose (mg/kg/day):	-	1	4	16	64

	Foetuses					Litters				
Group	1	2	3	4	5	1	2	3	4	5
Number examined	265	257	254	267	276	23	24	22	23	24
Number affected	4	2	1	1	4	4	2	1	1	4
Description	Incidence									
Microphthalmia		1			1		1			1
Microphthalmia and cardiac malformation					1					1
Cranial and cardiac malformation, situs inversus and anal atresia		1					1			
Cardiac malformation	1			1	1	1			1	1
Cardiac malformation and situs inversus	1					1				
Diaphragmatic hernia	1		1			1		1		
Umbilical hernia					1					1
Situs inversus	1					1				

TABLE 9

Skeletal variants - group mean incidence

Group:	1	2	3	4	5
Compound:	Control		Ziram		
Dose (mg/kg/day):	-	1	4	16	64

Group	Foetuses examined	Foetuses‡ with									
		Normal sternebrae		Unossified sternebrae		Reduced sternebrae		Asym./bip. sternebrae		Total variant sternebrae	
		No.	%	No.	%	No.	%	No.	%	No.	%
1	131	28	22.4	77	59.8	51	37.9	5	3.9	103	77.6
2	128	20	16.6	73	59.1	60	44.9	4	2.9	108	83.4
3	129	26	19.8	69	51.3	54	44.1	2	1.7	103	80.2
4	132	25	20.1	74	54.6	63	47.6	6	4.3	107	79.9
5	134	15	11.2	91	68.2	57	42.6	4	2.8	119	88.8

Statistical analysis by Jonckheere, Kruskal-Wallis or Fisher's Exact test where 75% of the values were tied, were non-significant (P>0.05)

‡ Malformed foetuses are excluded

Principal signs and autopsy findings - individual observations

Key

B	Brown salivation (post-dose)
C	Clear salivation (post-dose)
E	Eye: left, small and dark
H	Generalised hair loss
R	Noisy respiration

N}	No abnormality detected (autopsy findings)
-}	No abnormality detected (signs)

(Principal signs and autopsy findings - continued)

Group 1: Control

Animal no.	Clinical signs: Day observed	Principal autopsy findings	
		Females	Day 20 Foetus
	With live young		
1	-	N	N
2	-	N	N
3	-	N	N
4	-	N	N
5	-	N	One foetus with subcutaneous haemorrhage (~2 x 2 mm) left pinna
6	-	N	N
7	-	N	N
8	-	N	N
9	-	N	N
10	-	N	N
11	-	N	N
12	-	N	N
13	-	N	N
14	-	N	N
15	-	N	N
16	H:14-20	Skin; minimal patchy hair loss, dorsum	N
17	H:14-20	Skin; minimal patchy hair loss, dorsum	N
19	-	N	N
20	-	Skin; minimal hair loss, dorsocervical region	N
21	-	N	N
23	-	N	N
24	-	N	N
25	-	N	N
	Non pregnant*		
18	-	N	
22	-	N	

* Confirmed by Salewski test

(Principal signs and autopsy findings - continued)

Group 2: Ziram, 1 mg/kg/day

Animal no.	Clinical signs: Day observed	Principal autopsy findings	
		Females	Day 20 Foetus
	With live young		
26	-	N	N
27	-	Uterus; left horn, no* visible implantations	N
28	-	N	
29	-	N	N
30	-	N	N
31	-	N	N
32	-	Uterus; left horn, no* visible implantations	N
33	-	N	N
34	-	N	N
35	-	N	N
36	-	N	N
37	-	N	N
38	-	N	N
39	-	N	N
40	-	N	N
42	-	N	N
43	-	N	N
44	-	N	N
45	-	N	N
46	-	N	N
47	-	N	N
48	-	N	N
49	-	N	N
50	-	N	N
	Non-pregnant*		
41	-	N	

* Confirmed by Salewski test

(Principal signs and autopsy findings - continued)

Group 3: Ziram, 4 mg/kg/day

Animal no.	Clinical signs: Day observed	Principal autopsy findings	
		Females	Day 20 Foetus
	With live young		
51	-	N	N
53	-	N	N
54	-	Uterus; left horn, no* visible implantations	N
55	-	Kidney; right, minimal increased pelvic dilatation	N
56	-	N	N
57	-	N	N
58	-	N	N
59	-	N	N
60	-	N	N
61	-	N	N
62	-	N	N
63	-	Kidney; bilateral, moderate increased pelvic dilatation	N
64	-	N	N
65	-	N	N
67	-	N	N
68	-	N	N
69	-	Kidney; right, minimal increased pelvic dilatation	
70	-	N	N
71	-	N	N
73	-	N	N
74	-	N	N
75	-	N	N
	Non-pregnant*		
52	-	N	
66	-	N	
72	-	N	

* Confirmed by Salewski test

(Principal signs and autopsy findings - continued)

Group 4: Ziram, 16 mg/kg/day

Animal no.	Clinical signs: Day observed	Principal autopsy findings	
		Females	Day 20 Foetus
	With live young		
76	-	N	N
77	-	N	N
78	-	N	N
79	-	N	N
80	-	N	N
81	-	N	N
82	-	N	N
83	-	N	N
84	-	N	N
85	-	N	N
86	-	N	N
87	-	N	N
88	R:8,9 and 11	N	N
89	-	N	N
90	-	N	N
91	-	N	N
92	-	N	N
93	C:14	N	N
94	-	N	N
97	-	N	N
98	-	N	N
99	C:12-14	N	N
100	-	N	N
	Non-pregnant*		
95	-	N	
96	-	N	

* Confirmed by Salewski test

(Principal signs and autopsy findings - continued)

Group 5: Ziram, 64 mg/kg/day

Animal no.	Clinical signs: Day observed	Principal autopsy findings	
		Females	Day 20 Foetus
	With live young		
101	H:15-20	Skin; patchy hair loss, whole of dorsum	N
102	-	N	One foetus with a haemorrhage (4 x 3 mm) nasal region
103	H:15-20	Skin; minimal hair loss, whole of dorsum	N
104	H:20 B:15	Skin; generalised hair loss, dorsal lumbar region	One foetus with small subcutaneous haemorrhage (1 x 3 mm) dorsocervical region
105	-	N	N
106	H:15-20	Uterus; left horn, no visible implantations*	N
107	H:20	Skin; minimal general hair loss, whole of dorsum	N
108	H:20	Skin; minimal hair loss, whole of dorsum	N
109	-	N	N
110	-	N	N
111	-	N	N
112	-	N	One foetus with umbilical hernia, with small intestine exposed

* Confirmed by Salewski test

(Principal signs and autopsy findings - continued)

Group 5: Ziram, 64 mg/kg/day

Animal no.	Clinical signs: Day observed	Principal autopsy findings	
		Females	Day 20 Foetus
	With live young		
113	C:10	N	One foetus with suspected reduction of right eye
114	-	N	N
115	B:14 E:15-20	Eye; left slightly smaller than right, with possible haemorrhaging on retinal surface	One foetus with marked reduction/absence of left eye, reduced right eye
116	-	N	N
117	-	N	N
118	C:12,15 B:13,14	N	N
120	C:12-15	N	N
121	C:13,15	N	N
122	-	N	N
123	-	N	N
124	C:14	N	N
125	-	N	One foetus with diffuse subcutaneous haemorrhage left side of cranium
	Non-pregnant*		
119	C:13,14	N	

* Confirmed by Salewski test

Water consumption - individual cage values

Cage	Total water consumption (g) during Days								
	2	3 - 5	6 - 7	8 - 9	10 - 11	12 - 13	14 - 15	16 - 17	18 - 19
Group 1: Control									
1	159	401	276	266	285	306	343	378	361
2	164	459	306	321	361	370	386	418	445
3	129	420	293	298	330	353	392	410	419
4	105	435	315	314	323	343	371	404	401
5	122	426	299	297	300	322	362	397	400
Group 2: Ziram, 1 mg/kg/day									
6	172	475	325	316	360	379	409	455	478
7	155	442	300	282	337	349	376	429	436
8	154	409	280	300	322	350	362	450	440
9	109	433	302	316	377	378	389	433	406
10	111	442	298	312	324	343	384	432	431
Group 3: Ziram, 4 mg/kg/day									
11	183	469	391	348	402	402	451	454	444
12	157	427	314	312	369	374	401	415	418
13	152	384	299	292	328	337	374	402	429
14	135	466	345	363	398	410	413	441	432
15	115	410	331	347	350	375	415	450	430
Group 4: Ziram, 16 mg/kg/day									
16	152	451	454	465	492	636	586	501	481
17	154	482	477	478	495	520	511	452	436
18	127	362	314	314	350	377	374	407	411
19	118	463	420	544	511	583	485	465	463
20	116	451	393	433	414	446	450	435	403
Group 5: Ziram, 64 mg/kg/day									
21	141	414	390	435	359	330	483	468	508
22	137	436	414	506	534	468	520	453	492
23	136	414	375	433	421	393	433	403	400
24	100	379	478	491	468	429	452	345	335
25	135	488	516	597	605	540	492	519	499

Food consumption - individual cage values

Cage	Total food consumption - individual cage values								
	2	3 - 5	6 - 7	8 - 9	10 - 11	12 - 13	14 - 15	16 - 17	18 - 19
Group 1: Control									
1	110	340	240	240	255	255	270	280	285
2	105	350	240	260	280	270	275	300	310
3	95	320	235	230	250	255	270	280	285
4	85	375	260	265	250	275	285	315	305
5	95	345	265	265	250	265	285	290	295
Group 2: Ziram, 1 mg/kg/day									
6	110	360	240	235	255	295	290	305	300
7	95	345	235	230	250	260	275	285	280
8	110	360	240	245	255	270	275	295	290
9	120	350	240	255	260	280	280	300	280
10	80	355	255	280	290	330	305	325	315
Group 3: Ziram, 4 mg/kg/day									
11	105	325	235	220	240	245	275	285	285
12	90	320	220	225	245	240	260	275	270
13	105	325	230	235	250	255	260	295	275
14	95	380	260	275	270	275	275	320	320
15	85	330	230	245	250	250	255	285	280
Group 4: Ziram, 16 mg/kg/day									
16	100	335	165	210	235	235	255	295	295
17	100	335	170	185	205	225	240	280	280
18	100	325	180	180	205	220	225	275	280
19	85	345	175	200	215	215	230	275	280
20	85	370	180	225	240	250	250	290	295
Group 5: Ziram, 64 mg/kg/day									
21	95	335	150	165	170	185	195	265	290
22	100	330	125	155	180	200	200	265	280
23	100	350	150	160	195	200	195	255	265
24	90	340	160	190	195	200	215	245	285
25	90	365	120	195	200	195	230	280	285

APPENDIX 4

Bodyweights - individual values

Group 1: Control

Animal number	Bodyweight (g) at Day gestation									
	2	3	6	8	10	12	14	16	18	20
	Live young at Day 20									
1	181	189	217	218	227	241	256	268	297	323
2	167	185	202	211	222	230	243	258	286	310
3	191	195	224	231	246	254	268	285	317	349
4	187	206	230	235	246	256	269	278	296	319
5	178	194	221	226	233	245	253	270	298	321
6	183	201	226	230	256	268	276	298	324	355
7	179	190	223	230	245	261	273	288	320	357
8	185	191	213	219	236	248	262	273	300	327
9	170	179	201	209	222	237	251	266	297	327
10	193	204	231	237	253	267	281	297	325	356
11	180	196	217	225	237	254	265	282	307	336
12	189	204	223	233	248	260	273	293	316	348
13	199	209	230	248	263	275	286	299	328	356
14	176	184	210	218	232	239	249	268	298	319
15	180	188	206	211	229	236	244	257	275	299
16	190	192	212	233	245	258	273	302	325	368
17	204	202	228	237	236	277	288	312	344	380
19	198	199	216	231	236	254	266	286	310	339
20	213	211	237	255	278	281	299	314	342	385
21	185	190	204	224	229	252	262	284	309	340
23	202	206	223	241	256	268	281	298	323	346
24	193	197	219	233	250	260	277	293	313	337
25	211	216	230	243	258	274	291	313	335	362
	Non-pregnant									
18	184	192	208	217	222	219	221	217	225	231
22	194	196	214	227	237	235	247	243	245	245

APPENDIX 4

(Bodyweights - individual values - continued)

Group 2: Ziram, 1 mg/kg/day

Animal number	Bodyweight (g) at Day gestation									
	2	3	6	8	10	12	14	16	18	20
	Live young at Day 20									
26	188	202	237	242	256	263	282	297	330	361
27	181	190	210	219	232	241	251	262	280	294
28	180	193	218	225	236	246	260	270	292	310
29	172	183	215	220	235	246	254	273	299	329
30	193	208	234	243	257	268	277	290	316	342
31	181	188	225	237	255	273	292	313	345	375
32	194	199	223	233	242	252	261	274	288	304
33	176	184	203	210	222	233	244	255	278	302
34	188	196	233	234	251	265	272	291	316	336
35	176	185	200	206	210	221	232	243	267	291
36	184	197	219	228	241	247	261	271	293	313
37	175	185	212	220	233	244	256	267	297	324
38	193	202	227	232	245	258	271	283	317	339
39	177	193	222	236	250	260	273	288	323	350
40	186	206	241	242	266	281	293	320	346	377
42	187	191	214	223	243	256	270	290	311	337
43	213	210	234	246	267	276	294	321	348	393
44	191	197	223	233	250	266	286	310	338	378
45	204	209	226	237	250	266	273	290	318	350
46	190	193	209	219	234	244	257	277	306	333
47	195	196	216	225	242	261	277	295	320	353
48	189	190	206	233	230	251	267	286	320	354
49	207	204	222	225	250	266	283	307	327	358
50	210	213	231	247	262	278	293	306	342	367
	Non-pregnant									
41	195	198	199	206	200	211	218	223	231	227

APPENDIX 4

(Bodyweights - individual values - continued)

Group 3: Ziram, 4 mg/kg/day

Animal number	Bodyweight (g) at Day gestation 2	3	6	8	10	12	14	16	18	20
	Live young at Day 20									
51	180	194	214	220	234	245	255	276	300	324
53	187	200	221	235	248	264	271	288	322	350
54	191	207	232	235	243	256	268	282	302	323
55	183	190	213	220	235	245	256	268	302	331
56	176	187	210	212	230	236	249	265	289	313
57	190	198	220	230	239	253	260	277	300	325
58	181	194	223	224	245	256	265	284	310	337
59	173	181	205	211	225	236	245	261	293	320
60	193	198	226	235	250	261	271	286	314	343
61	192	211	229	242	260	267	278	295	328	366
62	176	188	208	216	237	238	249	267	298	331
63	184	194	213	224	239	247	258	270	300	327
64	188	196	216	227	239	250	264	283	314	345
65	173	181	194	199	212	217	226	239	263	287
67	208	213	238	254	268	281	298	316	355	402
68	189	202	229	239	257	268	291	308	340	380
69	211	214	233	246	261	274	288	313	342	379
70	194	206	226	242	260	274	289	306	339	369
71	217	220	241	243	267	290	302	324	358	395
73	204	205	222	230	239	255	262	280	310	336
74	195	200	212	225	237	251	265	280	305	338
75	191	198	216	226	240	264	265	281	304	327
	Non-pregnant									
52	167	185	201	212	215	215	214	228	228	238
66	192	192	210	213	223	221	228	231	238	242
72	179	188	208	214	221	223	230	237	249	253

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APPENDIX 4

(Bodyweights - individual values - continued)

Group 4: Ziram, 16 mg/kg/day

Animal number	Bodyweight (g) at Day gestation									
	2	3	6	8	10	12	14	16	18	20
	Live young at Day 20									
76	180	191	216	213	226	237	253	277	306	335
77	189	198	222	217	232	237	250	261	295	317
78	173	185	229	207	222	233	243	263	289	316
79	196	209	236	235	250	265	269	294	323	355
80	177	185	213	210	228	242	245	260	289	321
81	183	180	200	197	214	220	230	244	266	292
82	185	198	223	224	238	251	259	274	300	339
83	194	207	224	222	240	245	254	267	301	326
84	170	196	222	224	221	242	251	272	298	331
85	178	194	230	226	237	245	259	282	321	352
86	179	187	206	216	227	233	243	255	280	308
87	183	190	210	208	214	227	240	254	286	314
88	167	182	205	202	218	224	236	242	284	309
89	186	195	216	221	235	242	250	261	298	314
90	196	212	231	243	249	256	267	283	312	348
91	190	191	207	204	217	235	251	260	291	316
92	190	193	205	218	224	230	245	258	286	317
93	213	217	236	243	252	262	277	288	328	361
94	209	210	232	232	236	252	258	273	303	339
97	191	193	218	225	236	255	271	285	313	343
98	204	211	227	219	250	263	284	305	334	368
99	184	185	201	208	218	226	242	257	280	306
100	209	216	230	222	240	251	255	280	304	332
	Non-pregnant									
95	198	202	214	210	214	218	218	220	232	248
96	195	200	222	227	232	241	244	257	259	269

APPENDIX 4

(Bodyweights - individual values - continued)

Group 5: **Ziram**, 64 mg/kg/day

Animal number	Bodyweight (g) at Day gestation									
	2	3	6	8	10	12	14	16	18	20
	Live young at Day 20									
101	174	186	209	213	219	237	257	255	294	338
102	191	204	227	223	237	249	257	273	290	318
103	180	190	210	205	211	230	232	241	263	295
104	177	189	212	211	225	236	243	259	277	316
105	194	208	232	223	223	221	232	236	258	280
106	180	189	212	207	214	230	228	241	281	286
107	184	196	220	216	229	232	247	250	260	315
108	172	186	213	210	221	234	245	260	290	323
109	190	204	222	206	219	226	240	244	277	308
110	191	196	220	213	225	230	246	253	275	300
111	181	191	212	209	215	231	235	247	286	316
112	192	206	231	217	234	227	242	256	279	314
113	161	174	198	198	207	217	230	237	261	295
114	180	199	222	216	224	230	247	258	287	324
115	186	190	212	208	224	228	236	248	277	299
116	195	197	218	218	232	239	253	272	288	331
117	191	198	216	215	224	239	245	259	283	316
118	187	189	200	196	209	217	235	251	281	311
120	213	214	233	236	247	260	278	288	315	347
121	199	203	235	226	236	255	272	286	316	349
122	218	217	234	230	244	247	255	271	293	330
123	194	196	215	211	225	239	256	264	296	328
124	188	195	222	211	223	237	249	264	296	331
125	196	200	215	213	230	230	240	249	267	297
	Non-pregnant									
119	202	203	222	227	227	233	234	234	235	244

APPENDIX 5

Litter data - individual values

Group 1: Control

Animal number	Live young	Embryonic deaths Early	Late	Total	Implants	Corpora lutea	Pre-implant loss %	Post implant loss %	Litter wt.(g)	Mean foetal wt.(g)
	With live young									
1	12	0	0	0	12	13	7.7	0.0	39.99	3.33
2	13	0	0	0	13	13	0.0	0.0	41.12	3.16
3	15	0	1	1	16	16	0.0	6.3	47.20	3.15
4	7	0	1	1	8	15	46.7	12.5	21.52	3.07
5	13	0	0	0	13	14	7.1	0.0	41.76	3.21
6	13	0	0	0	13	13	0.0	0.0	42.79	3.29
7	11	1	0	1	12	12	0.0	8.3	41.59	3.78
8	12	1	0	1	13	15	13.3	7.7	35.10	2.93
9	11	0	0	0	11	11	0.0	0.0	40.14	3.65
10	14	0	0	0	14	14	0.0	0.0	44.71	3.19
11	14	0	0	0	14	14	0.0	0.0	44.32	3.17
12	12	0	0	0	12	15	20.0	0.0	44.03	3.67
13	13	0	0	0	13	14	7.1	0.0	45.21	3.48
14	6	2	0	2	8	11	27.3	25.0	21.42	3.57
15	10	1	0	1	11	11	0.0	9.1	29.69	2.97
16	11	0	0	0	11	11	0.0	0.0	37.14	3.38
17	13	0	0	0	13	13	0.0	0.0	45.18	3.48
19	10	1	0	1	11	11	0.0	9.1	33.27	3.33
20	11	2	0	2	13	16	18.8	15.4	34.18	3.11
21	13	0	0	0	13	13	0.0	0.0	45.62	3.51
23	11	2	0	2	13	13	0.0	15.4	35.99	3.27
24	6	0	0	0	6	14	57.1	0.0	23.40	3.90
25	10	0	0	0	10	12	16.7	0.0	34.86	3.49
	Non-pregnant									
18										
22										

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APPENDIX 5

(Litter data - continued)

Group 2: Ziram, 1 mg/kg/day

Animal number	Live young	Embryonic Deaths Early	Late	Total	Implants	Corpora lutea	Pre-implant loss %	Post implant loss %	Litter wt.(g)	Mean foetal wt.(g)
	With live young									
26	12	1	1	2	14	14	0.0	14.3	36.44	3.04
27	3	1	0	1	4	7	42.9	25.0	9.50	3.17
28	8	1	0	1	9	9	0.0	11.1	28.66	3.58
29	11	1	0	1	12	13	7.7	8.3	36.94	3.36
30	11	0	1	1	12	13	7.7	8.3	34.29	3.12
31	12	0	0	0	12	13	7.7	0.0	42.40	3.53
32	5	1	0	1	6	14	57.1	16.7	17.67	3.53
33	9	0	0	0	9	9	0.0	0.0	30.43	3.38
34	11	3	0	3	14	15	6.7	21.4	33.99	3.09
35	11	0	0	0	11	12	8.3	0.0	37.72	3.43
36	6	1	0	1	7	14	50.0	14.3	17.15	2.86
37	13	1	0	1	14	14	0.0	7.1	45.10	3.47
38	11	1	0	1	12	12	0.0	8.3	38.09	3.46
39	13	0	0	0	13	16	18.8	0.0	43.03	3.31
40	12	1	0	1	13	14	7.1	7.7	44.15	3.68
42	11	1	0	1	12	13	7.7	8.3	31.78	2.89
43	14	0	0	0	14	14	0.0	0.0	45.34	3.24
44	12	0	0	0	12	13	7.7	0.0	39.06	3.26
45	11	0	0	0	11	11	0.0	0.0	35.94	3.27
46	13	0	0	0	13	13	0.0	0.0	42.04	3.23
47	11	1	1	2	13	15	13.3	15.4	36.64	3.33
48	12	0	0	0	12	12	0.0	0.0	39.13	3.26
49	12	0	0	0	12	16	25.0	0.0	35.30	2.94
50	11	2	0	2	13	14	7.1	15.4	39.76	3.61
	Non-pregnant									
41										

APPENDIX 5

(Litter data - continued)

Group 3: Ziram, 4 mg/kg/day

Animal number	Live young	Embryonic deaths Early	Late	Total	Implants	Corpora lutea	Pre-implant loss %	Post implant loss %	Litter wt.(g)	Mean foetal wt.(g)
	With live young									
51	9	0	0	0	9	9	0.0	0.0	30.52	3.39
53	12	1	0	1	13	14	7.1	7.7	40.37	3.36
54	3	0	0	0	3	9	66.7	0.0	10.34	3.45
55	11	0	0	0	11	11	0.0	0.0	33.67	3.06
56	10	1	0	1	11	11	0.0	9.1	31.85	3.19
57	11	1	0	1	12	13	7.7	8.3	36.87	3.35
58	12	0	0	0	12	12	0.0	0.0	40.44	3.37
59	13	1	0	1	14	14	0.0	7.1	41.89	3.22
60	14	0	0	0	14	14	0.0	0.0	38.75	2.77
61	13	0	0	0	13	15	13.3	0.0	44.89	3.45
62	13	0	0	0	13	13	0.0	0.0	44.36	3.41
63	11	0	0	0	11	12	8.3	0.0	38.32	3.48
64	11	2	0	2	13	13	0.0	15.4	36.16	3.29
65	11	0	0	0	11	11	0.0	0.0	32.43	2.95
67	13	0	0	0	13	19	31.6	0.0	44.37	3.41
68	16	0	0	0	16	16	0.0	0.0	51.98	3.25
69	15	0	0	0	15	16	6.3	0.0	46.31	3.09
70	11	1	0	1	12	12	0.0	8.3	37.62	3.42
71	12	0	0	0	12	17	29.4	0.0	43.10	3.59
73	12	0	0	0	12	15	20.0	0.0	36.51	3.04
74	10	1	0	1	11	12	8.3	9.1	36.46	3.65
75	10	1	0	1	11	11	0.0	9.1	30.60	3.06
	Non-pregnant									
52										
66										
72										

APPENDIX 5

(Litter data - continued)

Group 4: Ziram, 16 mg/kg/day

Animal number	Live young	Embryonic deaths Early	Late	Total	Implants	Corpora lutea	Pre-implant loss %	Post Implant Loss %	Litter wt.(g)	Mean foetal wt.(g)
	With live young									
76	14	0	0	0	14	15	6.7	0.0	37.84	2.70
77	11	1	0	1	12	14	14.3	8.3	33.52	3.05
78	13	0	0	0	13	15	13.3	0.0	37.71	2.90
79	12	0	0	0	12	13	7.7	0.0	38.16	3.18
80	11	1	0	1	12	12	0.0	8.3	33.75	3.07
81	9	2	0	2	11	14	21.4	18.2	29.10	3.23
82	12	0	1	1	13	13	0.0	7.7	40.06	3.34
83	12	1	0	1	13	13	0.0	7.7	38.26	3.19
84	13	0	0	0	13	13	0.0	0.0	40.41	3.11
85	14	0	0	0	14	15	6.7	0.0	47.42	3.39
86	12	0	0	0	12	12	0.0	0.0	39.60	3.30
87	13	0	0	0	13	13	0.0	0.0	38.91	2.99
88	11	1	0	1	12	12	0.0	8.3	38.59	3.51
89	11	2	0	2	13	13	0.0	15.4	34.74	3.16
90	10	1	0	1	11	14	21.4	9.1	37.50	3.75
91	10	0	0	0	10	10	0.0	0.0	33.37	3.34
92	9	1	0	1	10	13	23.1	10.0	29.82	3.31
93	14	1	0	1	15	15	0.0	6.7	43.02	3.07
94	11	0	0	0	11	11	0.0	0.0	34.99	3.18
97	9	1	0	1	10	11	9.1	10.0	30.49	3.39
98	13	0	0	0	13	16	18.8	0.0	42.58	3.28
99	11	1	0	1	12	13	7.7	8.3	34.37	3.12
100	11	0	0	0	11	11	0.0	0.0	34.60	3.15
	Non-pregnant									
95										
96										

APPENDIX 5

(Litter data - continued)

Group 5: Ziram, 64 mg/kg/day

Animal number	Live young	Embryonic deaths			Implants	Corpora lutea	Pre-implant loss %	Post implant loss %	Litter wt.(g)	Mean foetal wt.(g)
		Early	Late	Total						
	With live young									
101	12	0	0	0	12	12	0.0	0.0	36.35	3.03
102	11	1	0	1	12	12	0.0	8.3	30.26	2.75
103	11	0	0	0	11	11	0.0	0.0	36.06	3.28
104	13	0	0	0	13	13	0.0	0.0	38.83	2.99
105	9	1	0	1	10	10	0.0	10.0	23.00	2.56
106	7	0	0	0	7	10	30.0	0.0	22.13	3.16
107	14	0	0	0	14	14	0.0	0.0	39.70	2.84
108	12	0	0	0	12	14	14.3	0.0	35.99	3.00
109	12	0	0	0	12	12	0.0	0.0	35.64	2.97
110	8	0	0	0	8	10	20.0	0.0	22.88	2.86
111	11	2	0	2	13	13	0.0	15.4	36.97	3.36
112	13	1	0	1	14	14	0.0	7.1	39.88	3.07
113	12	0	0	0	12	12	0.0	0.0	37.82	3.15
114	15	0	0	0	15	15	0.0	0.0	42.51	2.83
115	12	0	0	0	12	12	0.0	0.0	32.48	2.71
116	12	0	0	0	12	12	0.0	0.0	35.82	2.99
117	10	2	0	2	12	12	0.0	16.7	29.14	2.91
118	11	1	0	1	12	16	25.0	8.3	34.48	3.13
120	11	0	1	1	12	12	0.0	8.3	36.97	3.36
121	11	0	1	1	12	14	14.3	8.3	33.42	3.04
122	8	0	0	0	8	8	0.0	0.0	24.56	3.07
123	13	1	0	1	14	15	6.7	7.1	36.34	2.80
124	13	0	0	0	13	14	7.1	0.0	40.67	3.13
125	11	0	0	0	11	11	0.0	0.0	28.76	2.61
	Non-pregnant									
119										

Sex ratios - individual values

Group 1: Control

Animal no.	♂	♀	Total	% Males
With live young				
1	5	7	12	41.7
2	7	6	13	53.8
3	6	9	15	40.0
4	2	5	7	28.6
5	5	8	13	38.5
6	4	9	13	30.8
7	8	3	11	72.7
8	7	5	12	58.3
9	8	3	11	72.7
10	6	8	14	42.9
11	7	7	14	50.0
12	7	5	12	58.3
13	6	7	13	46.2
14	4	2	6	66.7
15	4	6	10	40.0
16	7	4	11	63.6
17	3	10	13	23.1
19	6	4	10	60.0
20	5	6	11	45.5
21	4	9	13	30.8
23	5	6	11	45.5
24	4	2	6	66.7
25	3	7	10	30.0
Non-pregnant				
18				
22				

(Sex ratios - continued)

Group 2: Ziram, 1 mg/kg/day

Animal no.	♂	♀	Total	% Males
With live young				
26	7	5	12	58.3
27	1	2	3	33.3
28	6	2	8	75.0
29	4	7	11	36.4
30	7	4	11	63.6
31	6	6	12	50.0
32	2	3	5	40.0
33	4	5	9	44.4
34	6	5	11	54.5
35	9	2	11	81.8
36	4	2	6	66.7
37	8	5	13	61.5
38	7	4	11	63.6
39	7	6	13	53.8
40	6	6	12	50.0
42	7	4	11	63.6
43	8	6	14	57.1
44	8	4	12	66.7
45	8	3	11	72.7
46	6	7	13	46.2
47	8	3	11	72.7
48	8	4	12	66.7
49	6	6	12	50.0
50	6	5	11	54.5
Non-pregnant				
41				

(Sex ratios - continued)

Group 3: Ziram, 4 mg/kg/day

Animal no.	♂	♀	Total	% Males
With live young				
51	5	4	9	55.6
53	4	8	12	33.3
54	0	3	3	0.0
55	7	4	11	63.6
56	4	6	10	40.0
57	6	5	11	54.5
58	5	7	12	41.7
59	7	6	13	53.8
60	6	8	14	42.9
61	6	7	13	46.2
62	8	5	13	61.5
63	7	4	11	63.6
64	5	6	11	45.5
65	8	3	11	72.7
67	7	6	13	53.8
68	8	8	16	50.0
69	10	5	15	66.7
70	6	5	11	54.5
71	5	7	12	41.7
73	6	6	12	50.0
74	7	3	10	70.0
75	4	6	10	40.0
Non-pregnant				
52				
66				
72				

(Sex ratios - continued)

Group 4: Ziram, 16 mg/kg/day

Animal no.	♂	♀	Total	% Males
With live young				
76	9	5	14	64.3
77	5	6	11	45.5
78	7	6	13	53.8
79	5	7	12	41.7
80	5	6	11	45.5
81	5	4	9	55.6
82	6	6	12	50.0
83	9	3	12	75.0
84	3	10	13	23.1
85	10	4	14	71.4
86	5	7	12	41.7
87	7	6	13	53.8
88	5	6	11	45.5
89	4	7	11	36.4
90	5	5	10	50.0
91	5	5	10	50.0
92	7	2	9	77.8
93	10	4	14	71.4
94	6	5	11	54.5
97	5	4	9	55.6
98	7	6	13	53.8
99	2	9	11	18.2
100	6	5	11	54.5
Non-pregnant				
95				
96				

(Sex ratios - continued)

Group 5: Ziram, 64 mg/kg/day

Animal no.	♂	♀	Total	% Males
With live young				
101	3	9	12	25.0
102	4	7	11	36.4
103	3	8	11	27.3
104	7	6	13	53.8
105	3	6	9	33.3
106	4	3	7	57.1
107	7	7	14	50.0
108	8	4	12	66.7
109	5	7	12	41.7
110	5	3	8	62.5
111	9	2	11	81.8
112	4	9	13	30.8
113	7	5	12	58.3
114	3	12	15	20.0
115	3	9	12	25.0
116	6	6	12	50.0
117	5	5	10	50.0
118	7	4	11	63.6
120	5	6	11	45.5
121	4	7	11	36.4
122	4	4	8	50.0
123	10	3	13	76.9
124	10	3	13	76.9
125	9	2	11	81.8
Non-pregnant				
119				

Individual incidence of malformations and anomalies

Group 1: Control

Litter no.	Malformations			Anomalies‡					
				Allocated for visceral examination			Allocated for skeletal examination		
	Foetuses examined	Foetuses affected	%	Foetuses examined	Foetuses affected	%	Foetuses examined	Foetuses affected	%
1	12	0	0.0	6	0	0.0	6	1	16.7
2	13	0	0.0	7	0	0.0	6	2	33.3
3	15	0	0.0	7	2	28.6	8	3	37.5
4	7	1	14.3	3	1	33.3	3	2	66.7
5	13	0	0.0	6	0	0.0	7	4	57.1
6	13	0	0.0	7	0	0.0	6	1	16.7
7	11	0	0.0	5	0	0.0	6	1	16.7
8	12	0	0.0	6	0	0.0	6	1	16.7
9	11	1	9.1	4	1	25.0	6	1	16.7
10	14	1	7.1	7	0	0.0	6	2	33.3
11	14	1	7.1	6	0	0.0	7	0	0.0
12	12	0	0.0	6	1	16.7	6	3	50.0
13	13	0	0.0	6	0	0.0	7	0	0.0
14	6	0	0.0	3	1	33.3	3	2	66.7
15	10	0	0.0	5	0	0.0	5	0	0.0
16	11	0	0.0	6	1	16.7	5	1	20.0
17	13	0	0.0	6	0	0.0	7	0	0.0
19	10	0	0.0	5	0	0.0	5	0	0.0
20	11	0	0.0	6	1	16.7	5	1	20.0
21	13	0	0.0	6	0	0.0	7	2	28.6
23	11	0	0.0	5	1	20.0	6	1	16.7
24	6	0	0.0	3	0	0.0	3	1	33.3
25	10	0	0.0	5	0	0.0	5	0	0.0

‡ Malformed foetuses are excluded

(Individual incidence of malformations and anomalies - continued)

Group 2: Ziram, 1 mg/kg/day

Litter no.	Malformations			Anomalies‡					
	Foetuses examined	Foetuses affected	%	Allocated for visceral examination			Allocated for skeletal examination		
				Foetuses examined	Foetuses affected	%	Foetuses examined	Foetuses affected	%
26	12	0	0.0	6	1	16.7	6	3	50.0
27	3	0	0.0	1	0	0.0	2	0	0.0
28	8	0	0.0	4	2	50.0	4	1	25.0
29	11	1	9.1	4	0	0.0	6	0	0.0
30	11	0	0.0	6	1	16.7	5	2	40.0
31	12	0	0.0	6	0	0.0	6	1	16.7
32	5	0	0.0	3	0	0.0	2	0	0.0
33	9	0	0.0	4	0	0.0	5	0	0.0
34	11	0	0.0	6	0	0.0	5	0	0.0
35	11	0	0.0	5	0	0.0	6	4	66.7
36	6	0	0.0	3	0	0.0	3	0	0.0
37	13	0	0.0	6	0	0.0	7	0	0.0
38	11	0	0.0	6	0	0.0	5	1	20.0
39	13	0	0.0	6	0	0.0	7	0	0.0
40	12	0	0.0	6	1	16.7	6	1	16.7
42	11	0	0.0	6	1	16.7	5	3	60.0
43	14	0	0.0	7	0	0.0	7	0	0.0
44	12	0	0.0	6	0	0.0	6	0	0.0
45	11	0	0.0	5	0	0.0	6	0	0.0
46	13	1	7.7	6	0	0.0	6	2	33.3
47	11	0	0.0	5	0	0.0	6	3	50.0
48	12	0	0.0	6	0	0.0	6	0	0.0
49	12	0	0.0	6	0	0.0	6	3	50.0
50	11	0	0.0	6	0	0.0	5	2	40.0

‡ Malformed foetuses are excluded

(Individual incidence of malformations and anomalies - continued)

Group 3: Ziram, 4 mg/kg/day

Litter no.	Malformations			Anomalies‡					
	Foetuses examined	Foetuses affected	%	Allocated for visceral examination			Allocated for skeletal examination		
				Foetuses examined	Foetuses affected	%	Foetuses examined	Foetuses affected	%
51	9	0	0.0	4	0	0.0	5	1	20.0
53	12	0	0.0	6	1	16.7	6	3	50.0
54	3	0	0.0	2	0	0.0	1	0	0.0
55	11	0	0.0	5	1	20.0	6	4	66.7
56	10	0	0.0	5	0	0.0	5	2	40.0
57	11	0	0.0	5	0	0.0	6	1	16.7
58	12	0	0.0	6	0	0.0	6	0	0.0
59	13	0	0.0	6	0	0.0	7	2	28.6
60	14	0	0.0	7	0	0.0	7	3	42.9
61	13	0	0.0	6	1	16.7	7	1	14.3
62	13	0	0.0	7	2	28.6	6	2	33.3
63	11	0	0.0	5	2	40.0	6	0	0.0
64	11	0	0.0	6	0	0.0	5	0	0.0
65	11	0	0.0	5	0	0.0	6	2	33.3
67	13	1	7.7	5	0	0.0	7	0	0.0
68	16	0	0.0	8	1	12.5	8	3	37.5
69	15	0	0.0	7	0	0.0	8	2	25.0
70	11	0	0.0	6	0	0.0	5	0	0.0
71	12	0	0.0	6	0	0.0	6	0	0.0
73	12	0	0.0	6	1	16.7	6	4	66.7
74	10	0	0.0	5	0	0.0	5	1	20.0
75	10	0	0.0	5	0	0.0	5	0	0.0

‡ Malformed foetuses are excluded

(Individual incidence of malformations and anomalies - continued)

Group 4: Ziram, 16 mg/kg/day

Litter no.	Malformations			Anomalies‡					
	Foetuses examined	Foetuses affected	%	Allocated for visceral examination			Allocated for skeletal examination		
				Foetuses examined	Foetuses affected	%	Foetuses examined	Foetuses affected	%
76	14	0	0.0	7	2	28.6	7	1	14.3
77	11	0	0.0	5	1	20.0	6	2	33.3
78	13	0	0.0	7	0	0.0	6	1	16.7
79	12	0	0.0	6	1	16.7	6	0	0.0
80	11	0	0.0	6	2	33.3	5	0	0.0
81	9	0	0.0	4	0	0.0	5	1	20.0
82	12	0	0.0	6	0	0.0	6	1	16.7
83	12	0	0.0	6	1	16.7	6	4	66.7
84	13	0	0.0	7	0	0.0	6	3	50.0
85	14	0	0.0	7	1	14.3	7	2	28.6
86	12	0	0.0	6	1	16.7	6	0	0.0
87	13	0	0.0	6	1	16.7	7	1	14.3
88	11	0	0.0	6	0	0.0	5	1	20.0
89	11	0	0.0	5	1	20.0	6	0	0.0
90	10	0	0.0	5	1	20.0	5	0	0.0
91	10	1	10.0	4	1	25.0	5	0	0.0
92	9	0	0.0	5	1	20.0	4	0	0.0
93	14	0	0.0	7	1	14.3	7	4	57.1
94	11	0	0.0	6	0	0.0	5	2	40.0
97	9	0	0.0	4	0	0.0	5	3	60.0
98	13	0	0.0	7	3	42.9	6	1	16.7
99	11	0	0.0	5	1	20.0	6	2	33.3
100	11	0	0.0	6	1	16.7	5	1	20.0

‡ Malformed foetuses are excluded

(Individual incidence of malformations and anomalies - continued)

Group 5: Ziram, 64 mg/kg/day

Litter no.	Malformations			Anomalies‡					
	Foetuses examined	Foetuses affected	%	Allocated for visceral examination			Allocated for skeletal examination		
				Foetuses examined	Foetuses affected	%	Foetuses examined	Foetuses affected	%
101	12	0	0.0	6	0	0.0	6	2	33.3
102	11	0	0.0	6	2	33.3	5	0	0.0
103	11	0	0.0	5	0	0.0	6	0	0.0
104	13	0	0.0	7	2	28.6	6	2	33.3
105	9	0	0.0	4	1	25.0	5	1	20.0
106	7	0	0.0	4	0	0.0	3	1	33.3
107	14	0	0.0	7	0	0.0	7	2	28.6
108	12	0	0.0	6	0	0.0	6	0	0.0
109	12	0	0.0	6	0	0.0	6	1	16.7
110	8	0	0.0	4	1	25.0	4	0	0.0
111	11	0	0.0	5	0	0.0	6	1	16.7
112	13	1	7.7	7	0	0.0	5	0	0.0
113	12	1	8.3	5	1	20.0	6	2	33.3
114	15	0	0.0	8	0	0.0	7	2	28.6
115	12	1	8.3	5	1	20.0	6	4	66.7
116	12	0	0.0	6	2	33.3	6	1	16.7
117	10	1	10.0	4	1	25.0	5	0	0.0
118	11	0	0.0	6	0	0.0	5	3	60.0
120	11	0	0.0	6	0	0.0	5	0	0.0
121	11	0	0.0	5	0	0.0	6	2	33.3
122	8	0	0.0	4	0	0.0	4	0	0.0
123	13	0	0.0	6	2	33.3	7	4	57.1
124	13	0	0.0	7	2	28.6	6	1	16.7
125	11	0	0.0	5	1	20.0	6	1	16.7

‡ Malformed foetuses are excluded

APPENDIX 8

Malformations and anomalies - individual descriptions

Group 1: Control

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			Foetus		Description	Foetus		Description
	M	A	No.	Code		No.	Code	
1	0	1	-	-		9	A	Reduced ossification of sacro-caudal vertebral arches and pubes.
2	0	2	-	-		4	A	Shortened 13th left rib.
						10	A	Reduced ossification of pubes.
3	0	5	14	A	Absent innominate artery.	15	A	Misshapen 12th thoracic centrum.
			6	A	Abnormal lobation of liver.	9	A	Reduced ossification of occipital, sacro-caudal vertebral arches and pubes.
						3	A	Reduced ossification of sacro-caudal vertebral arches.
4	1	3	1	M	Diaphragmatic hernia.	2	A	Fused/partially fused bilateral squamosal to jugal; hemicentric 2nd thoracic vertebra; partially fused 6th to 7th right, minimal distortions affecting 9th left, 10th right ribs; bipartite 1st and 11th to 13th thoracic centra; reduced ossification of pubes.
			7	A	Small (2.45 g) with subcutaneous oedema.			
						6	A	Reduced ossification of sacro-caudal vertebral arches.

M Malformation
A Anomaly

APPENDIX 8

(Malformations and anomalies - continued)

Group 1: (continued)

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			Foetus		Description	Foetus		Description
	M	A	No.	Code		No.	Code	
5	0	4	-	-		5	A	Absent 13th bilateral rib. [Abnormal lobation of liver*].
						11	A	Absent 13th right, shortened 12th left rib.
						3	A	Reduced ossification of sacro-caudal vertebral arches.
						9	A	Reduced ossification of sacro-caudal vertebral arches.
6	0	1	-	-		2	A	Reduced ossification of cranial centres.
7	0	1	-	-		3	A	Reduced ossification of sacro-caudal vertebral arches and right pubis.
8	0	1	-	-		6	A	Reduced ossification of cranial centres, sacro-caudal vertebral arches and left pubis; unossified 2nd metacarpals.
9	1	2	8	M	Retro-oesophageal right subclavian artery.	5	A	Shortened 13th right rib; reduced ossification of sacro-caudal vertebral arches.
			10	A	Left testis displaced to midline.			

M Malformation
A Anomaly

* Observed during processing for skeletal examination not included in calculation of totals and means

APPENDIX 8

(Malformations and anomalies - continued)

Group 1: (continued)

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			Foetus		Description	Foetus		Description
	M	A	No.	Code		No.	Code	
10	1	2	-	-		12	M	Situs inversus (thoracic and abdominal).
						2	A	Minimal distortion affecting 11th right rib; reduced ossification of sacro-caudal vertebral arches.
						4	A	Reduced ossification of sacro-caudal vertebral arches.
11	1	0	6	M	Atrial and interventricular septal defect with enlarged right, reduced left ventricles, incomplete inferior vena cava with venous drainage of lower body regions via persistent left posterior cardinal vein: situs inversus. Also, oesophagus displaced to left of trachea; abnormal lobation of lungs.			
12	0	4	9	A	Moderate subdural haemorrhage medulla oblongata region. (brain).	8	A	Shortened 13th bilateral rib; one less thoracic-lumber vertebra.
						12	A	Reduced ossification of sacro-caudal vertebral arches and pubes.
						2	A	Reduced ossification of left pubis.
13	0	0	-	-		-	-	

M Malformation
A Anomaly

APPENDIX 8

(Malformations and anomalies - continued)

Group 1: (continued)

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			Foetus		Description	Foetus		Description
	M	A	No.	Code		No.	Code	
14	0	3	5	A	Anterior displacement right testis.	2	A	Shortened 13th right rib; reduced ossification of sacro-caudal vertebral arches.
						6	A	Reduced ossification of sacro-caudal vertebral arches. (Abnormal lobation of liver*).
15	0	0	-	-		-	-	
16	0	2	11	A	Increased dilatation bilateral renal pelvis.	6	A	Shortened 13th bilateral rib.
17	0	0	-	-		-	-	
19	0	0	-	-		-	-	
20	0	2	9	A	Increased dilatation right renal pelvis.	10	A	Reduced ossification of sacro-caudal vertebral arches.
21	0	2	-	-		5	A	Small (2.04 g) with bilateral cervical rib; reduced ossification of sacro-caudal vertebral arches and left pubis.
						9	A	Shortened 13th right rib; reduced ossification of interparietal.

M Malformation
A Anomaly

* Observed during processing for skeletal examination not included in calculation of totals and means

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APPENDIX 8

(Malformations and anomalies - continued)

Group 1: (continued)

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			Foetus		Description	Foetus		Description
	M	A	No.	Code		No.	Code	
23	0	2	8	A	Minimal subdural haemorrhage left cerebral region (brain).	1	A	Reduced ossification of sacro-caudal vertebral arches.
24	0	1	-	-		6	A	Reduced ossification of sacro-caudal vertebral arches.
25	0	0	-	-		-	-	

M Malformation
A Anomaly

APPENDIX 8

(Malformations and anomalies - continued)

Group 2: Ziram, 1 mg/kg/day

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			Foetus		Description	Foetus		Description
	M	A	No.	Code		No.	Code	
26	0	4	9	A	Intra-abdominal haemorrhage.	6	A	Absent 13th left rib; reduced ossification of occipital and sacro-caudal vertebral arches.
						10	A	Reduced ossification of sacro-caudal vertebral arches.
						4	A	Unossified right pubis.
27	0	0	-	-		-	-	
28	0	3	5	A	Increased dilatation right renal pelvis.	8	A	Bipartite 10th thoracic centrum.
			7	A	Increased dilatation right renal pelvis.			

M Malformation
A Anomaly

APPENDIX 8

(Malformations and anomalies - continued)

Group 2: (continued)

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			Foetus		Description	Foetus		Description
	M	A	No.	Code		No.	Code	
29	1	0	2	M	Small (2.45 g), misshapen lower jaw with absent tongue and posterior palatine cleft; transposition of ascending aorta and narrow pulmonary trunk; atrial and interventricular septal defects; incomplete inferior vena cava with venous drainage of lower body regions via persistent right posterior cardinal vein; abdominal situs inversus; reduced genital papilla with anal atresia also, abnormal lobation of atelectatic lungs anterior displacement of right adrenal; increased dilatation bilateral renal pelvis; subcutaneous haemorrhage dorso-lumbar region; subcutaneous oedema.	-	-	
30	0	3	9	A	Increased dilatation right renal pelvis.	2	A	Reduced ossification of sacro-caudal vertebral arches.
						10	A	Reduced ossification of sacro-caudal vertebral arches.
31	0	1	-	-		3	A	Reduced ossification of sacro-caudal vertebral arches.
32	0	0	-	-		-	-	

M Malformation
A Anomaly

APPENDIX 8

(Malformations and anomalies - continued)

Group 2: (continued)

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			Foetus		Description	Foetus		Description
	M	A	No.	Code		No.	Code	
33	0	0	-	-		-	-	
34	0	0	-	-		-	-	
35	0	4	-	-		9	A	Butterfly-shaped 9th thoracic centrum.
						3	A	Bipartite thoracic 12th thoracic centrum; shortened 13th left rib.
						7	A	Reduced ossification of interparietal
36	0	0	-	-		11	A	Reduced ossification of interparietal
37	0	0	-	-		-	-	
38	0	1	-	-		4	A	Shortened 13th right rib.
39	0	0	-	-		-	-	
40	0	2	11	A	Increased dilatation right renal pelvis.	8	A	Reduced ossification of sacro-caudal vertebral arches.
42	0	4	7	A	Increased dilatation right renal pelvis.	4	A	Reduced ossification of sacro-caudal vertebral arches. [Left testis displaced to midline]*
						8	A	Reduced ossification of sacro-caudal vertebral arches.
						10	A	Reduced ossification of sacro-caudal vertebral arches.

M Malformation
A Anomaly

* Observed during processing for skeletal examination. Not included in calculation of total and means.

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APPENDIX 8

(Malformations and anomalies - continued)

Group 2: (continued)

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			Foetus		Description	Foetus		Description
	M	A	No.	Code		No.	Code	
43	0	0	-	-		-	-	
44	0	0	-	-		-	-	
45	0	0	-	-		-	-	
46	1	2	3	M	Small (2.32 g) with right microphthalmia	2	A	Reduced ossification of occipital.
						4	A	Reduced ossification of sacro-caudal vertebral arches and left pubis.
47	0	3	-	-		9	A	Shortened 13th left rib.
						1	A	Reduced ossification of sacro-caudal vertebral arches and right pubis.
						5	A	Reduced ossification of right pubis.
48	0	0	-	-		-	-	
49	0	3	-	-		5	A	Shortened 13th left rib.
						7	A	Reduced ossification of sacro-caudal vertebral arches.
						9	A	Reduced ossification of sacro-caudal vertebral arches.

M Malformation
A Anomaly

APPENDIX 8

(Malformations and anomalies - continued)

Group 2: (continued)

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			Foetus		Description	Foetus		Description
	M	A	No.	Code		No.	Code	
50	0	2	-	-		10 2	A A	Shortened 13th left rib. Reduced ossification of sacro-caudal vertebral arches.

M Malformation
A Anomaly

APPENDIX 8

(Malformations and anomalies - continued)

Group 3: Ziram, 4 mg/kg/day

Litter No.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			Foetus		Description	Foetus		Description
	M	A	No.	Code		No.	Code	
51	0	1	-	-		3	A	Bipartite 10th thoracic centrum.
53	0	4	8	A	Thinning of diaphragm with slight protrusion of right median liver lobe.	3	A	Minimal distortions affecting 6th, 10th and 11th bilateral, 7th and 8th left, 9th right ribs; reduced ossification of cranial centres and sacro-caudal vertebral arches.
						7	A	Butterfly-shaped 10th thoracic centrum.
						9	A	Shortened 13th right rib.
54	0	0	-	-		-	-	
55	0	5	10	A	Absent innominate artery.	9	A	Right cervical rib.
						3	A	Shortened 13th right rib.
						11	A	Reduced ossification of occipital.
						7	A	Reduced ossification of sacro-caudal vertebral arches.
56	0	2	-	-		6	A	Reduced ossification of sacro-caudal vertebral arches.
						10	A	Reduced ossification of sacro-caudal vertebral arches.
57	0	1	-	-		1	A	Bipartite 7th thoracic centrum.
58	0	0	-	-		-	-	

M Malformation
A Anomaly

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APPENDIX 8

(Malformations and anomalies - continued)

Group 3: (continued)

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			Foetus		Description	Foetus		Description
	M	A	No.	Code		No.	Code	
59	0	2	-	-		3	A	Reduced ossification of sacro-caudal vertebral arches and left pubis.
60	0	3	-	-		13	A	Reduced ossification of sacro-caudal vertebral arches.
						14	A	Shortened 13th right rib; reduced ossification of sacro-caudal vertebral arches.
						10	A	Shortened 13th right rib.
						6	A	Shortened 13th left rib.
61	0	2	12	A	Misshapen right median liver lobe.	1	A	Shortened 13th right rib.
62	0	4	3	A	Partial duplication of inferior vena cava.	10	A	Bipartite 11th thoracic centrum.
			13	A	Abnormal lobation of liver.	6	A	Reduced ossification of occipital and sacro-caudal vertebral arches.
63	0	2	8	A	Moderate subdural haemorrhage right cerebral region (brain).	-	-	
			10	A	Increased dilatation left renal pelvis.			
64	0	0	-	-		-	-	

M Malformation
A Anomaly

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APPENDIX 8

(Malformations and anomalies - continued)

Group 3: (continued)

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			Foetus		Description	Foetus		Description
	M	A	No.	Code		No.	Code	
65	0	2	-	-		3	A	Small (2.32 g) with bipartite 11th thoracic centrum.
						5	A	Reduced ossification of sacro-caudal vertebral arches.
67	1	0	6	M	Diaphragmatic hernia.	-	-	
68	0	4	13	A	Increased dilatation right renal pelvis.	16	A	Misshapen 12th thoracic centrum.
						4	A	Left lumbar rib.
						10	A	Reduced ossification of sacro-caudal vertebral arches and right pubis.
69	0	2	-	-		5	A	Reduced ossification of cranial centres, cervical and sacro-caudal vertebral arches.
						7	A	Reduced ossification of sacro-caudal vertebral arches.
70	0	0	-	-		-	-	
71	0	0	-	-		-	-	

M Malformation
A Anomaly

APPENDIX 8

(Malformations and anomalies - continued)

Group 3: (continued)

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			Foetus		Description	Foetus		Description
	M	A	No.	Code		No.	Code	
73	0	5	8	A	Increased dilatation right renal pelvis.	11	A	Bilateral cervical rib; absent 13th bilateral rib; asymmetric pelvic girdle.
						1	A	Reduced ossification of sacro-caudal vertebral arches.
						7	A	Reduced ossification of sacro-caudal vertebral arches.
						9	A	Reduced ossification of sacro-caudal vertebral arches.
74	0	1	-	-		8	A	Left lumbar rib.
75	0	0	-	-		-	-	

M Malformation
A Anomaly

APPENDIX 8

(Malformations and anomalies - continued)

Group 4: Ziram, 16 mg/kg/day

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			Foetus		Description	Foetus		Description
	M	A	No.	Code		No.	Code	
76	0	3	9	A	Absent innominate artery.	14	A	Small (2.11 g) with unossified 5th metatarsals.
			7	A	Increased dilatation left renal pelvis.			
77	0	3	6	A	Minimal haemorrhage anterior chamber left eye.	7	A	Reduced ossification of occipital, sacro-caudal vertebral arches and pelvic girdle; unossified 5th metatarsals.
						11	A	Small (2.30 g) with unossified 5th metatarsals.
78	0	1	-	-		12	A	Shortened 13th right rib.
79	0	1	8	A	Increased dilatation right renal pelvis.	-	-	
80	0	2	7	A	Thinning of diaphragm with slight protrusion of right median liver lobe.			
			11	A	Increased dilatation right renal pelvis			
81	0	1	-	-		1	A	Reduced ossification of occipital.
82	0	1	-	-		2	A	Shortened 13th right rib.

M Malformation
A Anomaly

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APPENDIX 8

(Malformations and anomalies - continued)

Group 4: (continued)

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			Foetus		Description	Foetus		Description
	M	A	No.	Code		No.	Code	
83	0	5	8	A	Minimal haemorrhage anterior chamber left eye.	9	A	Reduced ossification of cranial centres (minimal).
						1	A	Reduced ossification of sacro-caudal vertebral arches.
						5	A	Reduced ossification of sacro-caudal vertebral arches.
						7	A	Reduced ossification of sacro-caudal vertebral arches.
84	0	3	-	-		6	A	Reduced ossification of sacro-caudal vertebral arches.
						8	A	Reduced ossification of sacro-caudal vertebral arches.
						10	A	Reduced ossification of sacro-caudal vertebral arches.
85	0	3	12	A	Thinning of diaphragm with slight protrusion of median liver lobe.	13	A	Two less thoraco-lumbar vertebrae; absent 12th and 13th bilateral ribs.
						7	A	Connected 3rd and 4th sternebrae with asymmetric alignment of costal cartilage elements.
86	0	1	3	A	Posterior displacement of innominate artery.	6	-	[Abnormal lobation of liver]*
87	0	2	2	A	Moderate haemorrhage anterior chamber left eye.	9	A	Reduced ossification of sacro-caudal vertebral arches.
88	0	1	-	-		6	A	Misshapen 1st sternebra.

M Malformation
A Anomaly

* Observed during processing for skeletal examination, not included in calculation of totals and means

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APPENDIX 8

(Malformations and anomalies - continued)

Group 4: (continued)

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			Foetus		Description	Foetus		Description
	M	A	No.	Code		No.	Code	
89	0	1	6	A	Minimal haemorrhage anterior chamber right eye; thinning of diaphragm with slight protrusion of right median liver lobe.	-	-	
90	0	1	9	A	Moderate haemorrhage within 4th ventricle (brain).	-	-	
91	1	1	2	M	Atrial and interventricular septal defects; incomplete inferior vena cava with venous drainage of lower body regions via persistent left posterior cardinal vein. Also, oesophagus displaced to right of trachea; abnormal lobation of lungs; anterior displacement of left adrenal.	-	-	
			10	A	Minimal increased dilatation left lateral ventricle.			
92	0	1	1	A	Moderate subdural haemorrhage right cerebral region (brain); minimal subdural haemorrhage medulla oblongata region (brain).	-	-	

M Malformation
A Anomaly

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APPENDIX 8

(Malformations and anomalies - continued)

Group 4: (continued)

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			Foetus		Description	Foetus		Description
	M	A	No.	Code		No.	Code	
93	0	5	10	A	Anterior displacement left testis	9	A	Shortened 13th right rib.
						7	A	Reduced ossification of sacro-caudal vertebral arches and pubes.
						3	A	Reduced ossification of sacro-caudal vertebral arches.
						13	A	Reduced ossification of sacro-caudal vertebral arches.
94	0	2	-	-		2	A	Reduced ossification of cranial centres and sacro-caudal vertebral arches; unossified pubes.
						6	A	Reduced ossification of cranial centres, sacro-caudal vertebral arches and left pubis.
97	0	3	-	-		7	A	Shortened 13th right rib.
						3	A	Left lumbar rib; reduced ossification of sacro-caudal vertebral arches.
						9	A	Reduced ossification of sacro-caudal vertebral arches.
98	0	4	7	A	Thinning of diaphragm with slight protrusion of right median liver lobe.	6	A	Asymmetric alignment of costal cartilage elements.
			11	A	Thinning of diaphragm with slight protrusion of right median liver lobe.	2	-	[Right testis displaced to midline]*
			3	A	Increased dilatation right renal pelvis.			

M Malformation
A Anomaly

* Observed during processing for skeletal examination not included in calculation of totals and means

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APPENDIX 8

(Malformations and anomalies - continued)

Group 4: (continued)

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			Foetus		Description	Foetus		Description
	M	A	No.	Code		No.	Code	
99	0	3	10	A	Increased dilatation right renal pelvis.	7 11	A A	Reduced ossification of occipital. Reduced ossification of sacro-caudal vertebral arches.
100	0	2	1	A	Minimal haemorrhage within 4th ventricle (brain).	10	A	Bilateral lumbar rib.

M Malformation
A Anomaly

APPENDIX 8

(Malformations and anomalies - continued)

Group 5: Ziram, 64 mg/kg/day

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			Foetus		Description	Foetus		Description
	M	A	No.	Code		No.	Code	
101	0	2	-	-		9	A	Misshapen 7th left cervical vertebral arch.
						11	A	Reduced ossification of sacro-caudal vertebral arches.
102	0	2	7	A	Small right eye.	-	-	
			9	A	Marked subcutaneous haemorrhage nasal region.			
103	0	0	-	-		-	-	
104	0	4	5	A	Minimal subdural haemorrhage left cerebral region (brain).	2	A	Bipartite 11th thoracic centrum.
						10	A	Reduced ossification of sacro-caudal vertebral arches.
			7	A	Minimal subdural haemorrhage left cerebral region (brain).			
105	0	2	6	A	Abnormal lobation of liver.	7	A	Small (2.10 g) with shortened 13th bilateral rib; reduced ossification of sacro-caudal vertebral arches.
106	0	1	-	-		4	A	Bipartite 5th thoracic centrum.
107	0	2	-	-		13	A	Reduced ossification of cervical vertebral arches.
						7	A	Reduced ossification of sacro-caudal vertebral arches.
108	0	0	-	-		-	-	

M Malformation
A Anomaly

APPENDIX 8

(Malformations and anomalies - continued)

Group 5: (continued)

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			Foetus		Description	Foetus		Description
	M	A	No.	Code		No.	Code	
109	0	1	-	-		5	A	Shortened 13th right rib.
110	0	1	5	A	Thinning of diaphragm with slight protrusion of median liver lobe.	-	-	
111	0	1	-	-		3	A	Shortened 13th right rib.
112	1	0	-	-		1	M	Small (2.35 g) with umbilical hernia. Also, unossified 5th metatarsals.
113	1	3	4	M	Right microphthalmia; incomplete inferior vena cava with venous drainage of lower body regions via persistent left posterior cardinal vein. Also absent innominate artery; displaced left adrenal.	5	A	Absent 10th thoracic centrum with reduced 10th left thoracic vertebral arch.
						7	A	Absent 13th left, shortened right rib.
			12	A	Moderate haemorrhage 4th ventricle (brain); minimal subdural haemorrhage medulla oblongata region (brain).			
114	0	2	-	-		2	A	Absent 13th left, shortened 13th right rib.
						12	A	Shortened 13th right rib.

M Malformation
A Anomaly

APPENDIX 8

(Malformations and anomalies - continued)

Group 5: (continued)

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			Foetus		Description	Foetus		Description
	M	A	No.	Code		No.	Code	
115	1	5	12	M	Small (2.42 g) with bilateral microphthalmia.	9	A	Small (1.64 g) with shortened 13th left rib; reduced ossification of interparietal, occipital, sacro-caudal vertebral arches and pubes; unossified 5th metatarsals.
			10	A	Thinning of diaphragm with slight protrusion of right median liver lobe.	5	A	Shortened 13th right rib; reduced ossification of occipital.
						11	A	Reduced ossification of occipital and sacro-caudal vertebral arches.
						3	A	Reduced ossification of sacro-caudal vertebral arches.
116	0	3	3	A	Minimal haemorrhage within 4th ventricle (brain).	6	A	Reduced ossification of sacro-caudal vertebral arches.
			5	A	Increased dilatation right renal pelvis.			
117	1	1	8	M	Right sided aorta and pulmonary trunk/ductus arteriosus. Also, thinning of diaphragm with slight protrusion of right median liver lobe.	-	-	
			6	A	Increased dilatation right renal pelvis.			

M Malformation
A Anomaly

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APPENDIX 8

(Malformations and anomalies - continued)

Group 5: (continued)

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			Foetus		Description	Foetus		Description
	M	A	No.	Code		No.	Code	
118	0	3	-	-		4	A	Butterfly-shaped 12th thoracic centrum.
						10	A	Shortened 13th right rib.
						2	A	Reduced ossification of sacro-caudal vertebral arches.
						6	-	[Right testis displaced to midline]*
120	0	0	-	-		-	-	
121	0	2	-	-		9	A	Bipartite 11th and 13th thoracic centra.
						1	A	Bipartite 13th thoracic centrum.
122	0	0	-	-		-	-	
123	0	6	8	A	Thinning of diaphragm with slight protrusion of right median liver lobe.	5	A	Hemicentric 3rd thoracic vertebra.
						7	A	Bipartite 11th thoracic centrum.
						9	A	Butterfly-shaped 11th thoracic centrum.
			4	A	Small (2.48 g) with increased dilatation bilateral renal pelvis.	3	A	Small (2.01 g) with reduced ossification of right pubis; unossified 5th metatarsals.
124	0	3	13	A	Minimal subdural haemorrhage right cerebral region (brain).	12	A	Reduced ossification of sacro-caudal vertebral arches and right pubis.
			11	A	Thinning of diaphragm with slight protrusion of median liver lobe.			

M Malformation
A Anomaly

* Observed during processing for skeletal examination not included in calculation of totals and means

APPENDIX 8

(Malformations and anomalies - continued)

Group 5: (continued)

Litter no.	Foetuses affected		Allocated for visceral examination			Allocated for skeletal examination		
			Foetus		Description	Foetus		Description
	M	A	No.	Code		No.	Code	
125	0	2	8	A	Thinning of diaphragm with slight protrusion of median liver lobe.	7	A	Small (2.05 g) with reduced ossification of pubes; unossified 5th metatarsals.

M Malformation
A Anomaly

APPENDIX 9

Skeletal variants - individual incidence

Group 1: Control

Rat no.	Foetuses examined	Foetuses‡ with									
		Normal sternebrae		Unossified sternebrae		Reduced sternebrae		Asym./bip. sternebrae		Total variant sternebrae	
		No.	%	No.	%	No.	%	No.	%	No.	%
1	6	0	0.0	5	83.3	3	50.0	1	16.7	6	100.0
2	6	0	0.0	5	83.3	3	50.0	2	33.3	6	100.0
3	8	0	0.0	5	62.5	4	50.0	0	0.0	8	100.0
4	3	0	0.0	3	100.0	2	66.7	0	0.0	3	100.0
5	7	0	0.0	5	71.4	3	42.9	0	0.0	7	100.0
6	6	1	16.7	5	83.3	2	33.3	0	0.0	5	83.3
7	6	2	33.3	3	50.0	1	16.7	0	0.0	4	66.7
8	6	0	0.0	6	100.0	0	0.0	0	0.0	6	100.0
9	6	0	0.0	3	50.0	6	100.0	0	0.0	6	100.0
10	6	3	50.0	3	50.0	1	16.7	0	0.0	3	50.0
11	7	4	57.1	1	14.3	2	28.6	0	0.0	3	42.9
12	6	3	50.0	1	16.7	2	33.3	0	0.0	3	50.0
13	7	5	71.4	0	0.0	2	28.6	0	0.0	2	28.6
14	3	2	66.7	1	33.3	0	0.0	0	0.0	1	33.3
15	5	0	0.0	4	80.0	4	80.0	1	20.0	5	100.0
16	5	0	0.0	3	60.0	2	40.0	1	20.0	5	100.0
17	7	0	0.0	6	85.7	1	14.3	0	0.0	7	100.0
19	5	1	20.0	4	80.0	2	40.0	0	0.0	4	80.0
20	5	0	0.0	4	80.0	2	40.0	0	0.0	5	100.0
21	7	3	42.9	1	14.3	4	57.1	0	0.0	4	57.1
23	6	0	0.0	5	83.3	5	83.3	0	0.0	6	100.0
24	3	2	66.7	1	33.3	0	0.0	0	0.0	1	33.3
25	5	2	40.0	3	60.0	0	0.0	0	0.0	3	60.0

‡ Malformed foetuses are excluded

(Skeletal variants - continued)

Group 2: Ziram, 1 mg/kg/day

Rat no.	Foetuses examined	Foetuses‡ with									
		Normal sternebrae		Unossified sternebrae		Reduced sternebrae		Asym./bip. sternebrae		Total variant sternebrae	
		No.	%	No.	%	No.	%	No.	%	No.	%
26	6	0	0.0	6	100.0	3	50.0	1	16.7	6	100.0
27	2	0	0.0	2	100.0	0	0.0	0	0.0	2	100.0
28	4	0	0.0	4	100.0	2	50.0	1	25.0	4	100.0
29	6	2	33.3	3	50.0	3	50.0	0	0.0	4	66.7
30	5	0	0.0	5	100.0	3	60.0	0	0.0	5	100.0
31	6	1	16.7	3	50.0	2	33.3	0	0.0	5	83.3
32	2	2	100.0	0	0.0	0	0.0	0	0.0	0	0.0
33	5	0	0.0	4	80.0	3	60.0	0	0.0	5	100.0
34	5	0	0.0	4	80.0	4	80.0	0	0.0	5	100.0
35	6	0	0.0	2	33.3	4	66.7	0	0.0	6	100.0
36	3	0	0.0	3	100.0	2	66.7	0	0.0	3	100.0
37	7	2	28.6	2	28.6	4	57.1	1	14.3	5	71.4
38	5	0	0.0	3	60.0	2	40.0	0	0.0	5	100.0
39	7	1	14.3	2	28.6	5	71.4	1	14.3	6	85.7
40	6	2	33.3	3	50.0	2	33.3	0	0.0	4	66.7
42	5	0	0.0	4	80.0	2	40.0	0	0.0	5	100.0
43	7	1	14.3	4	57.1	2	28.6	0	0.0	6	85.7
44	6	1	16.7	4	66.7	2	33.3	0	0.0	5	83.3
45	6	1	16.7	3	50.0	3	50.0	0	0.0	5	83.3
46	6	3	50.0	0	0.0	3	50.0	0	0.0	3	50.0
47	6	1	16.7	3	50.0	2	33.3	0	0.0	5	83.3
48	6	1	16.7	3	50.0	3	50.0	0	0.0	5	83.3
49	6	0	0.0	5	83.3	2	33.3	0	0.0	6	100.0
50	5	2	40.0	1	20.0	2	40.0	0	0.0	3	60.0

‡ Malformed foetuses are excluded

APPENDIX 9

(Skeletal variants - continued)

Group 3: Ziram, 4 mg/kg/day

Rat no.	Foetuses examined	Foetuses‡ with									
		Normal sternebrae		Unossified sternebrae		Reduced sternebrae		Asym./bip. sternebrae		Total variant sternebrae	
		No.	%	No.	%	No.	%	No.	%	No.	%
51	5	1	20.0	4	80.0	3	60.0	0	0.0	4	80.0
53	6	1	16.7	5	83.3	1	16.7	0	0.0	5	83.3
54	1	0	0.0	0	0.0	1	100.0	0	0.0	1	100.0
55	6	0	0.0	6	100.0	2	33.3	0	0.0	6	100.0
56	5	0	0.0	3	60.0	2	40.0	0	0.0	5	100.0
57	6	2	33.3	2	33.3	3	50.0	0	0.0	4	66.7
58	6	2	33.3	2	33.3	2	33.3	0	0.0	4	66.7
59	7	0	0.0	3	42.9	6	85.7	0	0.0	7	100.0
60	7	0	0.0	6	85.7	1	14.3	0	0.0	7	100.0
61	7	3	42.9	4	57.1	2	28.6	0	0.0	4	57.1
62	6	1	16.7	4	66.7	2	33.3	0	0.0	5	83.3
63	6	4	66.7	0	0.0	2	33.3	0	0.0	2	33.3
64	5	3	60.0	1	20.0	1	20.0	0	0.0	2	40.0
65	6	0	0.0	5	83.3	2	33.3	0	0.0	6	100.0
67	7	1	14.3	3	42.9	4	57.1	0	0.0	6	85.7
68	8	2	25.0	4	50.0	3	37.5	0	0.0	6	75.0
69	8	1	12.5	3	37.5	4	50.0	0	0.0	7	87.5
70	5	3	60.0	2	40.0	1	20.0	0	0.0	2	40.0
71	6	2	33.3	4	66.7	1	16.7	0	0.0	4	66.7
73	6	0	0.0	4	66.7	4	66.7	1	16.7	6	100.0
74	5	0	0.0	3	60.0	3	60.0	0	0.0	5	100.0
75	5	0	0.0	1	20.0	4	80.0	1	20.0	5	100.0

‡ Malformed foetuses are excluded

(Skeletal variants - continued)

Group 4: Ziram, 16 mg/kg/day

Rat no.	Foetuses examined	Foetuses‡ with									
		Normal sternebrae		Unossified sternebrae		Reduced sternebrae		Asym./bip. sternebrae		Total variant sternebrae	
		No.	%	No.	%	No.	%	No.	%	No.	%
76	7	0	0.0	7	100.0	3	42.9	0	0.0	7	100.0
77	6	0	0.0	4	66.7	5	83.3	0	0.0	6	100.0
78	6	0	0.0	6	100.0	1	16.7	1	16.7	6	100.0
79	6	0	0.0	3	50.0	5	83.3	0	0.0	6	100.0
80	5	1	20.0	3	60.0	2	40.0	1	20.0	4	80.0
81	5	1	20.0	2	40.0	2	40.0	0	0.0	4	80.0
82	6	0	0.0	2	33.3	5	83.3	0	0.0	6	100.0
83	6	0	0.0	4	66.7	2	33.3	0	0.0	6	100.0
84	6	0	0.0	6	100.0	5	83.3	0	0.0	6	100.0
85	7	1	14.3	4	57.1	4	57.1	1	14.3	6	85.7
86	6	6	100.0	0	0.0	0	0.0	0	0.0	0	0.0
87	7	2	28.6	4	57.1	2	28.6	1	14.3	5	71.4
88	5	3	60.0	1	20.0	1	20.0	0	0.0	2	40.0
89	6	0	0.0	3	50.0	4	66.7	0	0.0	6	100.0
90	5	3	60.0	0	0.0	2	40.0	0	0.0	2	40.0
91	5	4	80.0	1	20.0	1	20.0	0	0.0	1	20.0
92	4	1	25.0	2	50.0	2	50.0	0	0.0	3	75.0
93	7	0	0.0	5	71.4	3	42.9	0	0.0	7	100.0
94	5	0	0.0	4	80.0	1	20.0	0	0.0	5	100.0
97	5	1	20.0	3	60.0	4	80.0	0	0.0	4	80.0
98	6	1	16.7	3	50.0	4	66.7	1	16.7	5	83.3
99	6	1	16.7	5	83.3	1	16.7	1	16.7	5	83.3
100	5	0	0.0	2	40.0	4	80.0	0	0.0	5	100.0

‡ Malformed foetuses are excluded

(Skeletal variants - continued)

Group 5: Ziram, 64 mg/kg/day

Rat no.	Foetuses examined	Foetuses‡ with									
		Normal sternebrae		Unossified sternebrae		Reduced sternebrae		Asym./bip. sternebrae		Total variant sternebrae	
		No.	%	No.	%	No.	%	No.	%	No.	%
101	6	1	16.7	5	83.3	1	16.7	0	0.0	5	83.3
102	5	0	0.0	5	100.0	1	20.0	0	0.0	5	100.0
103	6	4	66.7	2	33.3	1	16.7	0	0.0	2	33.3
104	6	1	16.7	3	50.0	3	50.0	0	0.0	5	83.3
105	5	0	0.0	5	100.0	1	20.0	0	0.0	5	100.0
106	3	1	33.3	2	66.7	1	33.3	0	0.0	2	66.7
107	7	1	14.3	4	57.1	3	42.9	0	0.0	6	85.7
108	6	0	0.0	4	66.7	2	33.3	0	0.0	6	100.0
109	6	0	0.0	3	50.0	3	50.0	1	16.7	6	100.0
110	4	0	0.0	3	75.0	2	50.0	0	0.0	4	100.0
111	6	3	50.0	2	33.3	3	50.0	0	0.0	3	50.0
112	5	0	0.0	3	60.0	3	60.0	0	0.0	5	100.0
113	6	0	0.0	4	66.7	4	66.7	0	0.0	6	100.0
114	7	0	0.0	6	85.7	4	57.1	0	0.0	7	100.0
115	6	1	16.7	3	50.0	2	33.3	0	0.0	5	83.3
116	6	1	16.7	5	83.3	2	33.3	0	0.0	5	83.3
117	5	0	0.0	4	80.0	2	40.0	0	0.0	5	100.0
118	5	0	0.0	5	100.0	1	20.0	0	0.0	5	100.0
120	5	1	20.0	2	40.0	3	60.0	0	0.0	4	80.0
121	6	0	0.0	4	66.7	3	50.0	1	16.7	6	100.0
122	4	0	0.0	2	50.0	3	75.0	0	0.0	4	100.0
123	7	0	0.0	5	71.4	3	42.9	0	0.0	7	100.0
124	6	1	16.7	4	66.7	3	50.0	1	16.7	5	83.3
125	6	0	0.0	6	100.0	3	50.0	1	16.7	6	100.0

‡ Malformed foetuses are excluded

Pre-treatment health check

Macroscopic examination revealed one female animal with increased bilateral renal pelvic dilatation. Histological examination confirmed this observation. There were no changes considered to be related to the presence of infectious disease. Subsequent veterinary inspection of remaining animals prior to commencement of treatment confirmed their general health and appearance to be satisfactory.

PART II

A PRELIMINARY STUDY OF THE EFFECT OF ZIRAM ON PREGNANCY OF THE RAT

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ADDENDUM

1. Pre-treatment health check 146

1. In this preliminary study of the effect of the fungicide, Ziram Technical on pregnancy of the rat, daily dosages 0(Control), 5, 20 and 80 mg/kg/day were administered by oral gavage to pregnant animals from Day 6 through to Day 15 of pregnancy inclusive. On Day 20 of pregnancy, the females were killed and subjected to post mortem examination. Litter parameters were determined and foetuses were examined for gross changes only.

2. Treatment at 80 mg/kg/day was associated with:

   (a) Post-dosing salivation on one or two days; hair loss in occasional animals.

   (b) Increase in water and decrease in food consumption.

   (c) Slight bodyweight loss to Day 8 of pregnancy; thereafter gain paralleled controls.

   (d) Increase in post implantation loss.

   (e) Reduced litter and mean foetal weight.

   Treatment at 20 mg/kg/day was associated with:

   (a) Hair loss in occasional animals.

   (b) Increase in water and decrease in food consumption.

   (c) Reduced weight gain to Day 8 of pregnancy.

   (d) Slightly reduced litter and mean foetal weight.

   Treatment at 5 mg/kg/day was associated with:

   (a) Increase in water consumption and marginal reduction in food consumption.

   (b) Slightly reduced weight gain to Day 8 of pregnancy, parity with control being regained by Day 20.

3. Treatment at 5 mg/kg/day was without adverse effect on mean litter parameters and treatment at all dosages did not produce any obvious structural abnormalities among foetuses at examination on Day 20.

4. On the basis of these findings it was considered that 80 mg/kg/day was too high for the main embryotoxicity study. A high dosage of 64 mg/kg/day was chosen since it was considered this would elicit sufficient toxicity in terms of retarded bodyweight gain. 5 mg/kg/day was not a NOEL therefore a low dosage of 1 mg/kg/day and intermediate dosages of 4 and 16 mg/kg/day were chosen.

This experiment describes the preliminary study performed to determine appropriate dosages of the fungicide, Ziram Technical, for future investigation of teratogenic potential in the rat. The test article was administered to pregnant animals from Day 6 to Day 15 of pregnancy inclusive by oral gavage; the potential route of operator and consumer exposure.

The dosages employed were chosen by the Sponsor based on data obtained in other toxicity studies.

Ziram Technical, Batch no. 8331 AA was received in these laboratories on 12 January 1989. It was a white powder with a melting point of 240°C and stated purity of 98.9%. It was stored at room temperature in the dark. It was known by the Sponsor that the material was stable for the duration of the study.

Stability of the test article in suspension was investigated prior to the start of dosing (see Part III).

KEY STUDY DATES

Date of approval by:

HRC Management:	27 February 1989.
Study Director:	24 February 1989.
Sponsor:	1 March 1989.
Date of arrival:	2 March 1989.
Date of first dose:	7 March 1989.
Date of last dose:	16 March 1989.
Date of final sacrifice:	21 March 1989.

Parent animals

Forty sexually mature (8-10 weeks old), Specific Pathogen Free female rats (Crl: CD R (SD) BR VAF/Plus strain) which were time mated to males of the same strain, were ordered from Charles River Portage, Michigan, USA. The day of mating, as judged by the appearance of sperm in the vaginal smear or by the presence of a vaginal plug, was considered as Day 0 of pregnancy. Six additional animals were ordered for health check purposes.

On arrival all animals were examined for abnormalities and for signs of overt ill health. Those designated as health check animals were killed within 24 hours after arrival at HRC and subjected to routine macroscopic examination. Any abnormalities seen were processed immediately and examined microscopically. Lungs, liver, kidneys, spleen and heart were preserved in fixative, but not processed further. Results are reported in Addendum 1.

The remaining animals were weighed on arrival and those in the weight range 161 g to 204 g were assigned to four groups by computerised stratified randomisation to give approximately equal initial mean bodyweights. Following allocation, the animals were earmarked to give individual identification. Prior to the commencement of treatment, all animals were inspected by a Veterinary Officer.

General animal management and accommodation

Animal room controls for temperature and relative humidity were set to maintain 21°C and 55% respectively, recorded range was 21.5 ± 3.5°C and 49 ± 5%. Lighting was controlled to give 12 hours light (8 am to 8 pm) and 12 hours dark per 24 hours.

The animals were housed five to a cage in suspended galvanised metal cages (Bowman R) equipped with solid sides and back, wire mesh front, floor and top.

Throughout the study each cage was identified by a label coloured according to the group and recording the study schedule number, animal numbers, details of treatment and the name of the Study Supervisor.

All animals were given free access to Labsure Laboratory Animal Diet No. 1 and to tap water.

There was no information available to the Study Director to indicate that any non-nutrient substance likely to influence the effect of the test compound could reasonably be expected to be present in the diet or water. (Quality Assurance Aspects for Food and Water are presented in Procedure Addenda 1 and 2). These analytical data are lodged in HRC Archives.

Experimental design

The experimental design was as follows:

Group: colour code	Treatment Ziram (mg/kg/ day)#	Concentration Ziram (% w/v)#	Vehicle	Dose volume (ml/100 g)	No. of rats ♀	Animal numbers
1: White	Control	Control	1% MC	0.5	10	1-10
2: Yellow	5	0.1	1% MC	0.5	10	11-20
3: Green	20	0.4	1% MC	0.5	10	21-30
4: Red	80	1.6	1% MC	0.5	10	31-40

Material as supplied
MC Methylcellulose

Method and frequency of dose preparation

Suspensions of the test article were prepared on a daily basis. On each occasion of formulation the highest required concentration was prepared by suspending a weighed quantity of the test article in the vehicle. The lower concentrations were prepared by serial dilution. Suspensions were placed on a magnetic stirrer immediately prior to, and throughout dosing.

Treatment

Treatment by oral gavage commenced on Day 6 of pregnancy and continued daily up to and including Day 15 of pregnancy.

Dosage volumes were calculated for individual animals on Day 6 of pregnancy and adjusted according to bodyweight on Days 8, 10, 12 and 14.

Observation

The following observations were made during the study:

1. Parent animals

(a) Signs

All animals were regularly handled and observed daily for obvious changes or signs of reaction to treatment.

(b) Food and water consumption

Food consumption was measured from weighday to weighday.

Daily measurement of water consumption was initiated on Day 8 of pregnancy through to termination due to an observed increase among treated animals.

(c) Bodyweights

All animals were weighed initially (=Day 1 of pregnancy) and on Days 4, 6, 8, 10, 12, 14, 16, 18 and 20.

2. Litter data and foetal examinations

On Day 20 of pregnancy the animals were killed by CO_2 asphyxiation, dissected and examined for congenital abnormalities and macroscopic pathological changes in maternal organs. The ovaries and uteri were examined immediately to determine:

(a) number of corpora lutea
(b) number and distribution of live young
(c) number and distribution of embryofoetal deaths
(d) individual foetal weight from which the litter weight is calculated
(e) foetal abnormalities

Embryofoetal deaths were classified as:

Early: only placenta visible at termination.

Late: both placental and embryonic remnants visible at termination.

Uteri or individual uterine horns without visible implantations were immersed in a 10% solution of ammonium sulphide to reveal evidence of embryonic death at very early stages of implantation[a].

Live young were examined externally and weighed. Half the foetuses in each litter were preserved in Bouin's solution; the remainder were fixed in 74 OP industrial methylated spirit.

All foetuses were sexed by gonadal inspection following preservation.

Further examinations were not performed.

Assessment of results

Individual litter values

In assessing litter parameters, pre-implantation loss was calculated as a percentage from the formula:

$$\frac{\text{(No. of corpora lutea - No. of implantations)}}{\text{No. of corpora lutea}} \times 100$$

Post implantation loss was similarly calculated from the formula:

$$\frac{\text{(No. of implantations - No. of live young)}}{\text{No. of implantations}} \times 100$$

Litter weight and mean foetal weight were calculated from individual foetal weight.

All values expressed as a percentage or ratio were first calculated within each litter and the group values calculated as a mean of the individual litter percentages.

Statistical analysis

Statistical analyses were performed on litter data. Significance tests were two-tailed.

The basic sample unit was the litter, and due to the preponderance of non-normal distribution, non-parametric analyses proved the most consistent.

Mean values of litter size, pre- and post implantation losses, litter weights and mean pup weight were analysed by the Kruskal-Wallis test[b]. Intergroup comparisons were made by the non-parametric equivalent of the 't' test together with the Jonckheere test (b) for an ordered series of treatments.

Where 75% of the values for a given variable consisted of one value, a Fisher's Exact test was used[c].

Location of study records

All specimens, raw data and other documents generated at HRC during the course of this study, together with a copy of the final report, are lodged in the Huntingdon Research Centre Ltd., Archives, Huntingdon, England.

Any such material arising from investigations made by the Sponsor or other laboratory, the findings of which are included in the final report, were retained either by the organisation conducting these investigations or by HRC.

References

a Salewski, E. 1964 — Farbemethode zum makroskopischen Nachweis von Implantationsstellen am Uterus der Ratte. Naunyn-Schmiedebergs Arch. exp. Pathol. Pharmakol. 247:367.

b Hollander, M & Wolfe, D.A., 1973 — Non-parametric statistical methods. Publ. J. Wiley & Sons, N.Y.

Kruskal-Wallis & Jonckheere tests: page 114 - 132.

Wilcoxon test: page 68 - 75.

c Fisher, R.A., 1950 — Fisher's exact test for 2 x 2 contingency table: 'Statistical Methods for Research Workers' para. 21-02. Oliver & Boyd, Edinburgh.

Composition and quality assurance aspects of rodent diet

Labsure LAD was a closed formula diet suitable for normal health, growth and reproduction of laboratory rats and mice. The standards of production adopted by the manufacturers have been approved by the HRC Quality Assurance Department.

Analyses were made of all batches of diet for most nutrients and for specified substances and micro-organisms likely to be present in feed ingredients or the finished diet and which, if in excess of specified amounts, might have had an undesirable effect on the test system. Although occasional slight deviation was permitted, batches of diet used conformed with the acceptable standards agreed by the Study Director and Department of Quality Assurance HRC as detailed below.

(A) Nutrients

		Target level	Maximum Tolerance (%)
Crude fat	%	3.7	±15
Crude protein	%	21.5	±10
Crude fibre	%	2.0	±40
Ash	%	5.5	±15
Moisture	%	9.5	10.5
Calcium	%	1.0	±20
Phosphorus	%	0.9	±20
Sodium	%	0.3	+100-50
Chlorine	%	0.5	+100-50
Potassium	%	0.8	+100-50
Magnesium	%	0.15	±50
Manganese	mg/kg	70	±50
Iron	mg/kg	220	±50
Copper	mg/kg	15	±50
Zinc	mg/kg	60	±50
Vitamin A	i.u./kg	12000	+50-20
Vitamin E	acceptable range 28 to 88 mg/kg		

(B) Contaminants

	Maximum allowable concentration (mg/kg except where stated)
Lead	2.5
Cadmium	0.5
Arsenic	1.5
Mercury	0.1
Selenium	0.6
Fluorine	40
Nitrates (as sodium nitrate)	200
Nitrites (as sodium nitrite)	10
PCBs	0.05
Total DDT	0.15
Dieldrin	0.05
Lindane	0.15
Heptachlor	0.05
Malathion	5.0
Total aflatoxins (B_1,B_2,G_1,G_2) µg/kg	5.0

(C) Microbiological content

Maximum count/g diet (at time of manufacture)

	LAD 1
Total viable organisms	10,000
Mesophilic spores	30,000
Presumptive *E. coli*	0
E. coli Type 1	0
Salmonella spp.	0
Fungal units	1,000
Antibiotic activity	0

Quality assurance aspects of water

Results of the routine physical and chemical examination of drinking water at source (Grafham Final Water) as conducted, usually weekly, by the supplier, Anglian Water Authority, were made available to HRC as quarterly summaries. Additionally, levels of specified substances known to be present from time to time in local water and which, if in excess of the maxima recommended (for humans) might have had undesirable effects on the test system, were determined in HRC tap water at approximately 6-monthly intervals.

Quarterly summary analyses of source water normally included levels of nitrites, nitrates, Ca, Mg, Na, K, P, Cl, Si, Fe.

Six-monthly analyses of HRC tap water included levels of As, Se, Ba, Ag, Sb, organophosphorus, organochlorine and other pesticides, haloforms, chlorophenols, polychlorinated biphenyls and polycyclic aromatic hydrocarbons.

(f) Terminal autopsy (Appendix 1)

Post mortem examination showed hair loss in 5 females at 20 mg/kg/day and 4 at 80 mg/kg/day confirming the clinical observation.

The incidence of occasional visceral macroscopic findings at terminal autopsy did not suggest a relationship with treatment.

2. LITTER DATA

(a) Litter size and post implantation loss (Table 5, Appendix 5)

Treatment at 80 mg/kg/day was associated with an increase in post implantation loss mainly due to the incidence of early embryonic deaths, ($P<0.05$), however the overall loss did not attain statistical significance ($P>0.05$). Since the implantation rate was also higher at this dosage the mean litter size remained comparable with controls.

(b) Litter and mean foetal weight and sex ratio (Tables 5 and 6, Appendices 5 and 6)

Treatment at 80 and 20 mg/kg/day was associated with a slight reduction in litter weight compared with the control. This was due to decreased mean foetal weights, the difference at 80 mg/kg/day attained statistical significance ($P<0.01$).

The mean value for the control litter data is atypical with regard to mean foetal weight. In six studies conducted at these laboratories this year the mean foetal weight has ranged between 3.18 g and 3.37 g, giving a mean of 3.30 g. The mean foetal weights at 5 and 20 mg/kg/day were 3.13 g and 2.90 g respectively, comparing these to the concurrent control shows little or no reduction but compared with the laboratory standard values does show that the mean foetal weight is depressed, an effect that was clearly demonstrated at 80 mg/kg/day.

Sex ratio, as expressed by the percentage of males per litter was essentially similar among both control and treated animals.

(c) Abnormalities among Day 20 foetuses (Appendix 1)

Macroscopic examination of foetuses at Day 20 did not reveal any obvious structural abnormalities. There was however an increased incidence of small foetuses (<2.5 g) 3/98, 0/113, 6/95 and 31/116 in Groups 1 to 4 respectively. At 80 mg/kg/day 8/10 litters were affected and this was reflected in the significantly reduced mean foetal weight ($P<0.01$).

FIGURE 1

Water and food consumption - group mean values

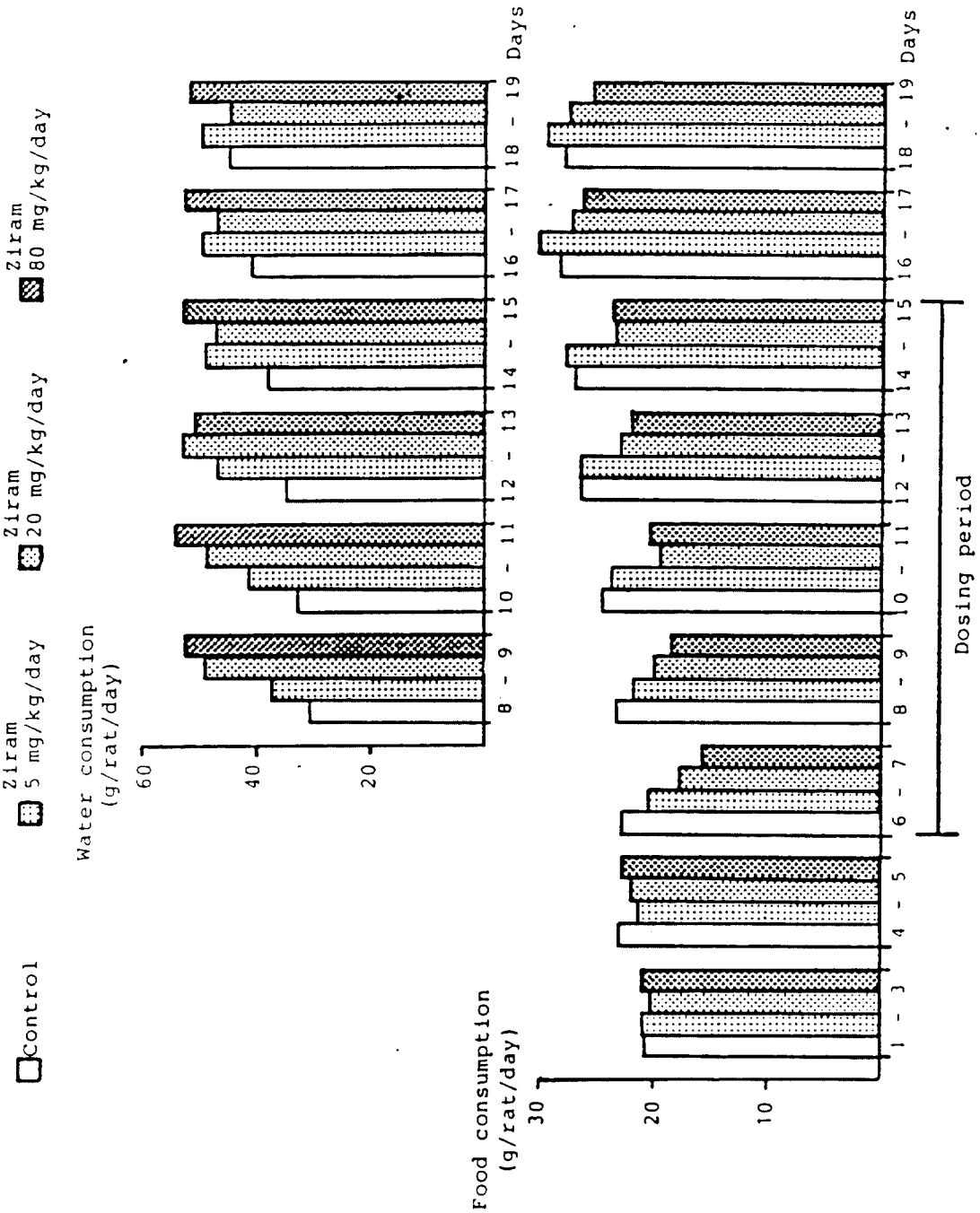

FIGURE 2

Bodyweight change of pregnant dams - group mean values

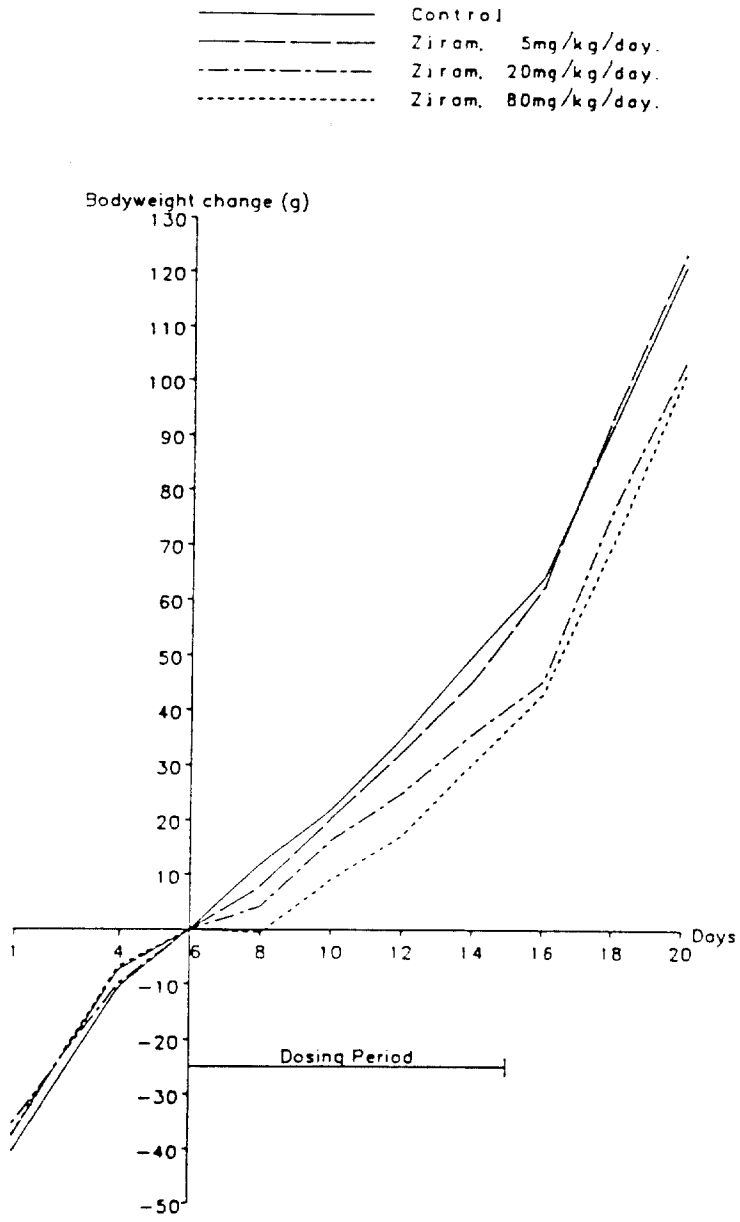

TABLE 1 ZIR 15/24/891371

Summary of adult performance

Group:	1	2	3	4
Compound:	Control		Ziram	
Dose (mg/kg/day):	-	5	20	80

Category	No. of animals in Group			
	1	2	3	4
Mated	10	10	10	10
Non-pregnant	1	0	1	0
With live young at Day 20	9	10	9	10
Pregnancy rate %	90	100	90	100

TABLE 2

Water consumption - group mean values

Group:	1	2	3	4
Compound:	Control		Ziram	
Dose (mg/kg/day):	-	5	20	80

Group	No. of animals	Mean water consumption (g/rat/day) during Days					
		8 - 9	10 - 11	12 - 13	14 - 15	16 - 17	18 - 19
1	10	30.9	33.2	34.6	38.1	41.1	45.1
2	10	37.5	41.5	47.4	49.2	49.9	50.1
3	10	49.3	49.2	53.4	47.7	47.2	44.7
4	10	53.0	54.4	51.3	53.2	53.2	52.1

TABLE 3

Food consumption - group mean values

Group:	1	2	3	4
Compound:	Control		Ziram	
Dose (mg/kg/day):	-	5	20	80

Group	No. of animals	Mean food consumption (g/rat/day) during Days								
		1 - 3	4 - 5	6 - 7	8 - 9	10 - 11	12 - 13	14 - 15	16 - 17	18 - 19
1	10	20.7	23.0	22.8	23.5	24.8	26.5	27.0	28.3	28.0
2	10	21.0	21.3	20.5	21.8	23.8	26.5	27.8	30.3	29.5
3	10	20.2	22.0	17.8	20.0	19.5	23.0	23.5	27.3	27.5
4	10	21.1	22.8	15.8	18.5	20.3	22.0	23.8	26.3	25.5

TABLE 4a

Bodyweights - group mean values

Group:	1	2	3	4
Compound:	Control		Ziram	
Dose (mg/kg/day):	-	5	20	80

Group	No of Animals	Bodyweight (g) at Day gestation									
		1	4	6	8	10	12	14	16	18	20
1	9	181.4	211.6	222.1	234.2	244.0	257.2	272.2	286.4	314.7	343.4
2	10	183.4	213.7	221.1	229.0	241.4	253.5	266.5	283.5	315.7	344.9
3	9	183.2	208.8	218.7	222.9	235.0	243.6	254.4	264.1	295.9	322.3
4	10	183.3	214.0	220.8	220.2	229.9	237.9	251.1	263.9	291.7	322.3

TABLE 4b

Bodyweights - group mean change values

Group:	1	2	3	4
Compound:	Control		Ziram	
Dose (mg/kg/day):	-	5	20	80

Group	No of Animals	Bodyweight change (g) at Day gestation									
		1	4	6	8	10	12	14	16	18	20
1	9	-40.7	-10.6	0.0	12.1	21.9	35.1	50.1	64.3	92.6	121.3
2	10	-37.7	-7.4	0.0	7.9	20.3	32.4	45.4	62.4	94.6	123.8
3	9	-35.4	-9.9	0.0	4.2	16.3	24.9	35.8	45.4	77.2	103.7
4	10	-37.5	-6.8	0.0	-0.6	9.1	17.1	30.3	43.1	70.9	101.5

TABLE 5

Litter data at Day 20 - group values

Group:	1	2	3	4
Compound:	Control		Ziram	
Dose (mg/kg/day):	-	5	20	80

Group	No. of anims.	Live young	Embryonic deaths Early	Embryonic deaths Late	Embryonic deaths Total	Implants	Corpora lutea	Pre-implant loss %	Post implant loss %	Litter wt.(g)	Mean foetal wt.(g)
1	9	10.9	0.2	0.1	0.3	11.2	12.0	6.5	2.7	33.02	3.04
2	10	11.3	0.2	0.2	0.4	11.7	13.1	11.0	3.3	35.47	3.13
3	9	10.6	0.7	0.1	0.8	11.3	11.9	4.5	6.3	30.54	2.90
4	10	11.6	* 1.1	0.1	(*) 1.2	(*) 12.8	13.5	4.2	9.5	31.01	** 2.68
Kruskal-Wallis 'H' statistic		NS	P<0.05	F	NS	NS	NS	NS	NS	NS	P<0.001
Jonckheere 'J' statistic		NS	P<0.05	NS	P<0.05	NS	NS	NS	NS	NS	P<0.001

Intergroup difference from control statistically significant: * P<0.05
** P<0.01

F Analysed by Fisher's exact test, differences from controls not significant (P>0.05)
NS Not significant (P>0.05)
() Not supported by significant 'H' statistic (P>0.05)

TABLE 6

Sex ratios - group mean values

Group:	1	2	3	4
Compound:	Control		Ziram	
Dose (mg/kg/day):	-	5	20	80

Group	No. of litters	No. of foetuses	Mean number of live young			% ♂ / litter
			♂	♀	Total	
1	9	98	5.7	5.2	10.9	51.4
2	10	113	6.0	5.3	11.3	53.9
3	9	95	5.6	5.0	10.6	53.2
4	10	116	6.3	5.3	11.6	54.6

APPENDICES

Principal signs and autopsy findings - individual observations

Key

F	Fur loss
C	Clear salivation
S	Scabbing on back
R	Noisy respiration

-	No abnormality detected (signs)
N	No abnormality detected (autopsy findings)

(Principal signs and autopsy findings - continued)

Group 1: Control

Animal no.	Clinical signs: days observed	Autopsy findings	
		Dam	Day 20 foetuses
	With live young		
1	-	N	One small foetus (2.47 g) with small subcutaneous haemorrhage (2 x 2 mm) between left orbit and nasals
2	-	N	N
3	-	N	One small foetus (2.40 g)
4	-	N	One small foetus (2.25 g)
6	-	N	N
7	-	N	N
8	-	N	N
9	-	N	N
10	-	N	N
	Non-pregnant		
5	-	N	-

Principal signs and autopsy findings - continued

Group 2: Ziram, 5 mg/kg/day

Animal no.	Clinical signs: days observed	Autopsy findings	
		Dam	Day 20 foetuses
	With live young		
11	-	N	N
12	-	N	N
13	-	N	N
14	-	N	N
15	-	N	N
16	-	N	N
17	-	N	N
18	-	N	N
19	F: 14,15	Right kidney; minimal increased pelvic dilatation	N
20	-	N	N

(Principal signs and autopsy findings - continued)

Group 3: Ziram, 20 mg/kg/day

Animal no.	Clinical signs: days observed	Autopsy findings	
		Dam	Day 20 foetuses
	With live young		
21	F: 16-20	Skin; slight generalised fur loss	One small foetus (2.32 g)
22	-	N	N
23	-	N	N
24	F: 16-20	Skin; dorsal lumbar/ thoracic region, minimal fur loss	Two small foetuses (2.47 and 2.43 g)
25	F: 14-20	Skin; dorsum, minimal fur loss	N
26	-	N	N
27	-	N	One with two small subcutaneous haemorrhages (1 x 1 and 2 x 1 mm) on right forepaw
28	-	N	N
30	F: 13-20	Skin; dorsum minimal fur loss	Three small foetuses (2.49, 2.41 and 2.28 g)
	Non-pregnant		
29	F: 14-20	Skin; dorsal lumbar and cervical region minimal diffuse fur loss	-

(Principal signs and autopsy findings - continued)

Group 4: Ziram, 80 mg/kg/day

Animal no.	Clinical signs: days observed	Autopsy findings	
		Dam	Day 20 foetuses
	With live young		
31	S: 1-5 C: 14	N	N
32	F: 12-20 C: 13 R: 12,14,15, 18-20	Skin; dorsal lumbar region fur loss Lungs; pale and oedematous Intestine; stomach and ileum, gaseous distension	All foetuses small (from 1.83 to 2.28 g)
33	-	N	Two small foetuses(2.48 g)
34	C: 13,14 F: 16-20	Skin; dorsum patchy minimal fur loss	One with subcutaneous haemorrhage in the dorsal thoracic region (1 x 4 mm). Nine small foetuses (from 2.26 to 2.49 g).
35	C: 13 F: 16-20	N	One with a diffuse haemorrhage in the cranium (5 x 5 mm)
36	C: 13,14	N	One small foetus (2.05 g)
37	F: 14-20 C: 13	Skin; dorsal lumbar region minimal fur loss	One small foetus (2.40 g)
38	C: 13	N	Four small foetuses (from 1.86 to 2.47 g)
39	F: 12-20 C: 13	Skin; dorsal abdominal region patches of fur loss	One small foetus (2.26 g)
40	C: 13	N	One small foetus (1.48 g)

Bodyweights - individual values (g)

Group 1: Control

Animal number	Bodyweight (g) at Day gestation									
	1	4	6	8	10	12	14	16	18	20
	Live young at Day 20									
1	175	200	214	229	237	251	266	279	303	341
2	190	220	228	228	254	268	279	290	321	351
3	181	208	217	234	246	260	273	288	321	343
4	173	209	221	243	246	260	280	293	320	348
6	176	202	217	225	231	244	260	275	305	332
7	161	211	222	236	244	258	271	292	317	343
8	192	215	222	238	240	254	266	281	309	339
9	184	217	224	234	242	256	270	283	311	338
10	201	222	234	241	256	264	285	297	325	356
	Non-pregnant									
5	204	246	257	270	283	297	296	290	294	297

(Bodyweights - continued)

Group 2: Ziram, 5 mg/kg/day

Animal number	Bodyweight (g) at Day gestation									
	1	4	6	8	10	12	14	16	18	20
	Live young at Day 20									
11	179	210	213	221	226	239	247	260	287	313
12	182	209	221	226	237	251	267	285	315	349
13	172	200	206	208	226	240	255	269	298	324
14	187	213	224	237	243	254	263	277	308	333
15	194	226	233	240	258	268	281	299	335	364
16	179	206	215	218	231	248	258	278	311	338
17	193	224	234	248	260	270	284	305	339	372
18	169	206	214	223	240	249	264	282	313	344
19	183	210	218	228	237	250	263	279	313	335
20	196	233	233	241	256	266	283	301	338	377

(Bodyweights - continued)

Group 3: Ziram, 20 mg/kg/day

Animal number	Bodyweight (g) at Day gestation									
	1	4	6	8	10	12	14	16	18	20
	Live young at Day 20									
21	185	209	214	211	237	247	254	267	298	328
22	179	205	217	230	240	246	252	259	290	316
23	186	210	220	223	236	244	257	271	304	334
24	164	200	207	216	226	236	243	253	280	304
25	198	220	234	241	248	255	265	272	309	333
26	180	207	216	210	226	231	251	259	292	317
27	170	195	209	214	224	234	244	251	280	307
28	196	223	231	243	250	261	273	284	320	351
30	191	210	220	218	228	238	251	261	290	311
	Non-pregnant									
29	186	195	205	200	213	209	221	217	227	228

(Bodyweights - continued)

Group 4: Ziram, 80 mg/kg/day

Animal number	Bodyweight (g) at Day gestation									
	1	4	6	8	10	12	14	16	18	20
	Live young at Day 20									
31	166	199	207	201	210	220	222	235	260	388
32	178	205	212	214	221	213	222	234	260	229
33	182	214	219	218	220	238	253	270	300	329
34	194	224	233	226	236	246	263	278	310	335
35	188	224	229	228	240	255	271	285	320	351
36	199	225	229	231	240	250	263	273	303	336
37	179	211	221	219	236	243	257	271	301	322
38	186	214	220	216	228	238	257	273	298	319
39	186	219	223	230	237	245	259	268	295	325
40	175	205	215	219	231	231	244	252	270	289

Litter data - individual values

Group 1: Control

Animal number	Live young	Embryonic deaths Early	Late	Total	Implants	Corpora lutea	Pre-implant loss %	Post implant loss %	Litter wt.(g)	Mean foetal wt.(g)
	With live young									
1	12	0	0	0	12	12	0.0	0.0	34.80	2.90
2	11	0	0	0	11	11	0.0	0.0	33.67	3.06
3	10	1	0	1	11	12	8.3	9.1	27.43	2.74
4	11	0	0	0	11	11	0.0	0.0	32.04	2.91
6	11	0	0	0	11	13	15.4	0.0	31.91	2.90
7	8	0	0	0	8	11	27.3	0.0	26.33	3.29
8	12	0	0	0	12	13	7.7	0.0	38.01	3.17
9	12	0	0	0	12	12	0.0	0.0	37.49	3.12
10	11	1	1	2	13	13	0.0	15.4	35.51	3.23
	Non-pregnant									
5										

(Litter data - continued)

Group 2: Ziram, 5 mg/kg/day

Animal number	Live young	Embryonic deaths			Implants	Corpora lutea	Pre-implant loss %	Post implant loss %	Litter wt.(g)	Mean foetal wt.(g)
		Early	Late	Total						
	With live young									
11	7	0	0	0	7	12	41.7	0.0	21.54	3.08
12	11	1	1	2	13	13	0.0	15.4	32.85	2.99
13	9	0	1	1	10	12	16.7	10.0	28.58	3.18
14	10	0	0	0	10	11	9.1	0.0	30.69	3.07
15	15	0	0	0	15	15	0.0	0.0	48.85	3.26
16	11	0	0	0	11	11	0.0	0.0	35.04	3.19
17	14	0	0	0	14	14	0.0	0.0	46.94	3.35
18	13	0	0	0	13	16	18.8	0.0	40.98	3.15
19	10	0	0	0	10	12	16.7	0.0	29.09	2.91
20	13	1	0	1	14	15	6.7	7.1	40.13	3.09

(Litter data - continued)

Group 3: Ziram, 20 mg/kg/day

Animal number	Live young	Embryonic deaths			Implants	Corpora lutea	Pre-implant loss %	Post implant loss %	Litter wt.(g)	Mean foetal wt.(g)
		Early	Late	Total						
	With live young									
21	11	0	0	0	11	11	0.0	0.0	31.37	2.85
22	11	1	0	1	12	13	7.7	8.3	32.59	2.96
23	13	0	0	0	13	13	0.0	0.0	36.86	2.84
24	9	0	0	0	9	9	0.0	0.0	23.29	2.59
25	9	0	1	1	10	10	0.0	10.0	27.02	3.00
26	10	4	0	4	14	14	0.0	28.6	29.80	2.98
27	9	1	0	1	10	12	16.7	10.0	28.03	3.11
28	11	0	0	0	11	12	8 3	0.0	33.41	3.04
30	12	0	0	0	12	13	7.[illegible]	0.0	32.45	2.70
	Non-pregnant									
29										

Sex ratios - individual values

Animal no.	Group 1 Control				Animal no.	Group 2 Ziram, 5 mg/kg/day			
	♂	♀	Total	% Males		♂	♀	Total	% Males
Live young at Day 20					Live young at Day 20				
1	8	4	12	66.7	11	6	1	7	85.7
2	7	4	11	63.6	12	6	5	11	54.5
3	4	6	10	40.0	13	4	5	9	44.4
4	4	7	11	36.4	14	4	6	10	40.0
6	7	4	11	63.6	15	7	8	15	46.7
7	3	5	8	37.5	16	5	6	11	45.5
8	6	6	12	50.0	17	9	5	14	64.3
9	6	6	12	50.0	18	6	7	13	46.2
10	6	5	11	54.5	19	5	5	10	50.0
					20	8	5	13	61.5
Non-pregnant									
5									

(Sex ratios - continued)

Animal no.	Group 3 Ziram, 20 mg/kg/day				Animal no.	Group 4 Ziram, 80 mg/kg/day			
	♂	♀	Total	% Males		♂	♀	Total	% Males
Live young at Day 20					Live young at Day 20				
21	6	5	11	54.5	31	8	3	11	72.7
22	5	6	11	45.5	32	5	7	12	41.7
23	7	6	13	53.8	33	6	5	11	54.5
24	7	2	9	77.8	34	9	4	13	69.2
25	5	4	9	55.6	35	8	6	14	57.1
26	1	9	10	10.0	36	8	5	13	61.5
27	7	2	9	77.8	37	5	6	11	45.5
28	5	6	11	45.5	38	5	5	10	50.0
30	7	5	12	58.3	39	4	9	13	30.8
					40	5	3	8	62.5
Non-pregnant									
29									

Pre-treatment health check

Macroscopic examination revealed one female animal with increased bilateral renal pelvic dilatation. Histological examination confirmed this observation. There were no changes considered to be related to the presence of infectious disease. Subsequent veterinary inspection of remaining animals prior to commencement of treatment confirmed their general health and appearance to be satisfactory.

PART III

THE ANALYSIS OF ZIRAM IN 1% METHYLCELLULOSE

THE ANALYSIS OF ZIRAM IN LIQUID FORMULATIONS

Authors:

Alan Anderson,
I. Suzanne Dawe,
Stephen J. Youthed.

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ADDENDUM

(continued)

INTRODUCTION

This addendum details the following:

1. The analytical method which is an abstract from HRC Analytical Procedure ZIR/2421/M16/89.

2. The analytical results obtained for:

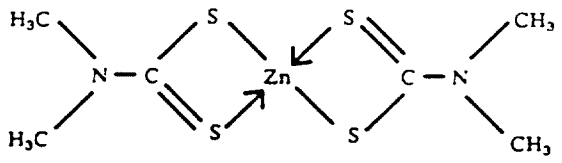
   2.1 The determination of concentrations of Ziram in dose formulations prepared for Day 2 of dosing.

   2.2 The determination of the physical stability (homogeneity and resuspendability) of Ziram in 1% MC formulations.

   2.3 The determination of the chemical stability of Ziram in 1% MC formulations.

   2.4 The validation of the method of analysis for the determination of Ziram in 1% MC formulations.

The formulations for this study were prepared as suspensions of Ziram in 1% MC in the Department of Formulation at Huntingdon Research Centre Ltd.



Ziram

1% MC : 1% aqueous methylcellulose

EXPERIMENTAL PROCEDURE

1. Apparatus and instrumentation

Spectrophotometer:	Shimadzu UV-260, variable wavelength double-beam spectrophotometer, equipped with a graphic recorder.
General laboratory glassware.	

2. Reagents

Ziram:	Ziram Task Force, UCB Chemical Corporation.
	Batch 8331 AA (stated purity 98.9%).
Tetrahydrofuran:	Fisons HPLC grade.
Methanol:	Fisons HPLC grade.

3. Method of analysis

A representative sub-sample (1 ml) of dose formulation was accurately weighed, dissolved in tetrahydrofuran (15 ml) and appropriately diluted using methanol to provide a solution containing Ziram at an expected concentration between 4 and 8 mcg/ml.

The concentration of Ziram was quantified spectrophotometrically using ultra-violet detection as detailed in section 4.

4. Typical spectrophotometric conditions

Pathlength:	1 cm.
Reference:	Methanol.
Absorbing wavelength:	UV, 256 nm.

Absorbance range: 0 - 1.5 absorbance units.

Wavelength range for spectral profile: 190 - 350 nm.

The spectral profile was recorded for each calibration, procedural recovery and sample solution following completion of the absorbance measurements.

5. Calibration

A primary standard was prepared for each analytical occasion by dissolving an accurately weighed quantity (50 mg) of Ziram in tetrahydrofuran (15 ml) and diluted to volume (50 ml) with methanol. The above solution was serially diluted using methanol to provide five standards for instrument calibration containing Ziram at concentrations of 2, 4, 6, 8 and 10 mcg/ml.

The absorbance of each calibration standard was measured, at the beginning and end of each sample analysis sequence, using the conditions described in section 4.

6. Method validation

The precision and accuracy of the analytical procedure was assessed by fortifying control vehicle (1 ml) with Ziram, to concentrations of 1 and 80 mg/ml, using either a solution of Ziram in tetrahydrofuran (inclusion levels <20 mg/ml) or using the neat standard Ziram (inclusion levels ≥20 mg/ml) and analysing as described in section 3.

Procedural recoveries were determined for each inclusion level and analysed concurrently with test formulations.

7. Determination of concentrations of Ziram in dose formulations prepared for Day 2 of dosing

Representative sub-samples (approximately 20 ml) of freshly prepared dose formulations, received from the Department of Formulation, were thoroughly mixed by shaking and duplicate sub-samples (1 ml) were removed for analysis as detailed in section 3.

8. Determination of the chemical and physical stability of Ziram in 1% MC formulations

8.1 Chemical stability

Freshly-prepared batches of test formulations, at nominal concentrations of 1 mg/ml and 80 mg/ml, were each sampled (6 replicates of approximately 1 ml) into pre-weighed volumetric flasks. Two samples were analysed immediately and the remaining samples were stored at ambient temperature in the dark for 4 hours (2 replicates) and at +4°C for 24 hours (2 replicates) before analysis.

Two samples from each formulation were analysed at each time-point, using the method described in section 3.

8.2 Physical stability

After sampling for chemical stability, the remainder of each test formulation was thoroughly mixed by repeated inversion and magnetically stirred for 5 minutes. Samples (approximately 1 ml) were removed for analysis from points approximately one-quarter, one-half and three-quarters the depth (representing the top, middle and bottom) of the formulation. Magnetic stirring was continued and samples were withdrawn for analysis, as above, at 0.5 hour and 1 hour.

The magnetic stirring was then discontinued and the remainder of each formulation was stored in the dark at ambient temperature for a further 3 hours and subsequently at +4°C for 24 hours. At time-points corresponding to 4 and 24-hour storage, each formulation was re-mixed and sampled, as above, for analysis.

The three samples removed from each formulation, at each time-point, were analysed using the method described in section 3.

9. Calculation

The absorbance of Ziram in each calibration standard was measured and calibration curves were constructed by linear regression of standard response versus standard concentration. The absorbance of each sample and procedural recovery solution was measured and the concentration of Ziram was calculated using the equation below:

$$\text{Concentration, mg/ml} = \frac{Y - I}{S} \times \frac{V}{W} \times D \times 10^{-3}$$

where Y = Absorbance of test solution
I = Intercept derived from linear regression of calibration data
S = Slope derived from linear regression of calibration data
V = Dilution volume of sample (ml)
W = Weight of sample (g)
D = Density (g/ml)

Results were corrected for the cumulative mean procedural recovery value at analysis.

10. Limit of detection

The limit of detection, defined as the concentration of Ziram in control matrix producing the minimum discernible spectral profile typical of Ziram, was determined as 0.025 mg/ml.

RESULTS AND DISCUSSION

The mean concentrations of Ziram in dose formulations analysed for the study and the deviation of mean results from nominal values are summarised in Table 1. Mean results were all within 9% of nominal concentrations. Individual analytical results and associated procedural recovery values are detailed in Table 2. The results in Tables 1 and 2 are corrected for the appropriate cumulative mean procedural recovery value at analysis (Table 2).

The results in Table 3 indicate that, at nominal concentrations of 1.0 mg/ml and 80 mg/ml, Ziram produces a homogeneous suspension in 1% MC formulations. The data confirm that homogeneity can be maintained by magnetic stirring for one hour and that the formulations can be successfully resuspended 4 hours after preparation during storage at ambient temperature and at 24 hours after storage at +4°C.

The results in Table 4 confirm the chemical stability of Ziram in 1% MC formulations, during storage at ambient temperature in the dark for 4 hours and at +4°C for 24 hours.

The procedural recovery data obtained during method validation are given in Table 5. The data confirm that the analytical method provides good extraction efficiency for Ziram from the 1% MC matrix. A mean procedural recovery value of 102.4% ± 2.01 SD (n = 8) was obtained at 1.0 mg/ml and 99.4% ± 0.58 SD (n = 8) at 80 mg/ml. The appropriate mean procedural recovery value in Table 5 was used to correct the results presented in Tables 3 and 4.

The density value obtained for each formulation was within the range 1.00 - 1.01 g/ml.

Typical ultra-violet spectra are shown in Figure 1.

(continued)

TABLE 1

Summary: mean concentrations of Ziram in dose formulations

Day of dosing	Group	Nominal inclusion (mg/ml)	Mean analysed concentration (mg/ml)	RME (%)
2	Control	0	ND	-
	2	1.0	1.04	+4.0
	3	4.0	3.66	-8.5
	4	16	14.8	-7.5
	5	64	58.8	-8.1

ND None detected
RME Relative mean error

(continued)

TABLE 2

Concentrations of Ziram in dose formulations
(individual values)

Day of dosing	Group	Nominal inclusion (mg/ml)	Analysed concentration (mg/ml)			Procedural recovery (%)	
			Analysis 1	Analysis 2	Mean	At analysis	Cumulative mean[1]
2	Control	0	ND	-	ND		
	2	1.0	1.02	1.07	1.04	105.4	102.8
	3	4.0	3.62	3.69	3.66	102.4	102.4
	4	16	14.8	14.8	14.8	102.1	102.1
	5	64	58.9	58.8	58.8	103.6	103.6

ND None detected
[1] Including data from Table 5, where appropriate

Results are corrected for the appropriate cumulative mean procedural recovery value given in this table

(continued)

TABLE 3

Physical stability of Ziram in 1% MC formulations

Nominal inclusion (mg/ml)	Storage time (hours)	Analysed concentration (mg/ml)				CV (%)	RME (%)
		Top	Middle	Bottom	Mean		
1	0	1.11	1.02	1.04	1.06	4.58	+6.0
	0.5	1.00	1.02	1.01	1.01	0.83	+1.0
	1	1.03	1.02	0.996	1.01	1.53	+1.0
	4	1.01	1.02	1.01	1.01	0.91	+1.0
	24	1.01	1.03	1.07	1.04	3.03	+4.0
80	0	77.8	77.0	77.7	77.5	0.61	-3.1
	0.5	78.2	78.1	78.2	78.2	0.06	-2.3
	1	77.6	78.0	78.1	77.9	0.32	-2.6
	4	78.1	78.0	78.6	78.2	0.40	-2.3
	24	76.7	76.5	76.4	76.5	0.21	-4.4

CV Coefficient of variation
RME Relative mean error

Results are corrected for the appropriate mean procedural recovery value in Table 5

(continued)

TABLE 4

Chemical stability of Ziram in 1% MC formulations

Nominal inclusion (mg/ml)	Storage time (hours)	Analysed concentration (mg/ml)			RME (%)
		Analysis 1	Analysis 2	Mean	
0	0	ND	ND	ND	-
	24	ND	ND	ND	-
1	0	1.01	0.994	1.00	0.0
	4	0.990	1.00	0.997	-0.3
	24	0.998	0.979	0.989	-1.1
80	0	78.3	76.0	77.1	-3.6
	4	77.5	77.4	77.4	-3.3
	24	79.7	78.0	78.8	-1.5

ND None detected
RME Relative mean error

Results are corrected for the appropriate mean procedural recovery value in Table 5

(continued)

TABLE 5

Procedural recovery data obtained during method validation and determination of stability

(results are expressed as percent recovery)

	Nominal fortification (mg/ml)	
	1	80
	103.2 103.7 103.1 104.0 103.7 103.1 98.4 100.2	99.9 99.1 99.3 99.4 99.3 100.1 99.7 98.2
Mean	102.4	99.4
SD (+/-)	2.01	0.58
RME (%)	+2.4	-0.6

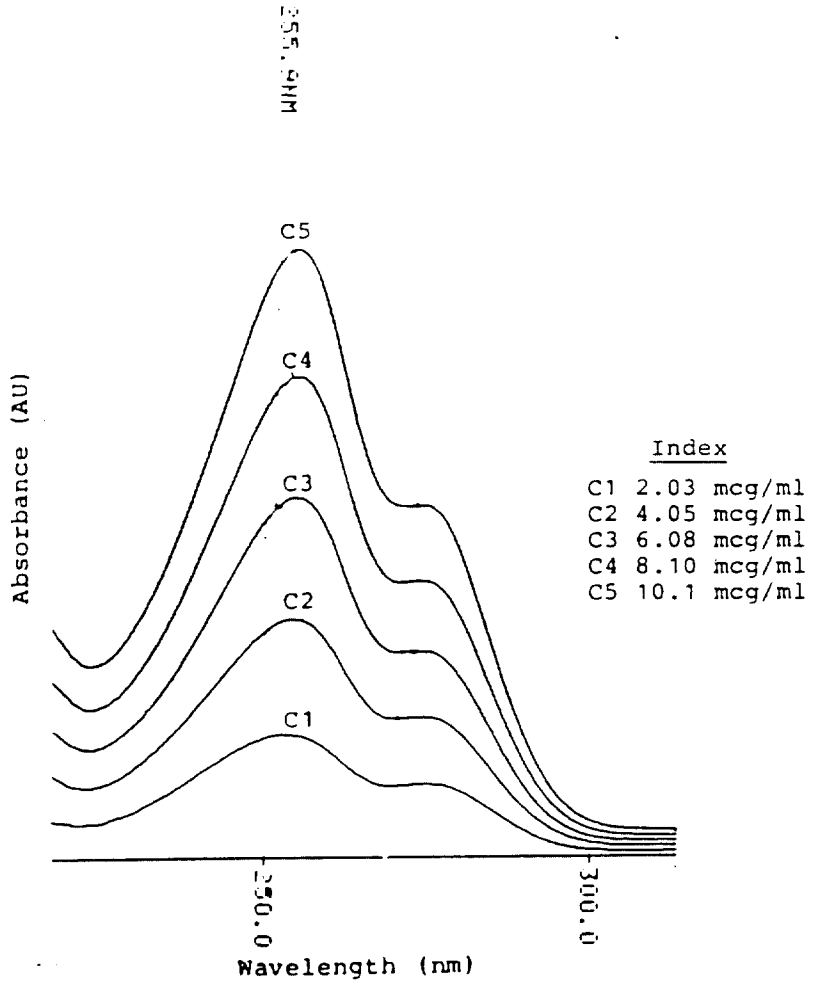
SD Standard deviation
RME Relative mean error

ADDENDUM

(continued)

FIGURE 1

Typical ultra-violet spectra



PART IV

THE ANALYSIS OF ZIRAM

(Data supplied by Sponsor)

CHEMISCHE SECTOR

MINERALE DIVISIE

VESTIGING TE GENT

Our ref. GM/8862

April 4th, 1990

Ziram technical grade: stability.

The lot number 8331AA, from which a sample has been sent to Huntingdon Research Center for toxicological studies, has been analysed at four different times in this laboratory.
On the other hand a 5 g sample sent back by HRC has also been analysed in this laboratory.
The method used for determinining the active ingredient content is the official CIPAC method [CIPAC 1; 31/LM.1.2].
The results are tabulated below:

Date of analysis	% ziram
March 88	99.0 %
Nov. 88	98.9 %
Jan. 89	98.5 %
Dec. 89*	98.8 %
March 90	98.5 %

The above results, which are within the accuracy limits of the method, show that the active ingredient content has been stable over the past two years.

G. Matthijs
Head of Laboratory

J. Swinnen
Head of Technical Department

*5 g sample sent back by HRC

ucb nv

Puntsersch[illegible]straat 207
Deenaven West
B-9000 Gent Belgie

Telefoon [illegible]
Telefax [illegible]
Telex [illegible] UCBGEN B

B.T.W. [illegible]
Bank [illegible]
P.R. [illegible]

HRC

HUNTINGDON RESEARCH CENTRE LTD
P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England.

Telephone: International +44 480 890 431 : National (0480) 890431
Facsimile : International +44 480 890 693 : National (0480) 890693
Telex : 32100 HRCUK G

TAM/mcc

3 February 1992

Dr M Giurgea
Ziram Task Force
c/o UCB SA
Chemical Sector
Avenue Louise 326
Box 7
B-1050 BRUSSELS
BELGIUM

Dear Dr Giurgea

ZIRAM : 52-week dietary toxicity study in beagle dogs (ZIR/10-G)

Please find enclosed a summary of the data generated to Week 52 (Week 49 for the replacement dog) for the above study. We hope you find this to your satisfaction.

Please contact us if you have any queries

With best wishes

Yours sincerely

Timothy A McLean
Study Director
Department of Non-rodent Toxicology

Encs: Summary and Data

cc : Mr P R Larson
Dr G Arce
Dr A Pelfrene

With Compliments

RECEIVED
FEB 11 1992
REGULATORY AFFAIRS

For the attention of:
Dr Gail T Arce

HUNTINGDON RESEARCH CENTRE Ltd
P.O. Box 2 ● Huntingdon ● Cambs ● PE18 6ES ● England
Tel: Huntingdon (0480) 890431 ● Telex: 32100
Fax: Huntingdon (0480) 890693 Gp.2 & Gp.3
A member of the HIH group of companies

Registered Office: Woolley Road, Alconbury, Huntingdon, Cambs
A member of the HIH

HRC

HUNTINGDON RESEARCH CENTRE

(TERMINAL) SYNOPSIS No. 11

STUDY SCHEDULE NUMBER: ZIR/10-G

STUDY TITLE: Ziram: Toxicity to Dogs by Repeated Dietary Administration for 52 Weeks.

STUDY DIRECTOR: T.A.McLean **STUDY SUPERVISOR:** T.G.Smith.

DATE OF COMMENCEMENT OF TREATMENT: 13 December 1990

WEEKS COVERED BY THIS SYNOPSIS: 1-52 (and 1-49 for replacement dog 112♀ at 500 (700) ppm).

DOSAGE LEVELS:

	Number of Dogs
Group 1: Control	4♂ + 4♀
Group 2: 50 ppm	4♂ + 4♀
Group 3: 185 ppm	4♂ + 4♀
Group 4: 500 (700) ppm *	4♂ + 4♀

* Dosage reduced from 700 to 500 ppm Day 3 of Week 12.

MORTALITIES: (Data were sent previously)

There was 1 death considered to be related to treatment:

Dog 368♀ (700 ppm) was found on the morning of Day 7 of Week 11 laterally recumbent and in a collapsed state, with excessive salivation, trembling and occasional champing of the jaws and jerking of limbs. The dog's condition worsened over the following 4 hours and the decision was taken to kill the animal for humane reasons. Data relating to this dog were sent in our fax of 28/01/91; histopathological findings are presented as Appendix 1. This mortality was considered to be related to treatment, and at the Sponsors request, the high dosage level was reduced to 500 ppm from Day 3 of Week 12.

In addition, Dog 372♀ (700 ppm) was killed on Day 1 of Week 6 due to suspected polyarteritis as previously discussed in our fax of 18/01/91. Histopathological findings indicated that polyarteritis was not present but the increased cellularity of the synovial membrane (indicative of mild synovitis) may correlate with some of the clinical signs reported. The demise of this animal was unrelated to treatment.

A replacement animal for Dog 372♀ (ie Dog 112♀) was obtained and after undergoing predose investigations commenced treatment on 07/02/91. It is running 8 weeks behind the main study animals.

HUNTINGDON RESEARCH CENTRE

(TERMINAL) SYNOPSIS No. 11

CLINICAL SIGNS:

With the exception of the clinical condition of Dog 368♀ described previously, there were no signs considered to be related to treatment. The replacement Dog 112♀ (500(700)ppm) has shown no signs considered to be related to treatment to Week 49.

BODYWEIGHT: (Figure 1, Table 1, Appendix 1)

Group mean bodyweight change values show slightly inferior overall bodyweight gains for females receiving 185 or 500(700)ppm compared with the Controls and the differences attained statistical significance. However, whilst the group mean change for males at 185 ppm was also slightly lower than for Controls, a similar difference was not apparent for the males at 500(700)ppm.

Bodyweight gain for the replacement Dog 112♀ (500(700)ppm) has been satisfactory to Week 49.

FOOD CONSUMPTION: (Table 2, Appendix 2)

Food consumption for the majority of surviving dogs was essentially maximal to Week 52. However, periods of slight inappetence were noted for several females receiving 185 or 500(700)ppm, particularly for Dog 366♀ which generally showed reduced consumption from Week 4. The significance of this finding is not clear.

Group mean food consumption values were slightly lower for females at 185 or 500(700)ppm compared with the Controls and the difference at 500(700)ppm attained statistical significance.

The possibility that the slightly decreased food consumption and consequent bodyweight loss in females at 500(700)ppm may be related to treatment cannot be ruled out at this stage.

Food consumption for the replacement dog 112♀ (500(700)ppm) was almost maximal over the first 49 weeks of treatment.

ACHIEVED INTAKES: (Table 3)

Weekly group mean achieved intakes of Ziram are shown in Table 3 and the overall mean values, Weeks 1 to 52, were as follows:

Diet concentration (ppm)	Males (mg/kg/day)	Females (mg/kg/day)
50	1.6	1.9
185	6.6	6.7
500(700)	17.4	19.7

OPHTHALMOSCOPY: (Appendix 3)

There were no changes at Weeks 13, 26 or 52 considered to be related to treatment.

NEUROLOGICAL EXAMINATIONS: (Data were sent previously)

The Week 50 examination for the replacement dog 112♀ was performed on 16 January 1992.

At the predose investigations and those performed during Weeks 34 and 50, examination for general physical condition and observation for behaviour and gait revealed no abnormalities. Tests for cranial nerve function, spinal reflexes and postural reactions elicited responses considered to be within the normal range. There was no evidence of neurological abnormality or deficit in any animal.

LABORATORY INVESTIGATIONS: (Tables 4, 5 & 6, Appendices 4, 5 and 6)

(a) Haematology:

Slightly raised reticulocyte counts, often with associated slight polychromasia, were recorded for 1 dog (376♂) at 500(700)ppm during Week 26, and 2 dogs (355♂ and 356♀) at 50 ppm, 1 dog (357♂) at 185 ppm, and 4 dogs (365♂, 367♂, 371♂ and 366♀) at 500(700)ppm during Week 52. In addition, compared with the concurrent Control values, there was a relatively small, but occasionally statistically significant decrease in RBC counts, with decreased MCHC and increased MCV at 500(700)ppm (and occasionally at 185 ppm, for RBC counts), apparent to varying degrees during Weeks 13, 26 and 52. Whilst the differences were relatively slight, were not consistent and, for the RBC counts, were also present in males at the pre-dose investigations, similar observations at 1000 ppm in a previous 13 Week study (ZIR/8-G) suggest that this may represent a marginal treatment-related effect.

The increase in group mean APTT observed at Week 13 for dogs receiving 185 or 500(700) ppm was less apparent at Week 26 and not present at Week 52.

There were no other changes considered indicative of a reaction to treatment.

(b) Biochemistry:

Group mean cholesterol values were increased for dogs of both sexes receiving 500(700) ppm at Weeks 13, 26 and 52, achieving statistical significance for male and combined data.

Group mean albumin values were slightly reduced for females receiving 500(700)ppm at Week 13 and for dogs of both sexes receiving 500(700)ppm at Weeks 26 and 52; the differences generally attaining statistical significance.

GPT values were clearly elevated at Week 26 for Dogs 359♂ (185ppm), 367♂ and 371♂ (500(700)ppm). The AP value for Dog 359♂ and the GOT value for Dog 367♂ were also slightly raised at this time. At Week 52 elevated GPT and AP values were noted for Dog 359♂ (185 ppm) and 371♂ (500(700)ppm).

Group mean T3 values were increased with the differences attaining statistical significance for males only receiving 500 (700)ppm) at Weeks 13, 26 and 52.

The high CPK group mean for males receiving 185 ppm at Week 52 was due to high individual values for Dogs 357♂ and 361♂. However, these 2 animals had slightly high CPK levels prior to the commencement of treatment, and therefore this finding is considered to be of no toxicological importance.

There were no other noteworthy findings.

(c) Urinalysis:

There were no changes at Weeks 13, 26 or 52 considered indicative of a reaction to treatment.

Results of laboratory investigations performed at Weeks 13 and 26 for the replacement dog 112♀ (500(700)ppm), show some of the trends described above for the other animals in this group.

Week 52 ophthalmoscopic and laboratory investigations for the replacement dog 112♀ are scheduled to be performed in the week commencing 30 January 1992.

HUNTINGDON RESEARCH CENTRE

(TERMINAL) SYNOPSIS No. 11

ANALYTICAL CHEMISTRY:

The analytical concentrations of ziram in the formulated diets were:

Nominal concentration (ppm)	Mean analysed concentration (ppm)			
	Week 1	Week 13	Week 26	Week 39
57.5	55.2	47.9	43.2	53.9
185	179	183	186	187
500	-	498	497	499
700	712	-	-	-

Results of the analyses performed on diets from Week 52 will be forwarded when they become available.

TERMINAL STUDIES: (Table 7, Appendices 7 & 8)

(a) Organ weights:

The group mean liver weight for males receiving 500(700)ppm was increased compared with the Control value, the difference attaining statistical significance. This appears largely due to the liver weight for 2 dogs (365♂ and 367♂) which were at, or approaching, our normally expected upper limit of 4% of terminal bodyweight.

Group mean prostate weights showed a dosage-related decrease compared with the Control value, although the differences did not attain statistical significance.

Uterus and ovary weights were also generally decreased in treated animals compared with Controls, however this may be related to the relative stages in the oestrus cycle of the individual animals which may also be clarified following histopathological examination.

Group mean heart weights were decreased at 500(700)ppm compared with the Controls, the difference attaining statistical significance for combined male and female data. This is possibly due to a fortuitous distribution, although this may also be clarified following the results histopathological analysis.

There were no other noteworthy findings.

HUNTINGDON RESEARCH CENTRE

(TERMINAL) SYNOPSIS No. 11

(b) Macroscopic pathology:

The only macroscopic findings considered to be noteworthy at this stage were:

- multiple red discoloured linear depressions upto 25x3mm on the mucosal surface throughout the stomach body and antrum for dog 364♀ (185ppm). Dark ingesta was also present throughout the gastro-intestinal tract for this dog, yet no dark stained faeces were noted in the week prior to post mortem.

- single oval, flat choleliths, which were firm but friable were present in the gall bladders of Dogs 359♂ and 363♂ (185ppm). Dimensions were 13x9x1mm and 5x3x1mm respectively.

There were no similar findings in dogs receiving 500(700)ppm. Results of histopathological investigation are awaited.

(c) Histopathology:

Data will be forwarded when they become available.

COMMENTS:

Dog 344♀ (Control) exhibited a similar clinical condition and haematological/biochemical profile during the 5th week of treatment to that observed for 372♀ (see Mortalities). A detailed case history was sent to you on 18/01/91. The dog appeared normal from the beginning of Week 6, however, during Week 13 the dog again appeared quiet, with a stiff posture and elevated temperature for 2 to 3 days. By Day 6 of Week 13 the dog had fully recovered (see our fax dated 12 March 1991). A similar, though less severe, episode again occured from Day 1 of Week 27, however, by the fourth day of that week the animal had again fully recovered. No further episodes have been noted to date.

A brief summary of findings for Dog 112♀ (500(700)ppm will be forwarded shortly after the post mortem.

---

03 February 1992

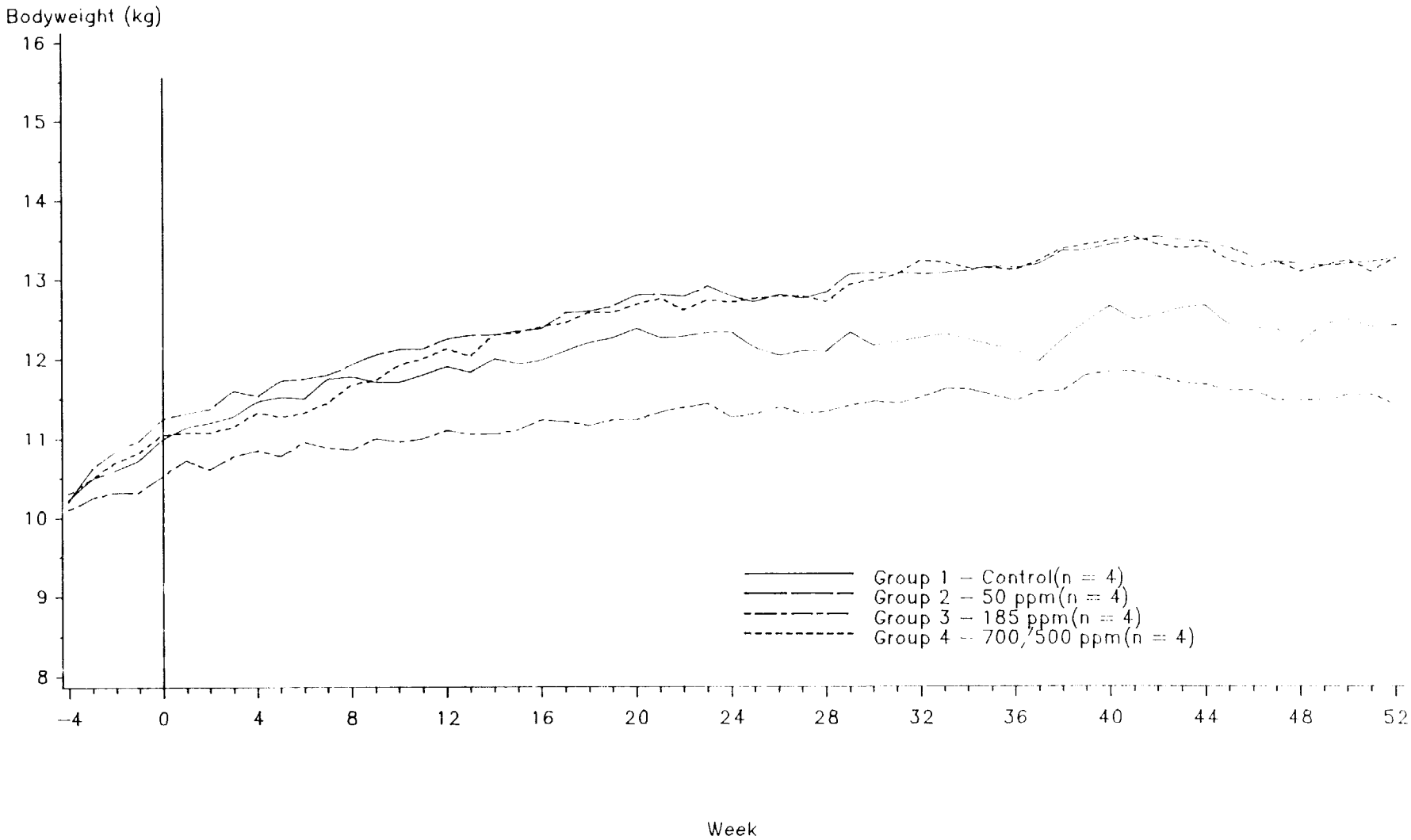
Prepared by : **T. G. Smith**
Study Supervisor

T. A. McLean
Study Director

Department of Non-rodent Toxicology

FIGURE 1a

Bodyweights – group mean values – males

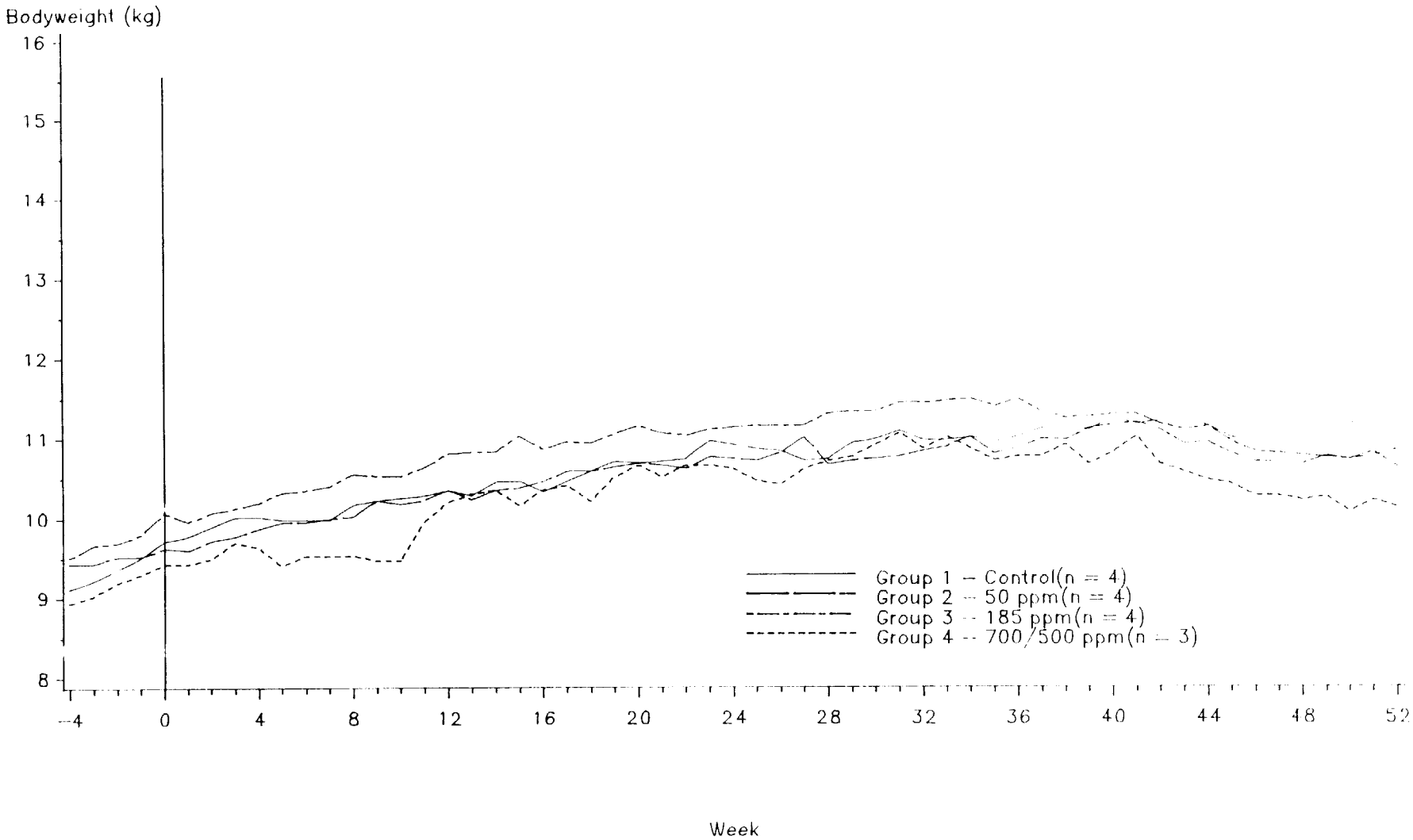


Group 4 – Dietary concentration reduced during week 12

FIGURE 1b

ZIR/10

Bodyweights – group mean values – females



Group 4 – Dietary concencentration reduced during week 12
Group 4 – Weeks -4 to 10 – n = 3, weeks 11 to 52 – n = 2
Group 4 – Replacement animal 112 excluded throughout and animal 368 killed in week 11

SPONSORS

Ziram Task Force,
c/o UCB Chemicals Corporation,
5505-A Robin Hood Road,
Norfolk, VA 23513,
U.S.A.

STUDY TITLE

13-week dietary toxicity study in beagle dogs

DATA REQUIREMENT

EPA FIFRA 82-1(b)

AUTHORS

Timothy A. McLean, Steven A. Horner, David P. Buist, David Crook, Rosemary M. Read, Chirukandath Gopinath, Alan Anderson, I. Suzanne Dawe, Steven F. Johnson, Sujit Patel.

STUDY COMPLETED ON

5 May 1992

PERFORMING LAB

Huntingdon Research Centre Ltd.,
P.O. Box 2,
Huntingdon,
Cambridgeshire,
PE18 6ES,
ENGLAND.

HRC STUDY REPORT NO.

ZIR 8/901813

FINAL REPORT DATE

5 May 1992

STATEMENT OF NO CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10(d) (1) (A), (B), or (C).

COMPANY:	Ziram Task Force c/o UCB Chemicals Corporation
COMPANY AGENT:	Paul R. Larson
DATE:	June 19, 1992
TITLE:	Manager Agricultural Products
SIGNATURE:	Paul R. Larson

These data are the property of the Ziram Task Force (Consortium No. 62405) and, as such, are considered to be confidential for all purposes other than compliance with FIFRA 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any other statute or in any other country.

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

HRC Report No. ZIR 8-G/901813

To the best of my knowledge and belief the Study described in this Report was conducted in compliance with the following Good Laboratory Practice Standards:

Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health & Social Security 1986 and subsequent revision, Department of Health, 1989.

United States Environmental Protection Agency, (FIFRA), Title 40 Code of Federal Regulations Part 160, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

Japan Ministry of Agriculture, Forestry and Fisheries, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984.

Organisation for Economic Co-operation and Development, ISBN 92-64-12367-9, Paris 1982.

Timothy A. McLean, B.Sc. (Hons.), M.Sc., C.Biol., M,I,Biol.,
Study Director,
Huntingdon Research Centre Ltd.

Date 5 May 1992.

Keith F. Rivett, B.Sc., C.Biol., M.I.Biol.,
For Laboratory Management

Date 7/6/92

Sponsor's Monitoring Scientist

Date 16 June 1992

Sponsor's Representative

Date 19 June 1992

We the undersigned, hereby declare that the work was performed under our supervision according to the procedures herein described, and that this report provides a correct and faithful record of the results obtained.

Timothy A. McLean, B.Sc. (Hons.), M.Sc., C.Biol., M,I,Biol.,
Study Director (from 29 May 1991),
Department of Non-Rodent Toxicology.

Steven A. Horner, B.Sc. (Hons.), M.Sc., Ph.D., C.Biol., M.I.Biol.,
Study Director (until 29 May 1991),
Department of Non-Rodent Toxicology

David P. Buist, B.Vet.Med., M.R.C.V.S.,
Veterinary Director,
Veterinary Services

David Crook, B.Sc. (Hons.), Ph.D.,
Head of Department of Clinical Pathology

Rosemary M. Read, B.V.Sc., M.R.C.V.S., Ph.D.,
Senior Pathologist

Chirukandath Gopinath, B.V.Sc., M.V.Sc., Ph.D., F.R.C.Path.,
Director of Pathology

FLAGGING STATEMENT

I have applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of the attached study. In my professional judgement, this study neither meets nor exceeds any of the applicable criteria.

Signature of Agent of Submitter/Sponsor

19 June 1992
Date

Michaela Giurgea, M.D.
Scientific Monitor for
Ziram Task Force

16 June 1992
Date

Submitter/Sponsor:

Ziram Task Force
(Consortium No. 62405)
5505-A Robin Hood Road
Norfolk, VA 23513

Ziram Task Force Members:

UCB Chemicals Corporation
(Company No. 45728)
Atochem North America, Inc.
(Company No. 4581)

DEPARTMENT OF QUALITY ASSURANCE

REPORT AUDIT STATEMENT

HRC REPORT No. ZIR 8/901813

This report has been audited by HRC Quality Assurance Department. It is considered to be an accurate description of the procedures and practices employed during the course of the study and an accurate presentation of the findings.

Date of reporting Audit Findings to the Study Director and HRC Management 18.02.91

5-5-92

Peter H.C.V. Richold, B.Sc.,
Senior Systems Compliance Auditor,
Department of Quality Assurance,
Huntingdon Research Centre Ltd.

P.R. QA.3 (1)

DEPARTMENT OF QUALITY ASSURANCE

DATES OF STUDY INSPECTIONS

HRC REPORT No. ZIR 8/901813

Inspections were made by the Quality Assurance Department of the various phases of the study described in this Report. The dates on which inspections were made and the dates on which the findings were reported to the Study Director and to HRC Management are given below.

Phase of Study	Date of Inspection*	Date of Reporting
Protocol Review	-	08.05.90
Pre-experimental Period	22.05.90	23.05.90
Experimental Period	29.05.90	04.06.90
	27.06.90	02.07.90
	24.07.90	27.07.90
	16.08.90	16.08.90
	22.08.90	23.08.90
	29.08.90	30.08.90

5.5.92

Peter H.C.V. Richold, B.Sc.,
Senior Systems Compliance Auditor,
Department of Quality Assurance,
Huntingdon Research Centre Ltd.

*Week beginning. This has been adopted to avoid the presentation of excessive dates in instances of an inspection being conducted over several days.

Dates on which any 'process-based' inspections were made while the study was in progress are not included in the above listing.

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SUMMARY

Test compound:	Ziram (Technical).
Test species:	Pure-bred beagle dogs (4 groups each of 4♂ and 4♀).
Route of administration:	By admixture with the diet.
Duration of treatment:	13 weeks.
Dietary concentration:	Control (untreated diet) 100 ppm 300 ppm 1000 ppm

Results

Mortality:	During Week 5, Dog 1035♂ (1000 ppm) suffered a seizure from which significant recovery was not apparent and therefore it was killed for humane reasons. This was considered to be related to treatment.
Clinical signs:	Convulsive episodes were noted for Dog 1035♂ (1000 ppm) during Weeks 2, 4 and 5. In addition, Dog 1034♀ (1000 ppm) was noted to be trembling on one occasion during Week 8.
Bodyweight and food consumption:	Inappetence and associated loss in bodyweight were recorded for Dog 1035♂ (1000 ppm) from Week 2 until its death in Week 5. Reduced food intake/bodyweight was also noted for animal 1036♀ at 1000 ppm from Weeks 3/4 to 6.

Ophthalmoscopy: There was no effect of treatment.

Laboratory investigations:

(a) Haematology: Treatment at 1000 ppm was considered to be associated with a slight lowering of group mean red cell indices (PCV, Hb, RBC) in males and females at Weeks 6 and 13, although differences from concurrent controls were not always statistically significant. In addition, slightly elevated circulating reticulocyte counts, with associated slight polychromasia, hypochromasia and/or moderate anisocytosis, were noted for animals 1033♂ and 1036♀ at 1000 ppm in Week 6. Slightly elevated reticulocyte count was also apparent for animal 1034♀ at 1000 ppm in Week 13.

There was a minimal, but dosage-related/statistically significant increase in group mean APTT in Weeks 6 and/or 13, for males receiving 300 or 1000 ppm. A similar change was apparent for females at 1000 ppm in Weeks 6 and 13, although these did not attain statistical significance.

(b) Biochemistry: High individual AP, GPT, GOT, CPK and cholesterol values were noted for animal 1035♂ (1000 ppm) killed for humane reasons in Week 5. High individual AP, GPT, GOT and OCT values were also recorded for animal 1036♀ at 1000 ppm during Week 6 only.

A statistically significantly elevated group mean AP value was noted for females at 1000 ppm in Week 13.

A dosage-related/statistically significant increase in group mean cholesterol was noted for animals receiving 300 or 1000 ppm during Weeks 6 and/or 13.

A statistically significant decrease in group mean albumin, with a corresponding increase in group mean globulin, was noted for animals at 1000 ppm during Week 6 and for animals from all treated groups during Week 13.

(c) Urinalysis:

High urinary SG and protein and traces of total reducing substances were noted in the urine of Dog 1035♂ (1000 ppm), killed for humane reasons in Week 5.

There were no other treatment-related effects.

Terminal studies:

(a) Bone marrow examination:

There were no treatment-related effects.

(b) Organ weights:

High liver weight for Dog 1035♂ (1000 ppm), killed in Week 5, high group mean liver weights for males and females at 1000 ppm and high liver weight for animal 1027♂ at 300 ppm were considered to be related to treatment.

Liver weight for animal 1020♀ at 100 ppm was also slightly higher than normal; however, in isolation this finding is of uncertain relationship to treatment.

(c) Macroscopic and microscopic findings:

The following changes were considered treatment-related:

<u>Heart</u>

Pallor of the endocardium/myocardium, red endocardial discolouration in the left ventricle or atrioventricular valve and focal myocardial necrosis with acute inflammatory cell infiltration and haemorrhage in the heart of Dog 1035♂ (1000 ppm), killed in Week 5.

These changes are considered to be related to the convulsions seen clinically for this animal.

Liver

Centrilobular hepatocyte degeneration in the liver of Dog 1035♂ (1000 ppm), was considered possibly secondary to the heart changes seen.

Multiple depressed pale areas on liver surface and focal necrosis, with loss of cells and dilated sinusoids, for animal 1036♀ at 1000 ppm. (Correlating with high AP, GPT, GOT and OCT values previously noted for this animal). Focal necrosis was also seen for animal 1027♂ at 300 ppm.

Minimal amounts of pigment in Kupffer cells of animals 1029♂ and 1028♀ at 300 ppm and animals 1033♂, 1037♂ and 1036♀ at 1000 ppm.

In addition, areas of polymorphonuclear leucocyte infiltration into the alveoli was seen for Dog 1035♂ (1000 ppm), killed Week 5, and minimal foci of eosinophilic homogeneous material was noted in the alveoli of animals 1021♂ and 1018♀ at 100 ppm, animal 1027♂ at 300 ppm and animal 1033♂ at 1000 ppm. There was no apparent dosage-relationship and therefore the toxicological significance of these findings is uncertain.

CONCLUSION

Following daily oral administration of Ziram, at dietary concentrations of 100, 300 and 1000 ppm, to beagle dogs for 13 weeks, changes considered to be conclusively related to treatment were noted at 300 and 1000 ppm. Consequently, 100 ppm is considered to be the no-effect level.

Several treatment-related changes, including minimal focal necrosis and pigmented Kuppfer cells, identify the liver as the target organ for toxicity at 300 and 1000 ppm. None of these findings would preclude the use of either dosage in a 52-week study but the treatment-related convulsions seen for 1 animal at 1000 ppm, requiring the animal to be killed for humane reasons in Week 5, indicate that this dietary concentration exceeds the MTD and is therefore unsuitable for use in a chronic study.

Based on these findings, dosages of 50, 185 and 700 ppm were selected for use in the 52-week dietary toxicity study in beagle dogs (HRC Study No. ZIR/10-G).

INTRODUCTION

The purpose of this study, performed at the Huntingdon Research Centre, England, was to assess the toxicity of the fungicide, Ziram, when administered orally to pure-bred beagle dogs over a period of 13 weeks, and to select dosages for use in a subsequent 52-week study in the beagle dog.

Four groups of animals (each containing 4♂ and 4♀) were allocated to this study. The test material was administered by admixture with the diet. Three groups received the test material at dietary concentrations of 100, 300 or 1000 ppm, while the fourth group received untreated diet and acted as controls. The dosages were selected by the Sponsor from available toxicological information, including a 4-week preliminary toxicity study in beagle dogs performed at the Huntingdon Research Centre (HRC Report No. ZIR 1-G/89637).

The procedural details and all relevant information obtained during the study are presented in this report.

RELEVANT CALENDAR DATES

Protocol Approval	
by Study Director:	3 May 1990.
by HRC Management:	3 May 1990.
by Sponsor:	7 May 1990.
Start of pre-dosing period:	25 April 1990.
Start of dosing period:	23 May 1990.
Post mortem examinations:	22 - 24 August 1990.

PROCEDURE

Animal management

Thirty-two pure-bred beagle dogs (16 males and 16 females) were supplied by Consort Limited, Harewood Park, Harewood End, Herefordshire, HR2 8TS.

Documentation provided by the Supplier included details of the litter of origin, ear tattoo, date of birth, confirmation of inoculation and details relating to any other treatment.

Shortly after arrival, each animal was examined by a veterinary surgeon. Dogs were acclimatised to conditions in the kennel unit for at least 4 weeks before treatment was started, during which time they were subject to our routine examination and acceptance procedures. During the acclimatisation period the dogs were re-inoculated against canine distemper virus, canine adeno virus (hepatitis), para influenza virus, canine parvovirus, Leptospira canicola, Leptospira icterohaemorrhagiae and Bordetella bronchiseptica. The animals also received a course of treatment with the anthelmintic piperazine.

During the study, all ailments were brought to the attention of a veterinary officer. The treatment of any such ailments was documented in the raw data.

The study was performed in the Department of Canine Toxicology, Building J24, Unit 19.

The dogs were housed in kennels within a building for which the environment was governed by a computer controlled building management system, which continually monitored the temperature and the operational state of all the air handling plant. Animal room temperature controls were set to maintain, as far as possible, a temperature range of 15 - 24°C. Lighting was controlled to give 12 hours of light and 12 hours of dark per 24-hour period. Air extraction was via a balanced system designed to provide approximately 12 air changes per hour.

The dogs were housed in kennels which were designed in accordance with the requirements of the United Kingdom Home Office Code of Practice for the Housing and Care of Animals used on Scientific Procedures. The kennels each have a floor area of 4.5 square metres and accommodated two animals of the same sex and dosage group. The kennels were sub-divided into two equal areas by means of a movable partition so that each day, from approximately 0900 to 1700 hours, the animals were segregated for assessment of individual clinical signs and food consumption. Both parts of the kennel were fitted with an automatic valve for the supply of drinking water.

Graded whitewood sawdust (Lignocel), supplied by R.S. Supplies, was used as litter and changed daily.

Details relating to the age and bodyweight of individual animals at the time dosing commenced have been presented in Table 1.

Diet

Each animal received 400 grams of a standard ground dry diet (Diet A: Special Diets Services) each day. Drinking water was available in the kennels at all times except those indicated under 'Laboratory investigations'.

There was no information available to indicate that any non-nutrient substance likely to influence the effect of the test compound could reasonably be expected to be present in the diet or drinking water, both of which were routinely subjected to chemical analysis. The results of these analyses have been lodged in HRC Archives.

Study design

Prior to the commencement of dosing, each dog was pseudo-randomly allocated to one of the treatment groups. Bodyweight and litter of origin are two factors taken into account during this procedure. The animals were given new identity numbers (odd numbers for males, even numbers for females). The identity number was displayed on a disc attached to a neck chain and was tattooed on the inner aspect of the left hind leg.

The animals in each treatment group were housed in a block of several adjacent kennels, rather than dispersed randomly throughout the kennel unit.

Each kennel was identified by a label coloured according to the occupant's group. Each label displayed the animal's identity number, dosage level and study schedule number.

The group identification was as follows:

Group	Dosage (ppm)	Colour code	Animal numbers
1	Control	White	♂: 1009,1011,1013,1015 ♀: 1010,1012,1014,1016
2	100	Yellow	♂: 1017,1019,1021,1023 ♀: 1018,1020,1022,1024
3	300	Green	♂: 1025,1027,1029,1031 ♀: 1026,1028,1030,1032
4	1000	Red	♂: 1033,1035,1037,1039 ♀: 1034,1036,1038,1040

Test compound

25 kg of the test material, Ziram (technical), Batch no. 8331 AA was supplied by UCB Chemicals, Belgium, and was received at the Huntingdon Research Centre on 12 January 1989. This was used for the duration of this study.

The test material, a known fungicide, was a white powder. The purity of the test material was stated by the Sponsor to be 98.5% and no correction for purity was made. The test material was stored at room temperature, protected from light and was stated by the Sponsor to be stable for the duration of the study. The stability of the test material was examined every 6 months by the Sponsor and the results of these analyses are included in this report in Addendum 2.

Administration of the test material

The test material was administered by admixture with the diet. Control animals received untreated diet. Each dog was offered 400 grams diet each day in the morning and any food uneaten removed at approximately 1700 hours. The residue was then recorded and discarded.

Fresh treated diet was prepared weekly, usually one day prior to the beginning of the dose week. The required amount of test compound was weighed and ground diet added until the desired concentration of pre-mix was achieved. This was blended in a Turbula mixer for a minimum of 2 minutes. Diets for each group were prepared by direct dilution of the pre-mix. Homogeneity of all diets was achieved by mixing for at least 7 minutes in a Gardner double cone blender.

Formulated diets were stored at -20°C, protected from light.

Formulation sampling

Prior to the commencement of the study, the proposed formulations were checked by chemical analysis to confirm that the method was acceptable and that the stability of the formulation was satisfactory.

Samples of the dietary formulations prepared in Weeks 1 and 13 were also analysed to check the accuracy of preparation.

In addition, samples of the 100 ppm formulation prepared in Week 1 were analysed following storage at room temperature for 8 hours. Chemical analyses were carried out by the Department of Analytical Chemistry, Huntingdon Research Centre and the results of these analyses are presented in Addendum 1.

Observations

The following observations were made during the course of the study:

Clinical signs

All signs of ill health or reaction to treatment were recorded.

Except on Saturdays, Sundays and public holidays, all animals were checked regularly throughout the working day from 0900 to 1700 hours. On Saturdays, Sundays and public holidays animals were checked regularly from 0900 to 1200 hours and again at 1700 hours. Dated and signed records of all activities relating to the day by day running and maintenance of the study within the animal unit, as well as to the group observations and examinations outlined below, were recorded in the Study Day Book. In addition, observations relating to individual animals made throughout the day were recorded on the kennel sheets.

Mortalities

The circumstances of any death were recorded in detail and a full post mortem examination was performed as described later. Prior to death, observations listed in this procedure relating to blood sampling and urinalysis were carried out.

Bodyweight

All animals were weighed, prior to feeding, once a week throughout the pre-dosing and dosing periods.

During the specified weeks, laboratory investigations were arranged so as to minimise any fluctuations in weight that might result from blood sampling or urinalysis.

Food consumption

The quantity of food left by individual animals was recorded daily throughout the experimental period.

Achieved intake of Ziram

From the food consumption data obtained, the quantity of Ziram ingested weekly by each group was calculated. The approximate dosage equivalents expressed as mg/kg/day were calculated at weekly intervals from the mean bodyweight data (obtained by averaging the values at the start and end of each week to give mid-week weights) using the following formula:

$$\frac{\text{Food consumption (g) x ppm}}{\text{mid-week bodyweight (g) x 7}}$$

Ophthalmoscopy

The eyes of each animal were examined by means of a Keeler indirect ophthalmoscope once before commencement of dosing and again during Week 13.

Prior to examination, the pupils of each animal were dilated using a Tropicamide ophthalmic solution ("Mydriacyl", Alcon Laboratories Inc.).

Laboratory investigations

Samples of venous blood were withdrawn from the jugular or cephalic vein from all animals in treatment and control groups, once before dosing commenced and then during dose Weeks 6 and 13.

The blood samples collected were divided into tubes as follows:

EDTA anticoagulant.....for haematological investigations
Citrate anticoagulant.....for coagulation tests
Fluoride anticoagulant.....for plasma glucose estimation
Plain tubes (no anticoagulant)..for the remaining biochemical tests

At the same intervals urine samples were obtained from the same animals over a collection period of approximately 16 hours. Water was removed from the kennels about 5 hours prior to the start of collection.

Animals were fasted overnight before collection of blood samples and during the period of urine collection.

At the discretion of the Study Director, investigation results found to be abnormal were checked by obtaining further samples of blood or urine and repeating the investigation.

The estimations performed on blood and urine samples have been listed below, together with an abbreviated title (used in Appendices and Tables), and the methods and the units of measurement used.

(a) Haematology

	Units
The following estimations were performed with an Ortho ELT 1500 analyser:	
Packed cell volume (PCV)	%
Haemoglobin (Hb)	g/dl
Red cell count (RBC)	$x10^6/mm^3$
Absolute indices calculated as follows:	
Mean corpuscular haemoglobin concentration (MCHC) Hb (g/dl) x 100 + PCV (%)	%
Mean corpuscular volume (MCV) PCV (%) x 10 + RBC ($x10^6/mm^3$)	fl
Total white cell count (WBC Total)	$x10^3/mm^3$
Platelet count (Plts)	$x10^3/mm^3$
The following were performed using the appropriate methodology, as described below:	
Reticulocyte count (Retic) - Method of Dacie, J.V. and Lewis, S.M. (Practical Haematology, 1966, 3rd edit. p28)	% (of red cells)
Differential WBC count - standard microscopy of blood smear, stained with modified Wright's stain, counting 100 cells	
Neutrophils (N))	
Lymphocytes (L))	
Eosinophils (E))	$x10^3/mm^3$
Basophils (B))	
Monocytes (M))	

	Units
The percentage distribution of each cell type was determined by microscopy, these values being converted to absolute values by computer. This inevitably involved a 'rounding off' in a proportion of the results and for this reason the reported total WBC may differ slightly from the differential cell total.	
Cell Morphology: If definitely abnormal cells were observed when examining any stained slide, such as the presence of Heinz bodies or abnormal lymphocytes etc., their presence or absence on each such slide examined was recorded and tabulated separately.	
Prothrombin Time (PT) - Method of Quick, A.J. ("The Haemorrhagic Diseases and the Physiology of Haemostasis" 1942, Thomas, Illinois)	s
Activated Partial Thromboplastin Time (APTT) - Method of Proctor, R.R. and Rapaport, S.I. (Am. J. Clin. Path. 1961, 36, 212)	s
(b) Biochemistry	
The following parameters were analysed with an Hitachi 737 Clinical Chemistry Analyser:	
Total Protein	g/dl
Albumin (Alb)	g/dl
Globulin (Glob) - By subtraction Total Protein (g/dl) minus Alb (g/dl)	g/dl
Urea	mg/dl
Creatinine	mg/dl
Sodium (Na)	mEq/l
Potassium (K)	mEq/l
Calcium (Ca)	mEq/l
Inorganic Phosphorus (P)	mEq/l
Chloride (Cl)	mEq/l

	Units
Total cholesterol (Chol)	mg/dl
Alkaline phosphatase (AP)	mU/ml
Total Bilirubin	mg/dl

The following parameters were analysed using a Roche Cobas centrifugal analyser using the appropriate BCL test kit:

Glucose - (Hexokinase mediated assay)	mg/dl
Glutamic-pyruvic transaminase (GPT), also known as 'alanine aminotransferase' Reaction temperature 30°C	mU/ml
Glutamic-oxaloacetic transaminase (GOT), also known as 'aspartate aminotransferase' Reaction temperature 30°C	mU/ml
Gamma-glutamyltransferase (γGT) - Reaction temperature 30°C	mU/ml
Creatine phosphokinase (CPK), also known as Creatine Kinase.	mU/ml

The following parameters were analysed as described:

Ornithine Carbamoyl transferase (OCT) - Method of Ceriotti, G. (Clin. Chim. Acta. 1973, 47, 97) Reaction temperature 37°C	mU/ml

(c) Urinalysis

Volume	ml
pH - by pH meter	
Specific Gravity (SG) - by refractometry, compared to water with a value of 1000	
Protein - Roche Cobas centrifugal analyser using modified method of Macart, M. and Gerbaut, L. (Clin. Chim. Acta, 1984 141, 77)	mg/dl

Qualitative Tests

Total reducing substances.....Clinitest
Glucose)
Ketones)
Bile pigments).....Multistix
Urobilinogen)
Haem pigments*)

Clinitest and Multistix are diagnostic reagents obtained from Ames Company, Stoke Poges, England and were used as qualitative indicators of analyte concentration. Results have been reported according to the following convention:

0	=	none
TR	=	'trace' of analyte
+	=	'small amount' of analyte
++	=	'moderate amount' of analyte
+++	=	'large amount' of analyte

* Reported as a positive or negative finding only

Microscopy

For microscopic examination, a portion of the urine sample was centrifuged at approximately 1500 'g' for 10 minutes and the resulting deposit spread on a microscope slide. The deposit was examined for the presence of the following:

Epithelial cells	(E)
Polymorphonuclear leucocytes	(P)
Mononuclear leucocytes	(M)
Erythrocytes	(R)
Organisms	(O)
Renal tubule casts	(C)
Other abnormal constituents	(A)

The grading of cell frequency in the centrifuged deposit was as follows:

0	=	none found in any field examined
1	=	few in some fields examined
2	=	few in all fields examined
3	=	many in all fields examined

Terminal studies

Bone marrow

Prior to terminal autopsy, bone marrow was obtained from each animal by sternal puncture and a smear prepared for subsequent staining and examination by our haematologist.

Post mortem examination

On completion of 13 weeks dosing each animal was killed by exsanguination under pentobarbitone anaesthesia (Expiral, 200 mg/ml pentobarbitone sodium). All animals were fasted overnight prior to post mortem examination.

Since the post mortem procedures took 3 days to complete, the dosing of individual treated animals continued until the day prior to their sacrifice.

At autopsy the macroscopic appearance of the tissues was noted and the weights of the following organs recorded (paired organs being weighed separately):

- adrenals
- brain
- heart
- kidneys
- liver
- lungs
- pancreas
- pituitary
- spleen
- testes or ovaries
- thymus
- thyroids (and parathyroids)
- uterus or prostate

Tissues for histopathological study

Samples (or the whole) of the following tissues from each animal were preserved together with any tissue showing macroscopic abnormality:

- adrenals
- alimentary tract (oesophagus, stomach body and antrum, duodenum, jejunum, ileum, caecum, colon, rectum)
- aorta (arch and abdominal)
- brain (cerebral cortex, thalamic nuclei, mid-brain, medulla and cerebellum)
- eyes
- femur and articular surface
- gall bladder
- heart
- kidneys
- liver
- lungs (with bronchi)
- lymph nodes (cervical and mesenteric)
- mammary gland
- ovaries
- pancreas
- pituitary
- prostate
- salivary gland (submandibular)
- sciatic nerve
- skeletal muscle (biceps femoris)
- skin
- spinal cord (cervical, thoracic and lumbar regions)
- spleen
- sternum
- tattoo*
- testes (and epididymis)
- thymus
- thyroids (and parathyroids)
- tongue
- trachea
- urinary bladder
- uterus
- vagina

* Preserved only

The fixative routinely used was 10% buffered formalin. The eyes were preserved in Davidson's fixative whilst additional pieces of liver and kidney were placed in formol calcium.

Prior to microscopic examination, tissues were embedded in 56°C M.P. paraffin wax and sections cut at 4 micron and stained with haematoxylin and eosin. Cryostat sections of liver previously fixed in formol calcium were cut at 12 micron and stained for fat with Oil Red O. Liver sections were stained using PAS for glycogen.

The tissues listed above were examined for all animals by light microscopy.

Statistical analysis

Data from male and female animals were analysed both together and separately.

Food consumption data were analysed as totals over selected time periods, expressed on a weekly basis. Bodyweight data were analysed using weight gains.

The following sequence of statistical tests was used for food consumption, bodyweight, organ weight and clinical pathology data:

(i) If the data consisted predominantly of one particular value (relative frequency of the mode exceeded 75%), the proportion of animals with values different from the mode was analysed by appropriate methods. Otherwise:

(ii) Bartlett's test (1) was applied to test for heterogeneity of variance between treatments. Where significant (at the 1% level) heterogeneity was found, a logarithmic transformation was tried to see if a more stable variance structure could be obtained.

(iii) If no significant heterogeneity was detected (or if a satisfactory transformation was found), a one-way analysis of variance was carried out. If significant heterogeneity of variance was present, and could not be removed by a transformation, the Kruskal-Wallis analysis of ranks (2) was used.

(iv) For pre-dose data, analyses of variance were followed by Student's 't' test. For data from the dosing period, analyses of variance were followed by Williams' test (4) for a dose-related response. The Kruskal-Wallis analyses were followed by the non-parametric equivalents of the 't' test and Williams' test (Shirley's test, (3)).

Where appropriate, analysis of covariance was used in place of analysis of variance in the above sequence.

In an attempt to allow for differences in bodyweight which might influence the organ weights, the final bodyweight was included as a covariate with organ weight data when the within-group relationship between organ weight and bodyweight was significant at the 10% level.

References

1. Bartlett, M.S. (1937), Proc. Roy. Soc. A., 160: 268 - 282.
2. Kruskal, W.H. and Wallis, W.A., (1952/3), J. Amer. Statist. Ass., 47: 583 - 621 and 48: 907 - 912.
3. Shirley, E., (1977), Biometrics, 33: 386 - 389.
4. Williams, D.A. (1971/2), Biometrics, 27: 103 - 117 and 28: 519 - 531.

Good laboratory practice

This study was conducted in compliance with the principles of Good Laboratory Practice as set forth in:

United States Environmental Protection Agency, Title 40 Code of Federal Regulations Part 160, Federal Register, 29 November 1983.

Organisation for Economic Co-operation and Development, ISBN 92-64-12367-9, Paris 1982.

Japan Ministry of Agriculture, Forestry and Fisheries, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984.

The United Kingdom Compliance Programme, Department of Health and Social Security, 1986.

Procedures

The procedures used during this study were documented in the relevant HRC Procedures Manual.

Quality Assurance Department Review

The Quality Assurance Department conducted inspections of the various phases of the study and of certain repetitive operations, at the intervals required by the EPA, OECD, JMAFF and DHSS Good Laboratory Practice Regulations. The dates on which the findings of these inspections were reported to the Study Director and to HRC Management have been specified in this final report.

The final report was reviewed by HRC's Quality Assurance Department, comparing individual findings against raw data and comparing the statements and results presented in the report with individual data presented in the Appendices of the report.

Staff safety

The standard safety precautions operating within the department were applied to this study.

Records and Archives

Records were maintained of all tests, measurements, investigations and analyses together with other records of observations and procedures associated with the study, as required by the official HRC Procedures Manual. This material was stored in the department during the conduct of the study.

All specimens, raw data and other documents generated at HRC during the course of this study, together with a copy of this final report, have been lodged in the Huntingdon Research Centre Archives, Huntingdon, England, to be retained for 10 years.

RESULTS

Treatment commenced:	23 May 1990.
Nominal dietary concentration:	Control 100 ppm 300 ppm 1000 ppm
Duration of treatment:	13 weeks.

MORTALITY

There was one mortality that was considered to be related to administration of Ziram:

Dog 1035♂ (1000 ppm) was killed for humane reasons on Day 6 of Week 5 (25 June 1990).

Throughout Day 6 of Week 5, this animal had a seizure (foaming at the mouth, champing of jaws, trembling/jerking of head and body, unawareness of surroundings and inability to stand), from which significant recovery was not apparent, and it was considered necessary to kill this animal for humane reasons. Prior to this seizure, convulsive episodes had previously been observed for this animal on Day 1 of Week 2 and on Day 5 of Week 4. These convulsions were of short duration (up to 4 minutes) followed by rapid recovery. There were no other clinical signs noted for this animal. Inappetence and bodyweight loss were noted for this animal from Week 2 until its sacrifice in Week 5.

Prior to sacrifice, blood samples were taken for haematology and biochemistry investigations. A sample of urine was also collected from the bladder at post mortem for urinalysis investigations. Individual results of these investigations have been presented in Appendices 4b, 5b and 6b.

The EDTA sample for haematological investigations was clotted and so no haematology results were obtained for this animal. Values for PT and APTT, however, were slightly lower than pre-dose values.

At Week 5, individual serum AP, GOT, CPK and cholesterol values for this animal were markedly higher, and serum GPT, sodium and chloride levels slightly higher than the corresponding pre-dose values. A high urinary SG and protein was also noted for this animal at post mortem and traces of total reducing substances were also noted in the urine sample.

At post mortem, all organ weights were considered to be within normally expected limits, with the exception of the liver weight, which at 4.33% of bodyweight exceeded our normal upper limit of 4.0%. Spleen and prostate weights were also considered to be lower than expected. Individual organ weights are presented in Appendices 7 and 8.

Macroscopic post mortem findings, presented in Appendix 9, were as follows:

Heart

Left ventricle; general pallor of endocardium, extending into myocardium, and several foci of red endocardial discolouration (up to 3 mm diameter).

Left atrioventricular valve; area of red endocardial discolouration (6 x 3 mm).

Liver

Pale with accentuated lobular marking, cut surface friable.

Lungs

Cardiac lobe and left intermediate lobe congested.

All histological changes for this animal are presented in Appendix 9. Principal findings were focal myocardial necrosis with acute inflammatory cell infiltration and haemorrhage with minimal centrilobular hepatocyte degeneration and the presence of moderate amounts of fat droplets in the periportal hepatocytes. The spleen was contracted, accounting for low spleen weight, but there were no changes to account for the low prostate weight seen for this animal.

The changes seen in the heart of Dog 1035♂ were considered likely to be related to the convulsions seen clinically and are not considered to be a direct effect of the test compound. It was also considered that the centrilobular hepatocyte degeneration noted for this animal was possibly secondary to the heart changes seen.

There were no other mortalities during the 13-week dosing period.

CLINICAL SIGNS

Convulsive episodes were noted for Dog 1035♂ (1000 ppm) as previously discussed. In addition, Dog 1034♀ (1000 ppm) was noted to be trembling on one occasion on Day 6 of Week 8.

There were no other clinical signs attributable to treatment.

BODYWEIGHT

Bodyweight data have been presented graphically in Figures 1 - 5(a - b); group mean bodyweight gain, and the results of statistical analysis, have been presented in Table 2 and individual bodyweights have been presented in Appendix 1(a - b).

Bodyweight gain was satisfactory for all dogs prior to the commencement of treatment.

Reductions in bodyweight were noted for Dog 1036♀ (1000 ppm) from Week 4 to 6, with subsequent recovery from Week 7 and for Dog 1035♂ (1000 ppm) from Week 2 until its death in Week 5, and were considered to be related to treatment.

Bodyweight loss and inappetence were also noted for Dog 1033♂ (1000 ppm) during Week 3 and 4, however as this was associated with a retropharyngeal abscess it was considered to be unrelated to treatment. With resolution of the abscess from Week 5, appetite restoration and bodyweight gain were noted for this animal.

It was concluded that treatment with Ziram produced a slight reduction in bodyweight gain for the 1 male and 1 female at 1000 ppm.

FOOD CONSUMPTION

Individual weekly food consumption values have been presented in Appendix 2 (a - b), with group mean values and the results of statistical analysis in Table 3.

Food consumption was satisfactory for all animals before treatment commenced.

From Weeks 3 to 5, a gradually reduced food intake was recorded for Dog 1036♀ (1000 ppm) with some subsequent recovery from Week 6. Inappetence was also noted for Dog 1035♂ (1000 ppm) from Week 2 until its death in Week 5. These changes were considered to be associated with treatment.

In addition, inappetence and associated bodyweight loss were noted for Dog 1033♂ (1000 ppm) during Weeks 3 and 4 and was associated with a retropharyngeal abscess, as previously discussed, and was considered to be unrelated to treatment.

It is concluded that treatment with Ziram was associated with a slightly reduced food intake for 1 male and 1 female receiving 1000 ppm.

ACHIEVED INTAKES OF TEST SUBSTANCE

Group mean weekly achieved intakes expressed as mg/kg/day, are presented in Table 4.

After 13 weeks of treatment the group mean achieved intakes of Ziram (Technical) were as follows:

Diet concentration ppm	100		300		1000	
	♂	♀	♂	♀	♂	♀
Achieved intake mg/kg/day	4.07	4.31	12.15	13.04	41.81	41.07
SD	0.39	0.33	1.09	1.14	3.37	4.20

SD Standard deviation

The dosage interval of 1:3:10 was closely maintained throughout the study.

OPHTHALMOSCOPY

The eyes of all dogs were examined once before dosing commenced and again during Week 13. Individual ophthalmoscopy findings are listed in Appendix 3 (a-b) and summarised in Table 5 (a-b).

There were no changes observed during Week 13 that were considered to be related to treatment.

LABORATORY INVESTIGATIONS

Laboratory investigations were performed once before the commencement of dosing and during Weeks 6 and 13 of dosing. Individual haematology, biochemistry and urinalysis results are presented in Appendices 4 - 6 (a-d) respectively and summarised in Tables 6 - 8 (a-c) respectively.

(a) Haematology

At Weeks 6 and 13, group mean red cell indices (PCV, Hb, RBC) were slightly reduced in comparison to concurrent control values, for males and females receiving 1000 ppm. However, these differences were not progressive and did not always attain statistical significance. In addition, during Week 6, slightly elevated circulating reticulocyte counts, with associated slight polychromasia, hypochromasia and/or moderate anisocytosis, were noted for 1033♂ and 1036♀ at 1000 ppm. Slightly elevated circulating reticulocyte count was also noted for Dog 1034♀ (1000 ppm) during Week 13.

Group mean APTT values for males receiving 300 ppm during Week 13 and 1000 ppm during Weeks 6 and 13 were slightly but statistically significantly higher than corresponding control values. A similar change was apparent for females at 1000 ppm during Weeks 6 and 13, although these did not attain statistical significance.

There were no other changes considered to be attributable to treatment.

(b) Biochemistry

Elevated serum AP, GPT, GOT, CPK, sodium, chloride and cholesterol values were noted for Dog 1035♂ (1000 ppm) prior to its death during Week 5, as previously discussed.

During Week 6, high bilirubin, AP, GPT, GOT, OCT and cholesterol values were recorded for Dog 1036♀ (1000 ppm), with the enzyme levels remaining clearly elevated, though to a lesser extent, on repeat investigation in Week 7. These values, however, were essentially unremarkable at Week 13, with the exception of AP which was still slightly elevated.

Mean AP values were increased among females receiving 1000 ppm at Weeks 6 and 13 in comparison with values for concurrent controls, attaining statistical significance in Week 13.

In addition, there was an apparent dosage-related increase in cholesterol for males and females at Weeks 6 and/or 13, attaining statistical significance at 300 and 1000 ppm.

Group mean albumin concentrations were slightly, but statistically significantly, lower than corresponding control values for animals at 1000 ppm during Week 6 and for animals from all treated groups during Week 13. This was associated with a corresponding increase in globulin concentrations, such that total protein levels were unaltered.

There were no other changes considered to be attributable to treatment.

(c) Urinalysis

A high urinary SG and protein and traces of total reducing substances were noted in the urine of Dog 1035♂ (1000 ppm), killed for humane reasons in Week 5.

There were no other effects on urinalysis parameters that were considered to be related to treatment.

TERMINAL STUDIES

On completion of 13 weeks dosing, all animals were killed on 22 - 24 August 1990.

(a) Bone marrow examination

There was a slight relative increase in myeloid cells in the smear from Dog 1011♂ (Control). For all other animals killed after 13 weeks of treatment, all smears were normal in cellularity, distribution or cell morphology.

(b) Organ weights

Individual organ weight values, expressed in grams and as a percentage of terminal bodyweight, are presented in Appendices 7 and 8. Group mean values, with the results of statistical analysis are presented in Table 9.

A high liver weight was seen for animal 1035♂ (1000 ppm), killed for humane reasons in Week 5, as previously discussed.

Group mean liver weights for males and females at 1000 ppm were statistically significantly higher than control values, with individual liver weights for animals 1033♂, 1037♂, 1039♂ and 1034♀ at this dosage exceeding our normal upper limit of 4.0% of bodyweight, and was considered to be related to treatment. A slightly high liver weight for animal 1027♂ at 300 ppm, equalling our normal upper limit of 4.0% bodyweight, was also considered to be related to treatment. The individual liver weight for animal 1020♀ at 100 ppm also exceeded our normal upper limit of 4.0% of bodyweight. In isolation this finding is considered to be of uncertain relationship to treatment.

In addition, a statistically significantly lower group mean heart weight for males at 1000 ppm and lower group mean lung weight for females at this dosage was also noted in comparison to controls. However, as differences were slight, not dosage-related and confined to 1 sex only and as all individual values were within normal limits for dogs of this age, these findings are considered to be incidental and unrelated to treatment.

(c) Macroscopic and microscopic pathology

Individual macroscopic and microscopic pathology findings are listed in Appendix 9 with histopathological findings summarised in Table 10.

The following comments are made in summary:

Changes considered to be related to treatment with Ziram were seen in the heart and liver.

Dog 1035♂ (1000 ppm) - killed for humane reasons in Week 5.

Macroscopically general pallor of the endocardium, extending into the myocardium of the left ventricle and an area of red endocardial discolouration on the left atrioventricular valve of the heart. Focal myocardial necrosis with acute inflammatory cell infiltration and haemorrhage was seen histologically.

Pallor of the liver, with accentuation of the lobular markings, and a friable cut surface, was seen grossly. Centrilobular hepatocyte degeneration was reported histologically. This was possibly secondary to the heart changes seen. These changes were considered likely to be related to convulsions seen clinically rather than a direct effect of the test compound.

Areas of polymorphonuclear leucocyte infiltration into the alveoli were also noted for this dog.

Liver

Macroscopically, multiple depressed pale areas were noted on the liver of animal 1036♀ receiving 1000 ppm. Focal necrosis, together with loss of cells and dilated sinusoids were seen histologically. This finding correlates with the high individual AP, GPT, GOT and OCT values previously noted for this dog. Focal necrosis was also seen in Dog 1027♂ treated with 300 ppm.

Minimal amounts of pigment were seen in the Kupffer cells of animals 1029♂ and 1028♀ receiving 300 ppm and for animals 1033♂, 1037♂ and 1036♀ at 1000 ppm.

These changes were not seen in control dogs or dogs receiving 100 ppm.

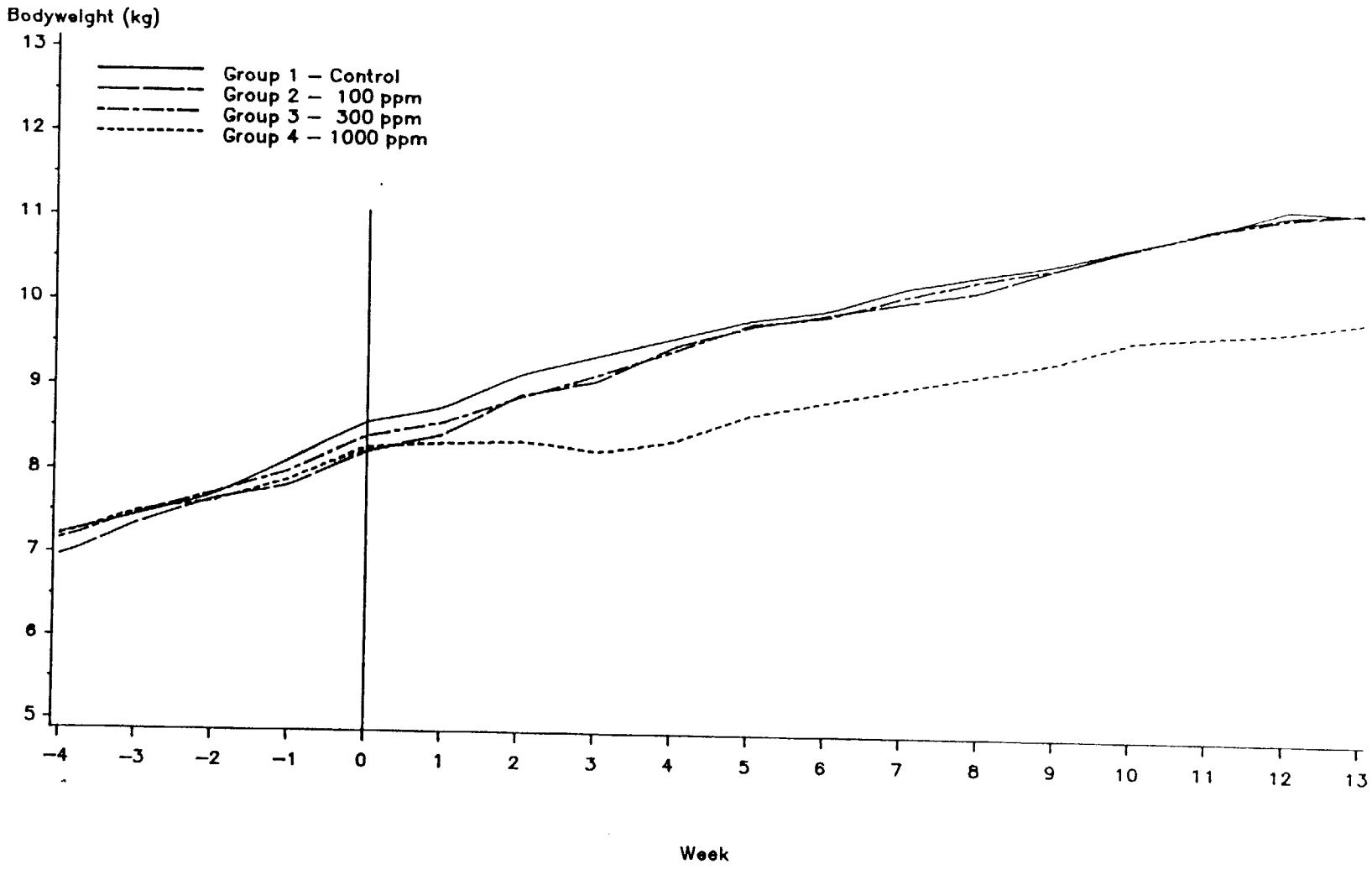
Lungs

Minimal foci of eosinophilic homogeneous material were seen in the alveoli of the lungs of animals 1021♂ and 1018♀ receiving 100 ppm, animal 1027♂ receiving 300 ppm and animal 1033♂ receiving 1000 ppm. In the male dog receiving 1000 ppm, this change was associated with acute inflammatory cell infiltration. The toxicological significance of these findings, which were not related to dosage, is not clear.

There were no other findings considered to be of toxicological importance, in particular no treatment-related changes were seen in the hearts of any dogs killed at termination.

FIGURE 1a

Bodyweights - group mean values - males



ZIR/8-G

FIGURE 1b

Bodyweights - group mean values - females

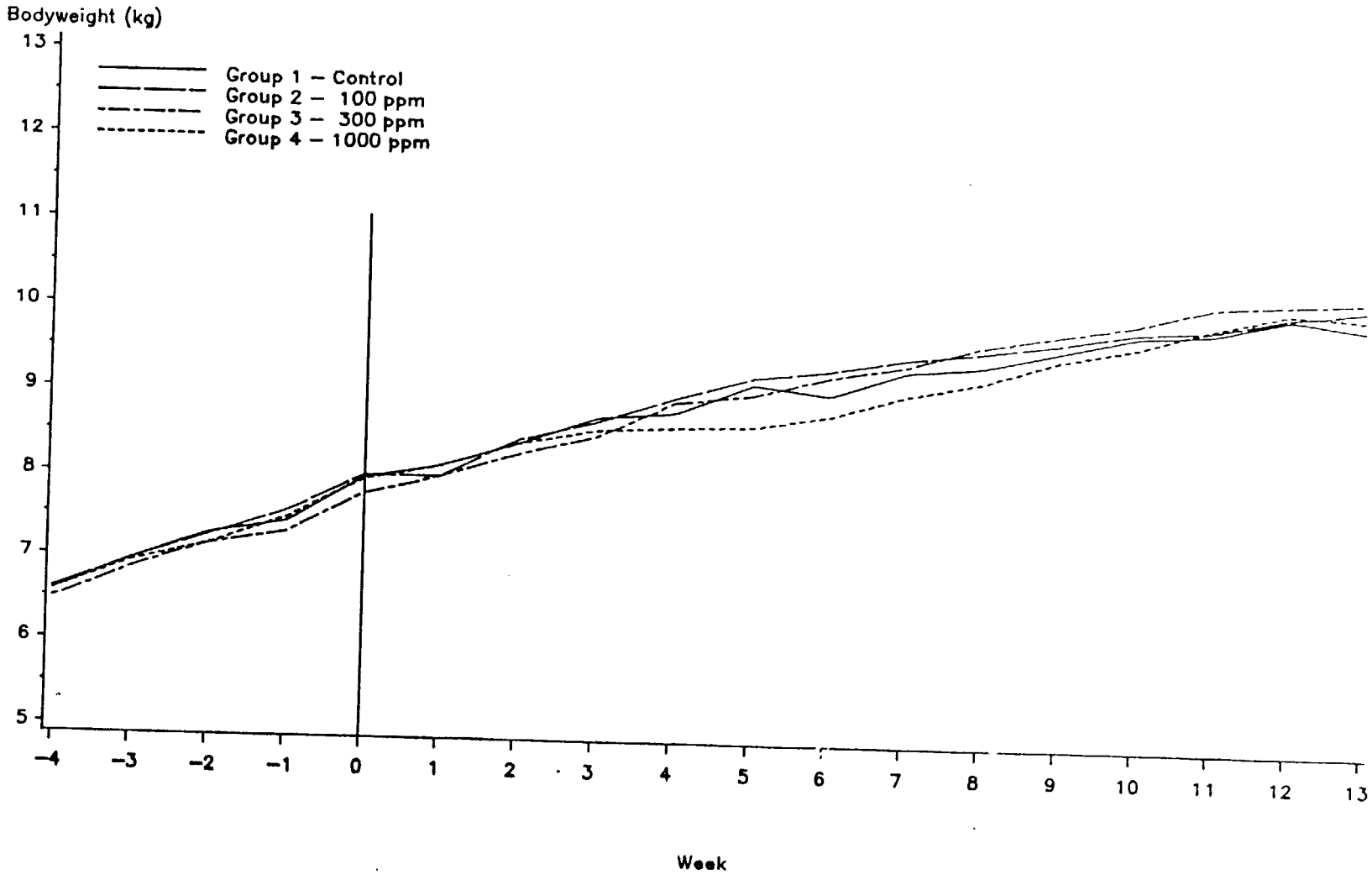


FIGURE 2a

Bodyweight - individual values - Group 1 - Control - males

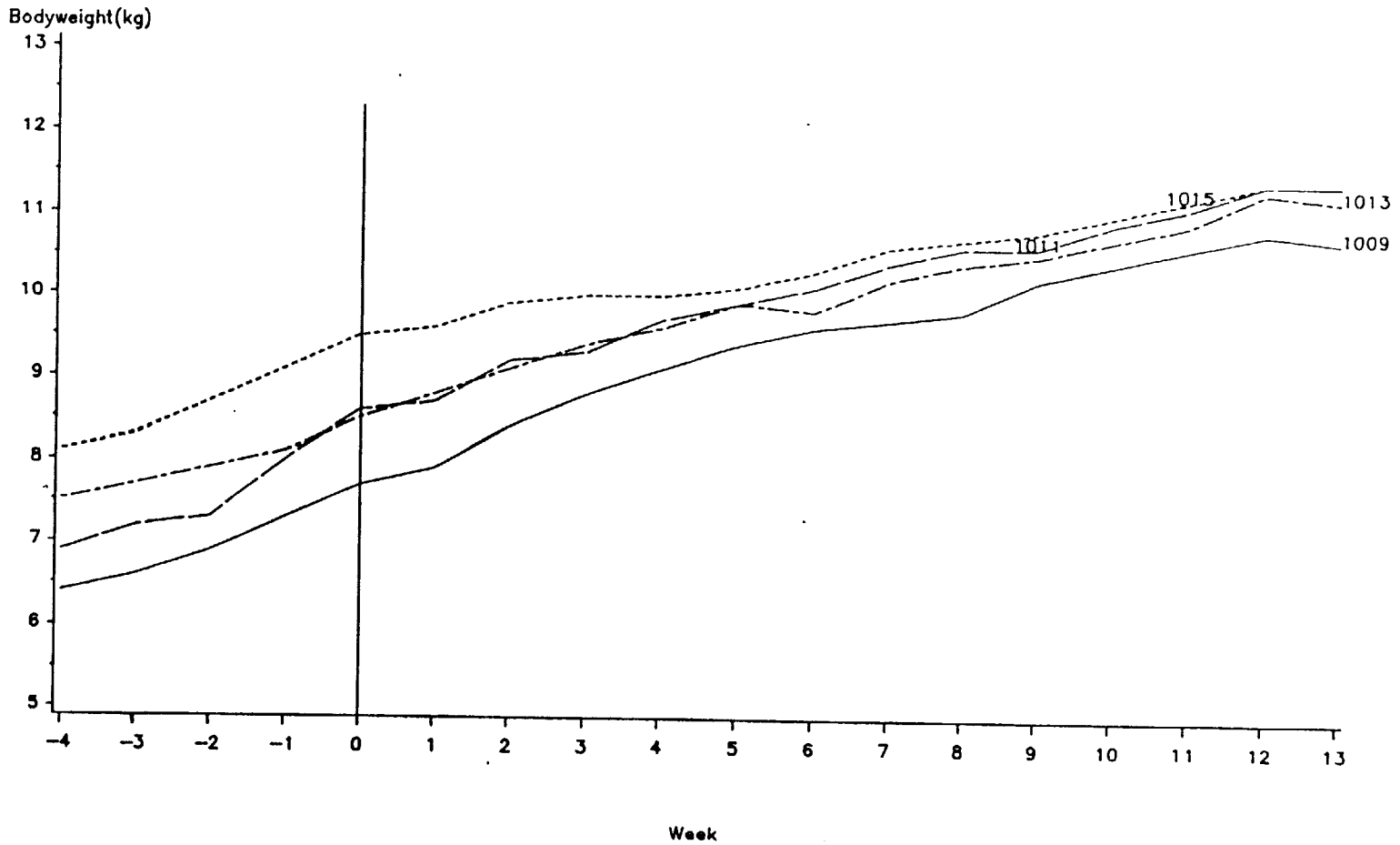


FIGURE 2b

Bodyweight - individual values - Group 1 - Control - females

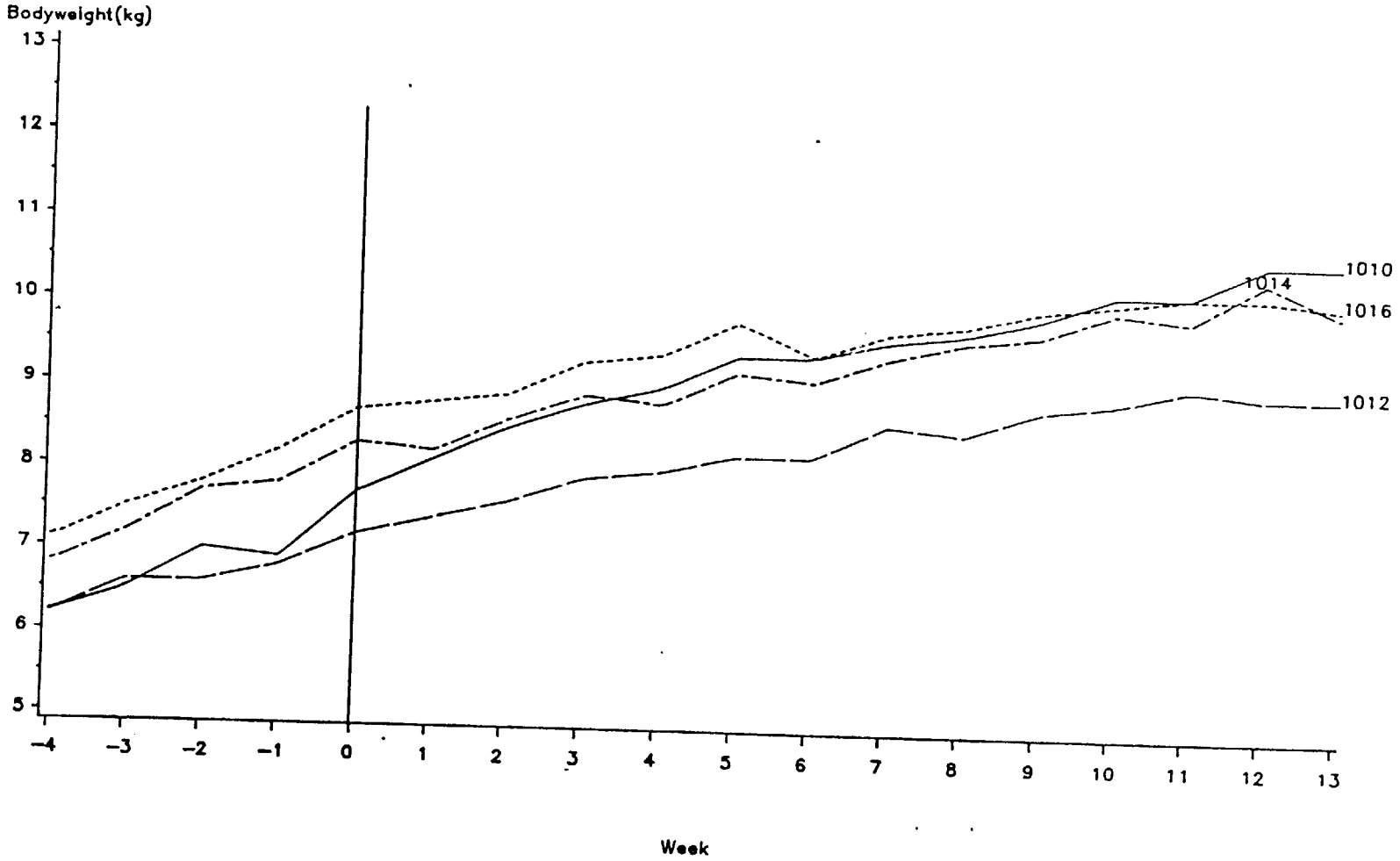


FIGURE 3a

Bodyweight - individual values - Group 2 - 100 ppm - males

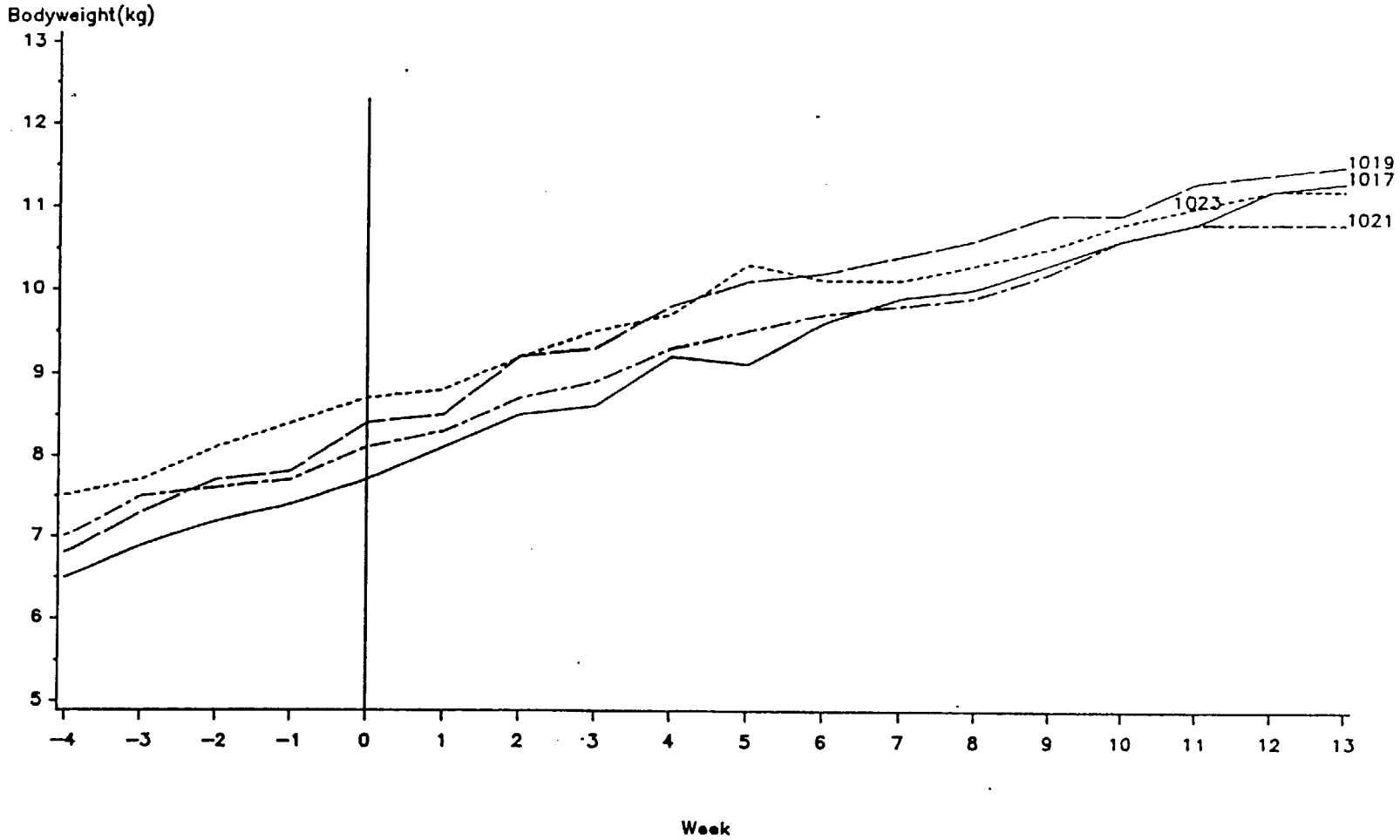


FIGURE 3b

Bodyweight - individual values - Group 2 - 100 ppm - females

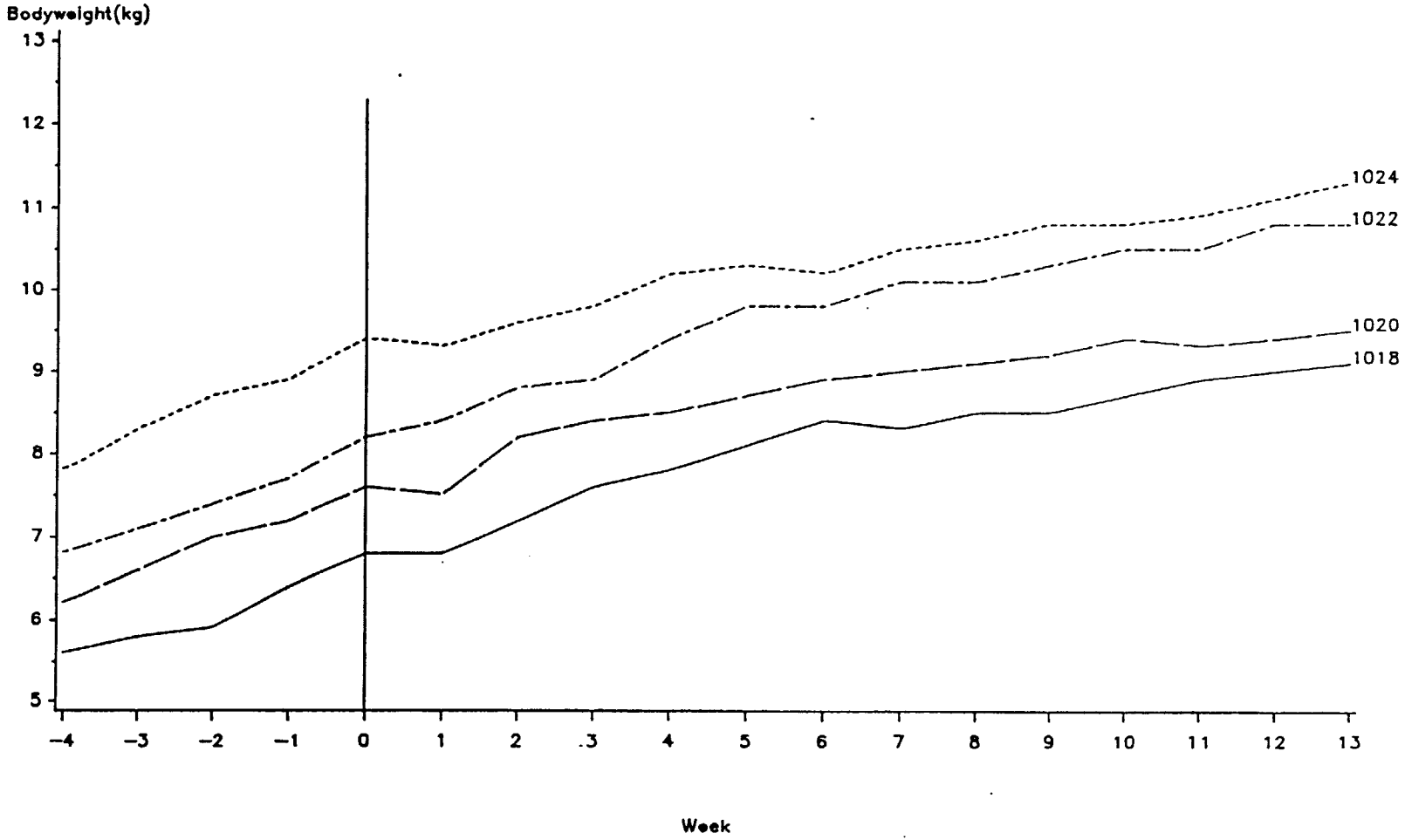


FIGURE 4a

Bodyweight - individual values - Group 3 - 300 ppm - males

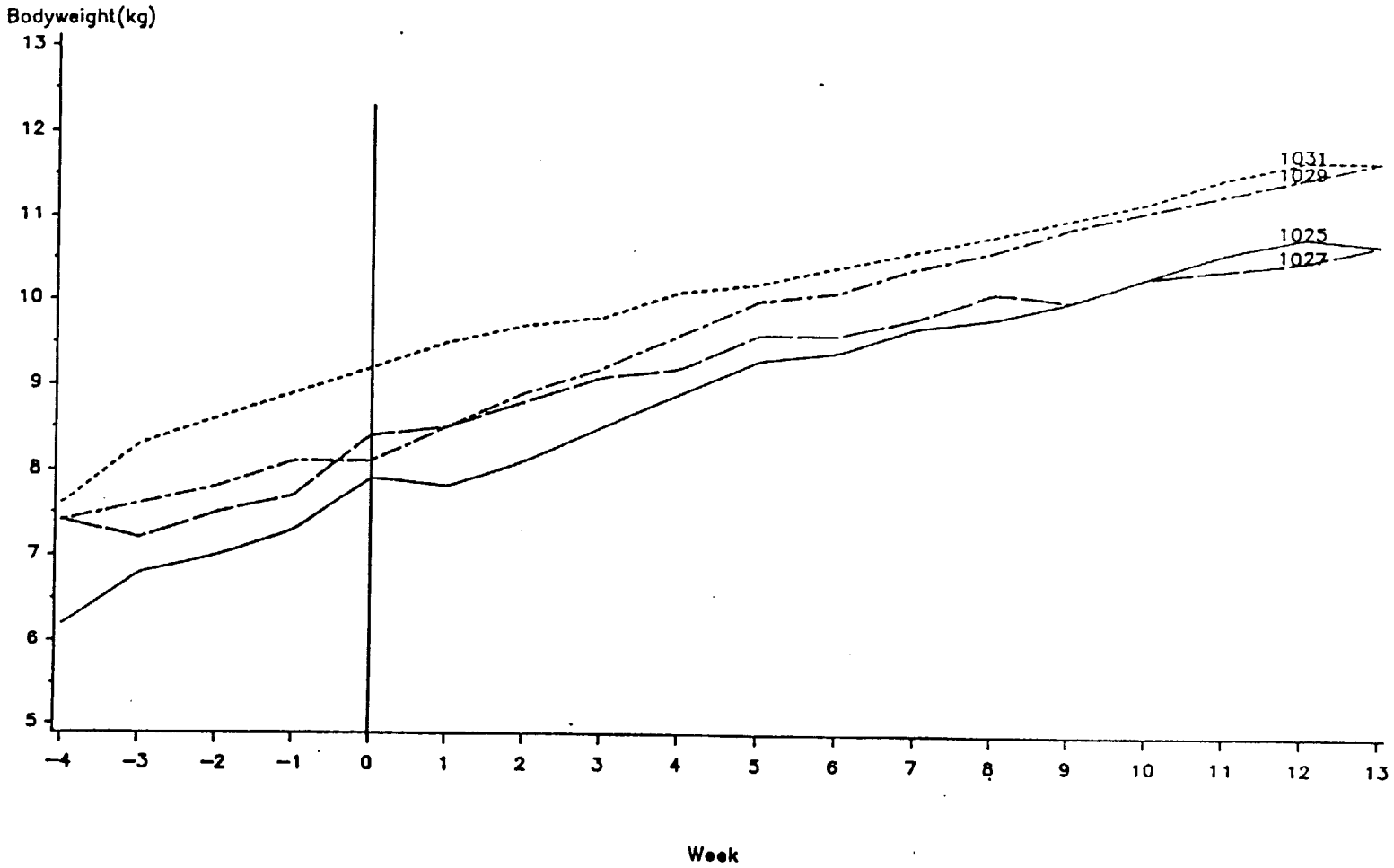


FIGURE 4b

Bodyweight - individual values - Group 3 - 300 ppm - females

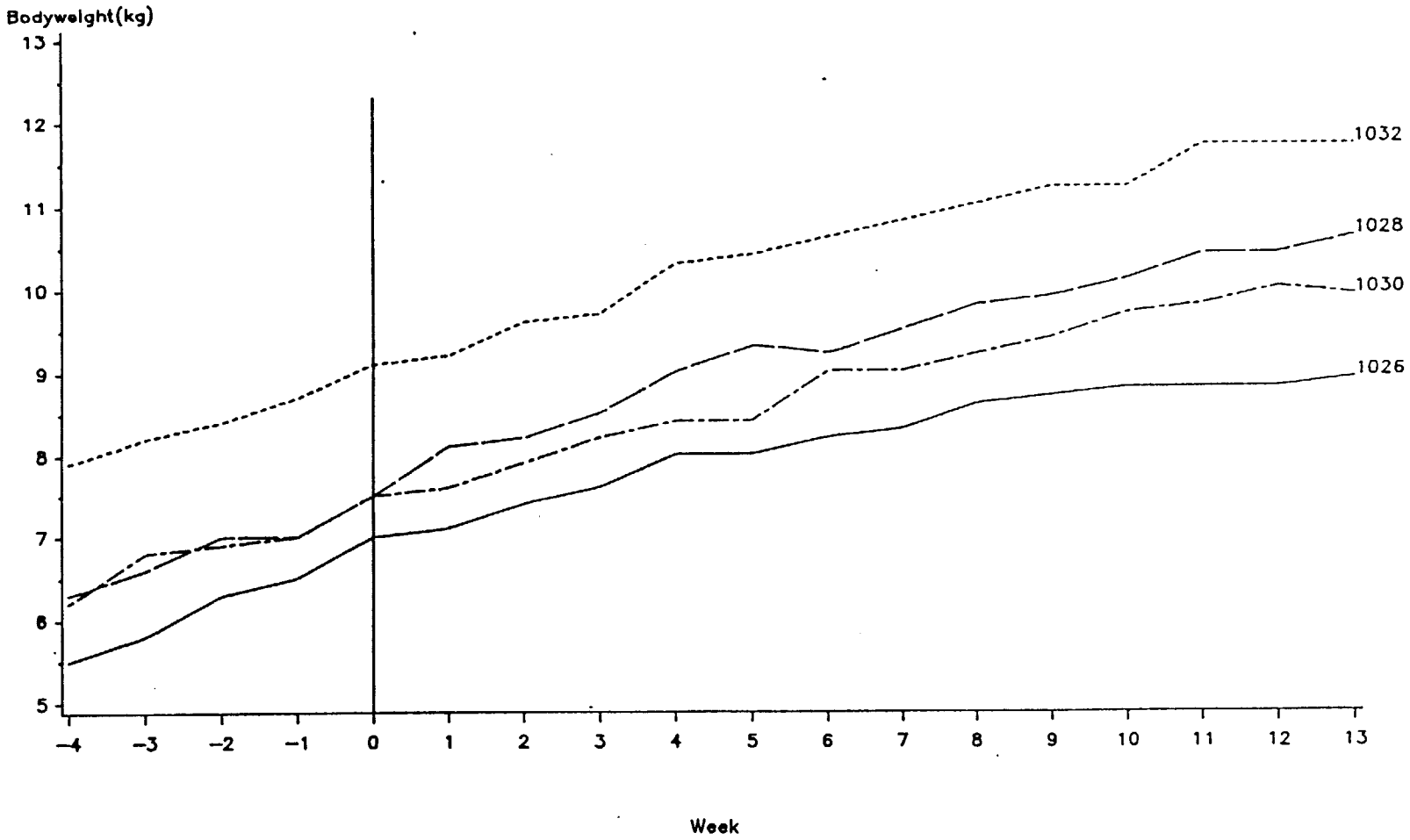


FIGURE 5a

Bodyweight - individual values - Group 4 - 1000 ppm - males

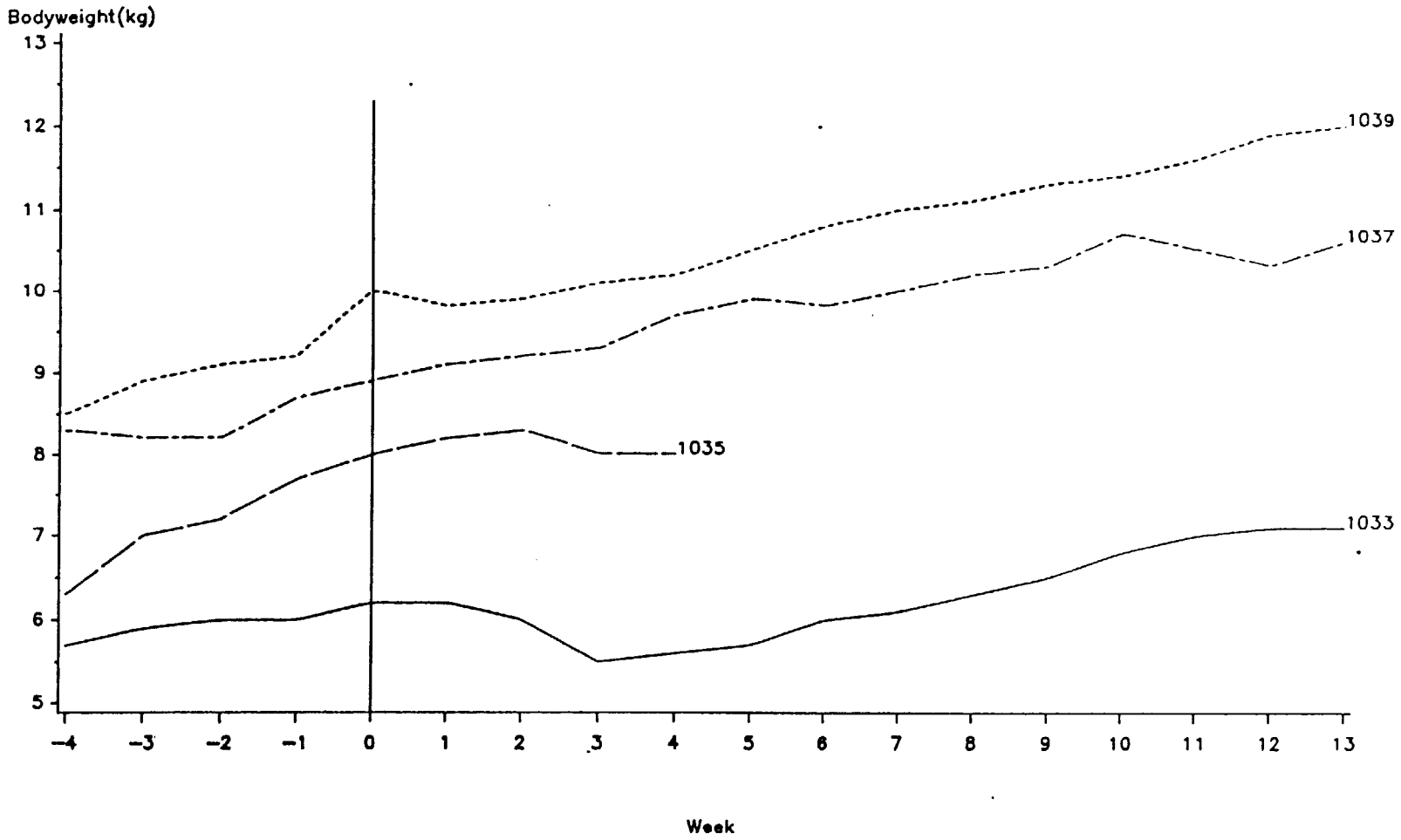


FIGURE 5b

Bodyweight - individual values - Group 4 - 1000 ppm - females

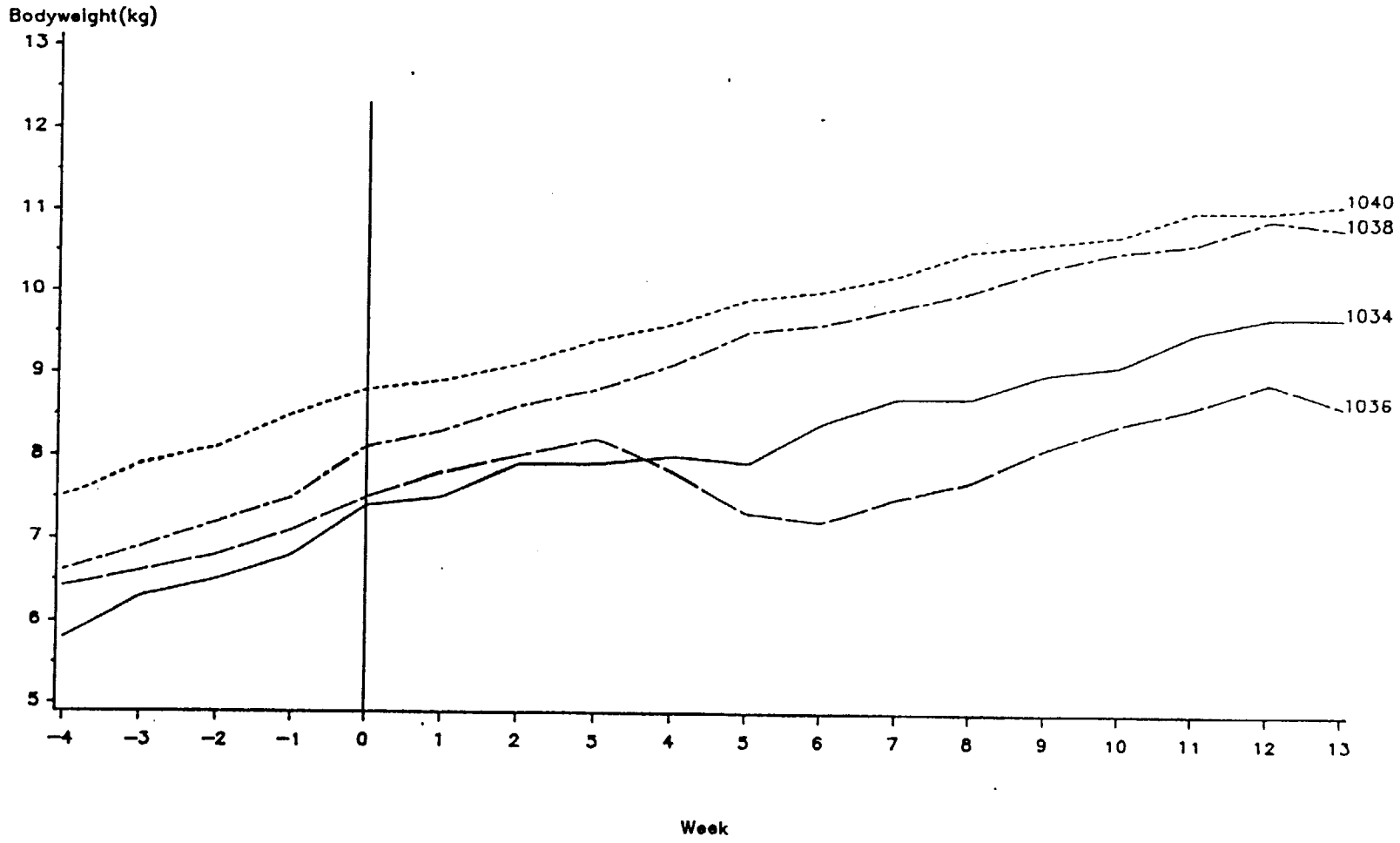


TABLE 1

Data relating to dogs at commencement of dosing (23 May 1990)

Diet concentration ppm	Males				Females			
	Dog no.	Ear no.	Age (weeks)	Bodyweight (kg)	Dog no.	Ear no.	Age (weeks)	Bodyweight (kg)
Control	1009	5241	21	7.7	1010	5229	21	7.7
	1011	5232	21	8.6	1012	5207	21	7.2
	1013	5216	21	8.5	1014	5249	20	8.3
	1015	5002	23	9.5	1016	5226	21	8.7
100	1017	5212	21	7.7	1018	5243	21	6.8
	1019	0101	20	8.4	1020	5236	21	7.6
	1021	5205	21	8.1	1022	5202	21	8.2
	1023	5223	21	8.7	1024	5219	21	9.4
300	1025	5228	21	7.9	1026	5242	21	7.0
	1027	5204	21	8.4	1028	0104	20	7.5
	1029	5213	21	8.1	1030	5220	21	7.5
	1031	5234	21	9.2	1032	5239	21	9.1
1000	1033	4908	24	6.2	1034	5230	21	7.4
	1035	5240	21	8.0	1036	5203	21	7.5
	1037	5217	21	8.9	1038	5105	22	8.1
	1039	5233	21	10.0	1040	5238	21	8.8
	Mean		21	8.4			21	7.9
	Range		21 - 24	6.2 - 10.0			20 - 21	6.8 - 9.4

TABLE 2

Bodyweight - group mean changes (kg)

Sex	Diet concentration ppm	Bodyweight change Weeks 0 - 13
Males	Control	2.6
	100	3.0
	300	2.8
	1000	1.5*
Females	Control	2.0
	100	2.2
	300	2.5
	1000	2.1
Males and Females	Control	2.3
	100	2.6
	300	2.7
	1000	1.9

Williams' test: * $P<0.05$

TABLE 3

Food consumption - group mean values (g/dog/week)

Sex	Diet concentration ppm	During the pre-dosing period	During Weeks 1 - 13 of dosing
Males	Control	2765	2800
	100	2800	2800
	300	2796	2799
	1000	2793	(2800)
Females	Control	2759	2798
	100	2668	2779
	300	2788	2795
	1000	2728	2588
Males and Females	Control	2762	2799
	100	2734	2789
	300	2792	2797
	1000	2761	2659

No significant intergroup differences, P>0.05
() Mean of 2 animals

TABLE 4

Achieved intakes of test substance (mg/kg/day) - group mean values

Week	Diet concentration ppm					
	100		300		1000	
	♂	♀	♂	♀	♂	♀
Dosing						
1	4.80	4.84	14.05	15.12	48.06	49.37
2	4.62	4.86	13.75	14.75	45.15	48.41
3	4.45	4.67	13.31	14.31	36.55	45.44
4	4.31	4.53	12.90	13.77	36.84	41.28
5	4.16	4.40	12.48	13.37	39.37	38.83
6	4.07	4.28	12.21	12.89	45.32	37.49
7	4.01	4.26	12.00	12.87	43.42	40.26
8	3.95	4.19	11.74	12.60	43.56	40.45
9	3.87	4.03	11.54	12.34	42.68	40.40
10	3.77	4.04	11.32	12.15	41.70	40.31
11	3.69	4.05	11.07	11.93	41.33	38.63
12	3.62	3.97	10.87	11.76	40.46	37.48
13	3.58	3.92	10.75	11.71	39.13	35.51
Mean 1 to 13	4.07	4.31	12.15	13.04	41.81	41.07
SD	0.39	0.33	1.09	1.14	3.37	4.20

SD Standard deviation

TABLE 5a

Summary of ophthalmoscopy findings

Before dosing commenced (4 May 1990)

Diet concentration ppm	Control		100		300		1000	
No. of animals examined	4♂	4♀	4♂	4♀	4♂	4♀	4♂	4♀
Conjunctiva								
Conjunctivitis	-	1	1	-	-	1	1	-
Lens								
Sutures	1	1	-	1	1	3	2	1
Vitreous								
Hyaloid remnants	1	1	-	-	1	3	2	1
Retina								
Pigment deficiency in NTF exposing choroidal vasculature	1	-	-	-	-	-	-	-

NTF Non-tapetal fundus

TABLE 5b

Summary of ophthalmoscopy findings

During dose Week 13 (16 August 1990)

Diet concentration ppm	Control		100		300		1000	
No. of animals examined	4♂	4♀	4♂	4♀	4♂	4♀	3♂	4♀
Conjunctiva								
Conjunctivitis	-	1	-	-	-	-	-	-
Lens								
Sutures	-	-	-	-	-	-	-	-
Posterior capsular scarring	-	-	-	1	-	-	-	-
Posterior capsular opacity	-	-	-	-	-	-	-	1
Vitreous								
Hyaloid remnants	1	1	1	-	-	2	1	1
Retina								
Pigment deficiency in NTF	1	-	-	-	-	-	-	-

NTF Non-tapetal fundus

TABLE 6a

Haematology - group mean values - males

Diet concn. ppm	PCV %	Hb g/dl	RBC x10⁶/mm³	MCHC %	MCV fl	WBC + Diff x10³/mm³ Total	N	L	E	B	M	Plts x10³/mm³	PT s	APTT s
Week -3 (3 May 1990)														
Control	38	11.5	5.0	30.0	76	15.5	10.27	4.41	0.12	0.00	0.74	570	5.8	13.4
100	39	11.7	5.1	29.9	77	13.5	8.02	4.57	0.30	0.00	0.56	531	6.1	13.3
300	38	11.5	5.0	30.2	76	16.0	10.24	4.85	0.27	0.00	0.67	432+	6.5	12.8
1000	38	11.3	5.0	29.8	76	16.4	12.30	3.60	0.25	0.00	0.25	439+	5.6	12.9
Week 6 (27 June 1990)														
Control	41	12.8	5.5	31.3	75	12.7	8.30	3.93	0.31	0.00	0.16	459	5.9	13.2
100	41	12.8	5.4	31.1	76	11.1	5.92	4.56	0.41	0.00	0.24	470	6.3	13.9
300	41	12.8	5.5	31.0	76	12.2	7.69	3.93	0.22	0.00	0.32	345	6.6	13.3
1000	36	11.3	4.8	31.0	75	11.1	6.68	4.20	0.16	0.00	0.10	466	5.7	15.1**
Week 13 (15 August 1990)														
Control	44	12.9	5.8	29.5	76	10.9	6.35	3.82	0.48	0.00	0.21	444	5.9	12.4
100	41	12.2	5.3	29.6	78	10.3	6.03	3.85	0.23*	0.00	0.15	463	6.1	12.5
300	42	12.4	5.4	29.6	78	12.7	7.48	4.94	0.09**	0.00	0.19	390	6.4	13.4*
1000	38	11.3	5.0*	29.5	77	13.0	9.22*	3.37	0.10**	0.00	0.31	461	5.5	14.7**

Levels of significance, Student's 't' test: + P<0.05
Williams' test: * P<0.05, in comparison with control
** P<0.01, in comparison with control

TABLE 6b

Haematology - group mean values - females

Diet concn. ppm	PCV %	Hb g/dl	RBC x10⁶/mm³	MCHC %	MCV fl	WBC + Diff x10³/mm³ Total	N	L	E	B	M	Plts x10³/mm³	PT s	APTT s
Week -3 (3 May 1990)														
Control	40	12.2	5.3	30.4	75	15.1	9.53	4.69	0.37	0.00	0.49	461	6.6	13.2
100	37	11.1	4.9	30.3	75	15.7	10.28	4.63	0.25	0.00	0.51	441	5.7	14.8
300	37	10.9	4.9	29.5	76	17.1	12.91	3.25	0.27	0.00	0.65	507	5.8	13.4
1000	40	12.3	5.3	30.7	75	15.0	9.75	4.40	0.21	0.00	0.61	379	5.8	13.7
Week 6 (27 June 1990)														
Control	44	13.7	6.0	31.1	73	11.5	6.76	4.30	0.17	0.00	0.25	360	6.6	13.2
100	40	12.6	5.5	31.2	74	13.1	7.71	4.90	0.36	0.00	0.13	418	5.9	14.6
300	42	13.2	5.6	31.1	75	13.9	8.48	5.21	0.12	0.00	0.04	454	5.9	13.4
1000	40	12.6	5.2*	31.5	78*	14.4	8.85	5.19	0.11	0.00	0.27	473*	5.9	14.3
Week 13 (15 August 1990)														
Control	48	14.0	6.3	29.3	75	11.4	6.30	4.76	0.20	0.00	0.11	337	6.7	12.8
100	42	12.4	5.5	29.9	76	12.6	7.88	4.43	0.16	0.00	0.09	401	5.9	14.1
300	44	13.1	5.6	29.7	78	11.8	7.41	4.26	0.03	0.00	0.06	469*	5.8	13.5
1000	46	13.6	5.8	29.6	79*	14.9*	9.43**	4.84	0.24	0.00	0.38	450*	5.8	14.8

Levels of significance, Williams' test: * P<0.05, in comparison with control
** P<0.01, in comparison with control

TABLE 6c

Haematology - group mean values - males and females combined

Diet concn. ppm	PCV %	Hb g/dl	RBC x10⁶/mm³	MCHC %	MCV fl	WBC + Diff x10³/mm³ Total	N	L	E	B	M	Plts x10³/mm³	PT s	APTT s
Week -3 (3 May 1990)														
Control	39	11.8	5.2	30.2	76	15.3	9.90	4.55	0.24	0.00	0.62	516	6.2	13.3
100	38	11.4	5.0	30.1	76	14.6	9.15	4.60	0.28	0.00	0.54	486	5.9	14.0
300	38	11.2	5.0	29.8	76	16.6	11.57	4.05	0.27	0.00	0.66	470	6.2	13.1
1000	39	11.8	5.2	30.3	76	15.7	11.02	4.00	0.23	0.00	0.43	409+	5.7	13.3
Week 6 (27 June 1990)														
Control	43	13.3	5.8	31.2	74	12.1	7.53	4.11	0.24	0.00	0.21	409	6.2	13.2
100	41	12.7	5.5	31.1	75	12.1	6.81	4.73	0.39	0.00	0.18	444	6.1	14.2
300	42	13.0	5.5	31.1	76	13.0	8.09	4.57	0.17	0.00	0.18	399	6.2	13.4
1000	38*	12.0	5.0**	31.3	76*	13.0	7.92	4.76	0.13	0.00	0.20	470	5.8	14.6*
Week 13 (15 August 1990)														
Control	45	13.4	6.0	29.4	76	11.1	6.33	4.23	0.36	0.00	0.16	398	6.3	12.6
100	41	12.3	5.4	29.7	77	11.4	6.95	4.14	0.19*	0.00	0.12	432	6.0	13.3
300	43	12.7	5.5	29.6	78*	12.2	7.44	4.60	0.06**	0.00	0.12	429	6.1	13.4
1000	42	12.5	5.4*	29.6	78*	14.0**	9.33**	4.11	0.17**	0.00	0.35	456	5.6	14.7**

Levels of significance, Student's 't' test: + P<0.05
Williams' test: * P<0.05, in comparison with control
** P<0.01, in comparison with control

TABLE 7a

Biochemistry - group mean values - males

Diet concn. ppm	Glucose mg/dl	Protein g/dl Total	Alb	Glob	Urea mg/dl	Creatinine mg/dl	AP mU/ml	GPT mU/ml	GOT mU/ml	γGT mU/ml	OCT mU/ml	CPK mU/ml	Bilirubin mg/dl	Na mEq/l	K mEq/l	Ca mEq/l	P mEq/l	Cl mEq/l	Chol mg/dl
Week -3 (3 May 1990)																			
Control	104	4.9	2.3	2.6	24	0.6	297	19	15	3	4.2	114	0.2	145	4.9	5.6	5.1	107	130
100	105	4.9	2.4	2.6	26	0.7	307	22	15	2	6.6	118	0.2	145	4.8	5.7	5.4	107	142
300	107	4.9	2.3	2.6	21	0.5	329	21	15	2	5.9	123	0.2	145	4.8	5.5	5.2	107	132
1000	103	4.8	2.4	2.5	27	0.6	260	20	16	3	6.0	113	0.2	145	5.1	5.6	5.0	108	128
Week 6 (27 June 1990)																			
Control	93	5.0	2.8	2.3	29	0.7	236	22	19	2	3.5	175	0.1	147	4.8	5.4	4.0	107	128
100	92	5.1	2.7	2.4	29	0.7	261	27	21	2	3.6	120	0.1	148	4.8	5.4	4.3	109	160
300	96	5.1	2.7	2.5	25	0.6	294	23	15	2	3.4	122	0.2	148	4.8	5.4	4.2	109	171
1000	92	5.0	2.4**	2.7	34	0.6	266	16	15	2	3.4	74	0.1	148	4.7	5.3	4.0	108	170
Week 13 (15 August 1990)																			
Control	95	5.1	2.8	2.3	28	0.7	168	23	20	2	5.1	113	0.1	146	4.6	5.3	3.5	108	122
100	93	5.0	2.5**	2.5	29	0.7	196	29	18	2	7.1	110	0.1	145	4.5	5.2	3.9	110	158
300	96	4.9	2.4**	2.5	23	0.6	211	25	20	2	5.8	110	0.1	146	4.4	5.1*	3.6	110	165
1000	94	4.9	2.3**	2.6	31	0.6	204	14*	15	2	5.4	80	0.1	146	4.4	5.1*	3.7	108	197**

Levels of significance, Williams' test: * $P<0.05$, in comparison with control
** $P<0.01$, in comparison with control

TABLE 7b

Biochemistry - group mean values - females

Diet concn. ppm	Glucose mg/dl	Protein g/dl Total	Alb	Glob	Urea mg/dl	Creatinine mg/dl	AP mU/ml	GPT mU/ml	GOT mU/ml	γGT mU/ml	OCT mU/ml	CPK mU/ml	Bilirubin mg/dl	Na mEq/l	K mEq/l	Ca mEq/l	P mEq/l	Cl mEq/l	Chol mg/dl
Week -3 (3 May 1990)																			
Control	105	5.0	2.3	2.6	25	0.6	323	19	15	2	5.9	103	0.2	145	4.6	5.7	5.3	108	133
100	103	5.0	2.3	2.7	22	0.6	307	18	15	2	2.9	129	0.1	145	4.6	5.6	4.9	107	126
300	96 +	4.9	2.2	2.7	23	0.6	263	16	13	2	2.3 +	92	0.2	145	4.6	5.6	5.1	108	128
1000	99	4.9	2.3	2.6	26	0.6	282	24	17	3	4.9	117	0.2	145	4.6	5.6	5.5	108	135
Week 6 (27 June 1990)																			
Control	94	5.2	2.9	2.3	27	0.6	260	25	21	<2	3.3	243	0.2	147	4.8	5.5	4.5	109	138
100	89	5.2	2.7	2.5	30	0.7	257	26	18	2	3.4	114	0.1	148	4.6	5.5	4.1	109	147
300	93	5.2	2.7	2.5	28	0.6	270	18	16	2	3.9	92	0.2	149	4.8	5.5	4.4	109	156
1000	92	5.3	2.6	2.7	25	0.7	565	72	24	2	12.3	113	0.2	149	4.7	5.5	4.4	109	210 **
Week 13 (15 August 1990)																			
Control	97	5.1	2.9	2.3	31	0.8	206	28	21	2	5.3	138	0.2	145	4.6	5.2	3.8	109	124
100	92	5.1	2.5 *	2.6	29	0.8	186	24	18	2	6.2	95	0.1	146	4.4	5.3	3.4	110	137
300	90	5.1	2.5 *	2.5	28	0.8	204	19	22	2	6.8	269	0.2	147	4.5	5.2	3.6	110	150
1000	95	5.2	2.5 *	2.7 *	26	0.7	294 *	27	20	1	5.2	88	0.2	147	4.6	5.2	4.3	108	200 **

Levels of significance, Student's 't' test: + P<0.05
Williams' test: * P<0.05, in comparison with control
** P<0.01, in comparison with control

TABLE 7c

Biochemistry - group mean values - males and females combined

Diet concn. ppm	Glucose mg/dl	Protein g/dl Total	Alb	Glob	Urea mg/dl	Creatinine mg/dl	AP mU/ml	GPT mU/ml	GOT mU/ml	γGT mU/ml	OCT mU/ml	CPK mU/ml	Bilirubin mg/dl	Na mEq/l	K mEq/l	Ca mEq/l	P mEq/l	Cl mEq/l	Chol mg/dl
Week -3 (3 May 1990)																			
Control	104	4.9	2.3	2.6	25	0.6	310	19	15	3	5.1	108	0.2	145	4.7	5.7	5.2	108	131
100	104	4.9	2.3	2.6	24	0.6	307	20	15	2	4.7	123	0.2	145	4.7	5.6	5.1	107	134
300	101	4.9	2.3	2.6	22	0.5	296	18	14	2	4.1	107	0.2	145	4.7	5.5	5.2	108	130
1000	101	4.8	2.3	2.5	26	0.6	271	22	16	3	5.4	115	0.2	145	4.8	5.6	5.2	108	131
Week 6 (27 June 1990)																			
Control	93	5.1	2.8	2.3	28	0.7	248	23	20	<2	3.4	L 209	0.2	147	4.8	5.4	4.2	108	133
100	91	5.2	2.7	2.5	29	0.7	259	26	19	2	3.5	117*	0.1	148	4.7	5.5	4.2	109	153
300	94	5.1	2.7	2.5	27	0.6	282	20	15	2	3.6	107*	0.2	149*	4.8	5.4	4.3	109	163*
1000	92	5.2	2.5*	2.7*	29	0.6	437	48	20	2	8.5	96**	0.2	148*	4.7	5.4	4.3	109	193**
Week 13 (15 August 1990)																			
Control	96	5.1	2.8	2.3	29	0.8	187	26	20	2	5.2	125	0.2	146	4.6	5.3	3.6	108	123
100	92	5.1	2.5**	2.5*	29	0.7	191	27	18	2	6.7	102	0.1	146	4.4	5.2	3.6	110	148
300	93	5.0	2.5**	2.5*	26	0.7	208	22	21	2	6.3	101	0.1	146	4.5	5.1	3.6	110	157**
1000	94	5.1	2.4**	2.7**	28	0.6*	255**	21	18	1	5.3	82**	0.1	146	4.5	5.2	4.1	108	199**

Levels of significance, Williams' test: * P<0.05, in comparison with control
** P<0.01, in comparison with control

L Analysis performed on log transformed data

TABLE 8a

Urinalysis - group mean values - males

Diet concn. ppm	Volume ml	pH	SG	Protein mg/dl
Week -3 (4 May 1990)				
Control	58	5.8	1039	37
100	70	5.6	1039	49
300	106 +	5.9	1038	34
1000	86	5.3 +	1039	39
Week 6 (28 June 1990)				
Control	112	6.5	1038	29
100	163	5.7	1026	20
300	322	5.7	1020	15
1000	192	5.7	1030	21
Week 13 (16 August 1990)				
Control	140	6.3	1037	31
100	187	6.1	1035	37
300	82	6.2	1032	57
1000	139	6.6	1033	40

Level of significance:
Student's 't' test: + $P<0.05$

TABLE 8b

Urinalysis - group mean values - females

Diet concn. ppm	Volume ml	pH	SG	Protein mg/dl
Week -3 (4 May 1990)				
Control	231	5.7	1025	22
100	118	5.8	1037	30
300	210	5.6	1028	46
1000	197	5.7	1028	26
Week 6 (28 June 1990)				
Control	250	6.1	1030	25
100	259	5.9	1028	20
300	194	5.8	1027	22
1000	160	6.0	1035	27
Week 13 (16 August 1990)				
Control	154	6.3	1038	28
100	223	6.1	1033	32
300	196	6.3	1033	49
1000	139	6.4	1035	22

No significant intergroup differences, P>0.05

TABLE 8c

Urinalysis - group mean values - males and females combined

Diet concn. ppm	Volume ml	pH	SG	Protein mg/dl
Week -3 (4 May 1990)				
Control	145	5.8	1032	30
100	94	5.7	1038	39
300	158	5.7	1033	40
1000	142	5.5	1033	32
Week 6 (28 June 1990)				
Control	181	6.3	1034	27
100	211	5.8	1027	20
300	258	5.8	1023	18
1000	173	5.9	1033	24
Week 13 (16 August 1990)				
Control	147	6.3	1037	30
100	205	6.1	1034	34
300	139	6.3	1032	53
1000	139	6.5	1034	30

No significant intergroup differences, P>0.05

TABLE 9a

Absolute organ weights - group mean values - males

Diet. concn. ppm	Body wt. kg	Brain g	Pitu-itary mg	Heart g	Lungs g	Liver g	Spleen g	Panc-reas g	Thymus g	Prostate /Uterus g	Kidneys g	Thyr-oids g	Adre-nals g	Gonads g
Control	11.1	81.7	68	77.5	90.8	374.3	63.1	20.4	14.7	2.95	53.8	1.03	1.53	15.43
100	11.1	78.8	68	78.5	98.3	373.2	50.6	23.8	18.2	4.94	54.5	0.90	1.59	20.60
300	11.1	81.2	72	82.0	92.9	410.6	60.6	24.9	15.1	4.86	56.0	0.89	1.58	21.88
1000	9.9	75.2	70	64.7	80.1	430.4	76.5	22.4	13.8	4.56	53.7	1.03	1.62	20.26
Adjusted means#														
Control	-	80.8	67	76.7	89.0	366.2	-	-	14.1	-	53.0	1.00	-	-
100	-	77.9	67	77.8	96.6	365.9	-	-	17.7	-	53.8	0.87	-	-
300	-	80.2	70	81.2	91.1	402.4	-	-	14.5	-	55.1	0.86	-	-
1000	-	78.9	76	67.7*	87.2	461.8*	-	-	16.0	-	56.9	1.13	-	-

Adjusted for bodyweight as covariate when the within-group relationship between bodyweight and organ weight was significant at the 10% level

Level of significance: Williams' test: * $P<0.05$ in comparison with control value

TABLE 9b

Absolute organ weights - group mean values - females

Diet. concn. ppm	Body wt. kg	Brain g	Pitu-itary mg	Heart g	Lungs g	Liver g	Spleen g	Panc-reas g	Thymus g	Prostate /Uterus g	Kidneys g	Thyr-oids g	Adre-nals g	Gonads g
Control	9.9	74.8	73	73.6	95.5	331.2	68.6	21.2	13.2	3.83	49.8	0.91	1.39	1.01
100	10.1	76.2	75	68.7	86.9	360.8	86.3	20.8	17.2	3.93	49.1	1.03	1.47	0.93
300	10.3	77.9	69	73.1	87.1	365.6	77.8	22.0	18.3	3.10	52.9	0.99	1.51	1.04
1000	10.0	72.1	72	72.8	79.3	388.6	81.7	21.2	16.9	3.89	51.7	1.00	1.39	0.98
Adjusted means#														
Control	-	-	-	-	96.2	335.7	71.2	-	13.8	3.71	50.5	-	-	-
100	-	-	-	-	86.9	360.8	86.3	-	17.2	3.93	49.1	-	-	-
300	-	-	-	-	86.2	359.8	74.5	-	17.5	3.25	51.9	-	-	-
1000	-	-	-	-	79.5**	389.9*	82.5	-	17.1	3.86	51.9	-	-	-

Adjusted for bodyweight as covariate when the within-group relationship between bodyweight and organ weight was significant at the 10% level

Level of significance: Williams' test: * P<0.05 in comparison with control value
** P<0.01 in comparison with control value

TABLE 9c

Absolute organ weights - group mean values - males and females combined

Diet. concn. ppm	Body wt. kg	Brain g	Pitu-itary mg	Heart g	Lungs g	Liver g	Spleen g	Panc-reas g	Thymus g	Kidneys g	Thyr-oids g	Adre-nals g
Control	10.5	78.3	71	75.5	93.2	352.8	65.9	20.8	13.9	51.8	0.97	1.46
100	10.6	77.5	72	73.6	92.6	367.0	68.4	22.3	17.7	51.8	0.96	1.53
300	10.7	79.5	70	77.5	90.0	388.1	69.2	23.4	16.7	54.4	0.94	1.54
1000	10.0	73.4	71	69.4	79.6	406.5	79.5	21.7	15.6	52.6	1.01	1.49
Adjusted means#												
Control	-	78.2	70	75.3	92.9	351.3	-	-	13.8	51.6	0.97	1.46
100	-	77.2	71	73.2	91.9	363.3	-	-	17.4	51.3	0.95	1.52
300	-	78.9	70	76.6	88.7	380.7	-	-	16.1	53.5	0.92	1.53
1000	-	74.6	73	71.1	82.3*	420.9**	-	-	16.7	54.3	1.04	1.51

Adjusted for bodyweight as covariate when the within-group relationship between bodyweight and organ weight was significant at the 10% level

Level of significance: Williams' test: * P<0.05 in comparison with control value
** P<0.01 in comparison with control value

TABLE 10

Relative organ weights - group mean values
(expressed as a percentage of final bodyweight)

Diet. concn. ppm	Body wt. kg	Brain	Pituitary	Heart	Lungs	Liver	Spleen	Panc-reas	Thymus	Prostate /Uterus	Kidneys	Thyroids	Adrenals	Gonads
Males														
Control	11.1	0.73	0.00062	0.70	0.82	3.37	0.57	0.19	0.13	0.027	0.48	0.0092	0.0137	0.1386
100	11.1	0.71	0.00062	0.71	0.89	3.37	0.45	0.21	0.16	0.045	0.49	0.0081	0.0143	0.1857
300	11.1	0.73	0.00065	0.74	0.84	3.70	0.54	0.22	0.14	0.044	0.50	0.0080	0.0142	0.1972
1000	9.9	0.79	0.00072	0.67	0.81	4.40	0.79	0.24	0.14	0.046	0.56	0.0102	0.0171	0.2095
Females														
Control	9.9	0.76	0.00074	0.75	0.97	3.36	0.69	0.21	0.13	0.039	0.51	0.0093	0.0141	0.0103
100	10.1	0.76	0.00075	0.69	0.87	3.58	0.85	0.21	0.17	0.041	0.49	0.0102	0.0145	0.0094
300	10.3	0.76	0.00067	0.72	0.85	3.56	0.76	0.22	0.18	0.031	0.51	0.0097	0.0148	0.0101
1000	10.0	0.73	0.00073	0.73	0.80	3.89	0.80	0.21	0.17	0.040	0.52	0.0099	0.0140	0.0099
Males and females combined														
Control	10.5	0.75	0.00068	0.72	0.89	3.36	0.63	0.20	0.13	-	0.49	0.0092	0.0139	-
100	10.6	0.74	0.00068	0.70	0.88	3.47	0.65	0.21	0.17	-	0.49	0.0091	0.0144	-
300	10.7	0.75	0.00066	0.73	0.84	3.63	0.65	0.22	0.16	-	0.51	0.0088	0.0145	-
1000	10.0	0.75	0.00073	0.70	0.80	4.11	0.80	0.22	0.16	-	0.54	0.0101	0.0153	-

TABLE 11

Microscopic pathology incidence summary

	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 1	GROUP 2	GROUP 3	GROUP 4
	MALES				FEMALES			
ANIMALS ON STUDY	4	4	4	4	4	4	4	4
ANIMALS COMPLETED	4	4	4	4	4	4	4	4
AORTA								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	4	4	4	4	4	4
TRACHEA								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	3	4	4	2	4	4	4
Tracheitis (TOTAL)	0	1	0	0	0	0	0	0
Minimal	0	1	0	0	0	0	0	0
Inflammatory cell infiltration into lamina propria (TOTAL)	0	0	0	0	2	0	0	0
Minimal	0	0	0	0	2	0	0	0
LUNGS								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	3	2	0	4	3	4	4
Polymorphonuclear leucocyte infiltration into alveoli	0	0	0	1	0	0	0	0
Focal acute inflammatory cell infiltration some associated with homogeneous eosinophilic material (TOTAL)	0	0	0	1	0	0	0	0
Minimal	0	0	0	1	0	0	0	0
Foci of homogeneous eosinophilic material in some alveoli (TOTAL)	0	1	1	0	0	1	0	0
Minimal	0	1	1	0	0	1	0	0
Bronchopneumonia (TOTAL)	0	1	1	0	0	0	0	0
Minimal	0	0	1	0	0	0	0	0
Focal pneumonitis (TOTAL)	0	0	1	0	0	1	0	0
Minimal	0	0	1	0	0	1	0	0
Pleural adhesions	0	1	0	0	0	0	0	0
Subpleural interstitial fibrosis with epithelialisation	0	0	0	1	0	0	0	0

TABLE 11

(Microscopic pathology incidence summary - continued)

	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 1	GROUP 2	GROUP 3	GROUP 4
	MALES				FEMALES			
ANIMALS ON STUDY	4	4	4	4	4	4	4	4
ANIMALS COMPLETED	4	4	4	4	4	4	4	4
LUNGS	(CONTINUED)							
Vacuolated alveolar macrophages.....	0	0	0	1	0	0	0	0
Alveolar macrophages (TOTAL).....	0	0	0	1	0	0	0	0
Minimal.....	0	0	0	1	0	0	0	0
HEART								
EXAMINED.....	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED.....	3	4	2	3	4	4	4	4
Focal myocardial necrosis with acute inflammatory cell infiltration and haemorrhage.....	0	0	0	1	0	0	0	0
Mucoid degeneration of atrioventricular valve.....	0	0	1	0	0	0	0	0
Haemocyst.....	0	0	1	0	0	0	0	0
Area of recent haemorrhage into endocardium and superficial myocardium.....	1	0	0	0	0	0	0	0
THYMUS								
EXAMINED.....	4	4	4	4	4	3	4	4
MISSING.....	0	0	0	0	0	1	0	0
NO ABNORMALITIES DETECTED.....	4	4	4	3	4	3	4	4
Involution (TOTAL).....	0	0	0	1	0	0	0	0
Minimal.....	0	0	0	1	0	0	0	0
LYMPH NODES - CERVICAL								
EXAMINED.....	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED.....	4	4	4	3	4	4	4	4
Sinus histiocytosis.....	0	0	0	1	0	0	0	0
LYMPH NODES - MESENTERIC								
EXAMINED.....	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED.....	4	4	4	4	3	4	4	4

TABLE 11

(Microscopic pathology incidence summary - continued)

	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 1	GROUP 2	GROUP 3	GROUP 4
	MALES				FEMALES			
ANIMALS ON STUDY	4	4	4	4	4	4	4	4
ANIMALS COMPLETED	4	4	4	4	4	4	4	4
LYMPH NODES - MESENTERIC	(CONTINUED)							
Erythrophagocytosis.....	0	0	0	0	1	0	0	0
LYMPH NODES - SUBMANDIBULAR								
EXAMINED.....	0	0	0	1	0	0	0	0
NO ABNORMALITIES DETECTED.....	0	0	0	1	0	0	0	0
SPLEEN								
EXAMINED.....	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED.....	3	3	4	2	4	4	4	4
Contracted.....	0	0	0	1	0	0	0	0
Haemosiderosis (TOTAL).....	1	0	0	1	0	0	0	0
Minimal.....	1	0	0	1	0	0	0	0
Areas of marked fibrous thickening of - capsule.....	0	1	0	0	0	0	0	0
LIVER								
EXAMINED.....	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED.....	2	4	2	1	3	3	2	1
Centrilobular hepatocyte degeneration - (TOTAL).....	0	0	0	1	0	0	0	0
Minimal.....	0	0	0	1	0	0	0	0
Focal necrosis (TOTAL).....	0	0	1	0	0	0	0	1
Minimal.....	0	0	1	0	0	0	0	1
Pigment in Kupffer cells (TOTAL).....	0	0	1	2	0	0	1	1
Minimal.....	0	0	1	2	0	0	1	1
Inflammatory cell infiltration - adjacent to central veins.....	0	0	0	0	0	0	0	1
Loss of cells with dilated sinusoids..	0	0	0	0	0	0	0	1
Foci of degenerate hepatocytes with - inflammatory cell infiltration - (TOTAL).....	2	0	0	0	1	1	0	2
Minimal.....	2	0	0	0	1	1	0	2

TABLE 11

(Microscopic pathology incidence summary - continued)

	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 1	GROUP 2	GROUP 3	GROUP 4
	MALES				FEMALES			
ANIMALS ON STUDY	4	4	4	4	4	4	4	4
ANIMALS COMPLETED	4	4	4	4	4	4	4	4
LIVER (CONTINUED)								
Subcapsular focus - inflammatory cells	0	0	0	0	0	0	1	0
LIVER (ORO STAIN)								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	4	3	4	4	4	4
Fat droplets in periportal - hepatocytes (TOTAL)	0	0	0	1	0	0	0	0
Moderate	0	0	0	1	0	0	0	0
LIVER (PAS STAIN)								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	2	4	4	4	4	4
Reduced glycogen in periportal - hepatocytes	0	0	1	0	0	0	0	0
Reduced glycogen in centrilobular - hepatocytes	0	0	1	0	0	0	0	0
GALL BLADDER								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	4	4	4	4	3	4
Mucosal inflammatory cell - infiltration (TOTAL)	0	0	0	0	0	0	1	0
Moderate	0	0	0	0	0	0	1	0
Mucosal hyperplasia (TOTAL)	0	0	0	0	0	0	1	0
Moderate	0	0	0	0	0	0	1	0
PANCREAS								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	4	4	4	4	4	4
KIDNEYS								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	4	2	2	4	4	4

TABLE 11

(Microscopic pathology incidence summary - continued)

	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 1	GROUP 2	GROUP 3	GROUP 4
	MALES				FEMALES			
ANIMALS ON STUDY	4	4	4	4	4	4	4	4
ANIMALS COMPLETED	4	4	4	4	4	4	4	4
KIDNEYS	(CONTINUED)							
Granulomatous lesion(s).....	0	0	0	0	1	0	0	0
Focal interstitial inflammatory cell - infiltration (TOTAL).....	0	0	0	0	1	0	0	0
Minimal.....	0	0	0	0	1	0	0	0
Groups of dilated cortical tubules - with associated primitive glomeruli...	0	0	0	1	0	0	0	0
Foci of mineralisation in the papilla.	0	0	0	1	0	0	0	0
URINARY BLADDER								
EXAMINED.....	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED.....	4	4	4	4	3	4	3	4
Cystitis.....	0	0	0	0	1	0	1	0
UTERUS								
EXAMINED.....	0	0	0	0	4	4	4	4
NO ABNORMALITIES DETECTED.....	0	0	0	0	4	4	4	4
VAGINA								
EXAMINED.....	0	0	0	0	4	4	4	4
NO ABNORMALITIES DETECTED.....	0	0	0	0	4	4	4	4
OVARIES								
EXAMINED.....	0	0	0	0	4	4	4	4
NO ABNORMALITIES DETECTED.....	0	0	0	0	4	4	4	4
PROSTATE								
EXAMINED.....	4	4	4	4	0	0	0	0
NO ABNORMALITIES DETECTED.....	4	4	4	3	0	0	0	0
Prominent alveolar activity.....	0	0	0	1	0	0	0	0
TESTES								
EXAMINED.....	4	4	4	4	0	0	0	0
NO ABNORMALITIES DETECTED.....	2	4	4	3	0	0	0	0

TABLE 11

(Microscopic pathology incidence summary - continued)

	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 1	GROUP 2	GROUP 3	GROUP 4
	MALES				FEMALES			
ANIMALS ON STUDY	4	4	4	4	4	4	4	4
ANIMALS COMPLETED	4	4	4	4	4	4	4	4
TESTES (CONTINUED)								
Immature	1	0	0	0	0	0	0	0
Atrophic tubules within marked interstitial cell hyperplasia	1	0	0	0	0	0	0	0
Arrest of spermatogenesis	0	0	0	1	0	0	0	0
EPIDIDYMIDES								
EXAMINED	0	0	1	1	0	0	0	0
No spermatazoa in tubules	0	0	0	1	0	0	0	0
Vasculitis	0	0	1	0	0	0	0	0
THYROIDS								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	4	4	4	4	4	3
Ectopic thymic tissue	0	0	0	0	0	0	0	1
PARATHYROIDS								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	4	4	4	4	3	4
Cyst	0	0	0	0	0	0	1	0
ADRENALS								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	4	4	4	4	4	4
PITUITARY								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	3	3	4	2	4	4	2
Cyst(s) in pars anterior	0	1	1	0	2	0	0	2
SALIVARY GLAND (SUB.MANDIB)								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	4	4	4	4	4	4
MUSCLE (BICEPS FEMORIS)								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	4	4	4	4	4	4

TABLE 11

(Microscopic pathology incidence summary - continued)

	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 1	GROUP 2	GROUP 3	GROUP 4
	MALES				FEMALES			
ANIMALS ON STUDY	4	4	4	4	4	4	4	4
ANIMALS COMPLETED	4	4	4	4	4	4	4	4
TONGUE								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	4	4	4	4	4	4
OESOPHAGUS								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	4	4	4	4	4	4
STOMACH								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	3	4	4	4	4	4	4	4
Foci of mineralisation in mucosa	1	0	0	0	0	0	0	0
DUODENUM								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	4	4	3	3	4	4
Congestion of capillaries of villi	0	0	0	0	1	1	0	0
JEJUNUM								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	4	4	4	4	4	4
ILEUM								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	4	4	4	4	3	4
Congestion of capillaries of the villi	0	0	0	0	0	0	1	0
CAECUM								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	4	4	4	4	4	4
COLON								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	4	4	4	4	4	4

TABLE 11

(Microscopic pathology incidence summary - continued)

	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 1	GROUP 2	GROUP 3	GROUP 4
	MALES				FEMALES			
ANIMALS ON STUDY	4	4	4	4	4	4	4	4
ANIMALS COMPLETED	4	4	4	4	4	4	4	4
RECTUM								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	4	4	4	4	4	4
MAMMARY GLAND								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	4	4	3	4	4	4
Dilated sweat glands	0	0	0	0	1	0	0	0
SKIN								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	4	4	4	4	4	4
EYES								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	4	4	4	4	4	3
Conjunctivitis (TOTAL)	0	0	0	0	0	0	0	1
Minimal	0	0	0	0	0	0	0	1
SPINAL CORD								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	4	4	4	4	4	4
SCIATIC NERVE								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	4	4	4	4	4	4
BRAIN								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	4	4	4	4	4	4
STERNUM								
EXAMINED	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED	4	4	3	4	1	4	2	3

TABLE 11

(Microscopic pathology incidence summary - continued)

	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 1	GROUP 2	GROUP 3	GROUP 4
	MALES				FEMALES			
ANIMALS ON STUDY	4	4	4	4	4	4	4	4
ANIMALS COMPLETED	4	4	4	4	4	4	4	4
STERNUM (CONTINUED)								
Focal haemorrhage.....	0	0	0	0	2	0	2	0
Area of bone remodelling.....	0	0	1	0	1	0	0	1
FEMUR & JOINT								
EXAMINED.....	4	4	4	4	4	4	4	4
NO ABNORMALITIES DETECTED.....	4	4	4	4	4	4	4	4
* PATHOLOGIST'S COMMENTS :								
EXAMINED.....	0	0	0	1	0	0	0	0
The changes seen in the heart were - considered to be sufficient to - account for some of the clinical - signs observed.....	0	0	0	1	0	0	0	0

APPENDIX 1a

Bodyweights - individual values (kg)

Week	Control							
	1009♂	1011♂	1013♂	1015♂	1010♀	1012♀	1014♀	1016♀
Pre-dose								
-4	6.4	6.9	7.5	8.1	6.2	6.2	6.8	7.1
-3	6.6	7.2	7.7	8.3	6.5	6.6	7.2	7.5
-2	6.9	7.3	7.9	8.7	7.0	6.6	7.7	7.8
-1	7.3	8.0	8.1	9.1	6.9	6.8	7.8	8.2
Dosing								
0	7.7	8.6	8.5	9.5	7.7	7.2	8.3	8.7
1	7.9	8.7	8.8	9.6	8.1	7.4	8.2	8.8
2	8.4	9.2	9.1	9.9	8.5	7.6	8.6	8.9
3	8.8	9.3	9.4	10.0	8.8	7.9	8.9	9.3
4	9.1	9.7	9.6	10.0	9.0	8.0	8.8	9.4
5	9.4	9.9	9.9	10.1	9.4	8.2	9.2	9.8
6	9.6	10.1	9.8	10.3	9.4	8.2	9.1	9.4
7	9.7	10.4	10.2	10.6	9.6	8.6	9.4	9.7
8	9.8	10.6	10.4	10.7	9.7	8.5	9.6	9.8
9	10.2	10.6	10.5	10.8	9.9	8.8	9.7	10.0
10	10.4	10.9	10.7	11.0	10.2	8.9	10.0	10.1
11	10.6	11.1	10.9	11.2	10.2	9.1	9.9	10.2
12	10.8	11.4	11.3	11.4	10.6	9.0	10.4	10.2
13	10.7	11.4	11.2	11.4	10.6	9.0	10.0	10.1
0 to 13	3.0	2.8	2.7	1.9	2.9	1.8	1.7	1.4

APPENDIX 1a

(Bodyweights - continued)

Week	100 ppm							
	1017♂	1019♂	1021♂	1023♂	1018♀	1020♀	1022♀	1024♀
Pre-dose								
-4	6.5	6.8	7.0	7.5	5.6	6.2	6.8	7.8
-3	6.9	7.3	7.5	7.7	5.8	6.6	7.1	8.3
-2	7.2	7.7	7.6	8.1	5.9	7.0	7.4	8.7
-1	7.4	7.8	7.7	8.4	6.4	7.2	7.7	8.9
Dosing								
0	7.7	8.4	8.1	8.7	6.8	7.6	8.2	9.4
1	8.1	8.5	8.3	8.8	6.8	7.5	8.4	9.3
2	8.5	9.2	8.7	9.2	7.2	8.2	8.8	9.6
3	8.6	9.3	8.9	9.5	7.6	8.4	8.9	9.8
4	9.2	9.8	9.3	9.7	7.8	8.5	9.4	10.2
5	9.1	10.1	9.5	10.3	8.1	8.7	9.8	10.3
6	9.6	10.2	9.7	10.1	8.4	8.9	9.8	10.2
7	9.9	10.4	9.8	10.1	8.3	9.0	10.1	10.5
8	10.0	10.6	9.9	10.3	8.5	9.1	10.1	10.6
9	10.3	10.9	10.2	10.5	8.5	9.2	10.3	10.8
10	10.6	10.9	10.6	10.8	8.7	9.4	10.5	10.8
11	10.8	11.3	10.8	11.0	8.9	9.3	10.5	10.9
12	11.2	11.4	10.8	11.2	9.0	9.4	10.8	11.1
13	11.3	11.5	10.8	11.2	9.1	9.5	10.8	11.3
0 to 13	3.6	3.1	2.7	2.5	2.3	1.9	2.6	1.9

APPENDIX 1a

(Bodyweights - continued)

Week	300 ppm							
	1025♂	1027♂	1029♂	1031♂	1026♀	1028♀	1030♀	1032♀
Pre-dose								
-4	6.2	7.4	7.4	7.6	5.5	6.3	6.2	7.9
-3	6.8	7.2	7.6	8.3	5.8	6.6	6.8	8.2
-2	7.0	7.5	7.8	8.6	6.3	7.0	6.9	8.4
-1	7.3	7.7	8.1	8.9	6.5	7.0	7.0	8.7
Dosing								
0	7.9	8.4	8.1	9.2	7.0	7.5	7.5	9.1
1	7.8	8.5	8.5	9.5	7.1	8.1	7.6	9.2
2	8.1	8.8	8.9	9.7	7.4	8.2	7.9	9.6
3	8.5	9.1	9.2	9.8	7.6	8.5	8.2	9.7
4	8.9	9.2	9.6	10.1	8.0	9.0	8.4	10.3
5	9.3	9.6	10.0	10.2	8.0	9.3	8.4	10.4
6	9.4	9.6	10.1	10.4	8.2	9.2	9.0	10.6
7	9.7	9.8	10.4	10.6	8.3	9.5	9.0	10.8
8	9.8	10.1	10.6	10.8	8.6	9.8	9.2	11.0
9	10.0	10.0	10.9	11.0	8.7	9.9	9.4	11.2
10	10.3	10.3	11.1	11.2	8.8	10.1	9.7	11.2
11	10.6	10.4	11.3	11.5	8.8	10.4	9.8	11.7
12	10.8	10.5	11.5	11.7	8.8	10.4	10.0	11.7
13	10.7	10.7	11.7	11.7	8.9	10.6	9.9	11.7
0 to 13	2.8	2.3	3.6	2.5	1.9	3.1	2.4	2.6

APPENDIX 1a

(Bodyweights - continued)

Week	1000 ppm							
	1033♂	1035♂	1037♂	1039♂	1034♀	1036♀	1038♀	1040♀
Pre-dose								
-4	5.7	6.3	8.3	8.5	5.8	6.4	6.6	7.5
-3	5.9	7.0	8.2	8.9	6.3	6.6	6.9	7.9
-2	6.0	7.2	8.2	9.1	6.5	6.8	7.2	8.1
-1	6.0	7.7	8.7	9.2	6.8	7.1	7.5	8.5
Dosing								
0	6.2	8.0	8.9	10.0	7.4	7.5	8.1	8.8
1	6.2	8.2	9.1	9.8	7.5	7.8	8.3	8.9
2	6.0	8.3	9.2	9.9	7.9	8.0	8.6	9.1
3	(5.5)	8.0	9.3	10.1	7.9	8.2	8.8	9.4
4	(5.6)	8.0#	9.7	10.2	8.0	7.8	9.1	9.6
5	5.7		9.9	10.5	7.9	7.3	9.5	9.9
6	6.0		9.8	10.8	8.4	7.2	9.6	10.0
7	6.1		10.0	11.0	8.7	7.5	9.8	10.2
8	6.3		10.2	11.1	8.7	7.7	10.0	10.5
9	6.5		10.3	11.3	9.0	8.1	10.3	10.6
10	6.8		10.7	11.4	9.1	8.4	10.5	10.7
11	7.0		10.5	11.6	9.5	8.6	10.6	11.0
12	7.1		10.3	11.9	9.7	8.9	10.9	11.0
13	7.1		10.6	12.0	9.7	8.6	10.8	11.1
0 to 13	0.9		1.7	2.0	2.3	1.1	2.7	2.3

() Value excluded from means and statistical analysis - animal 1033♂ - bodyweight loss in Weeks 3 and 4 associated with retropharyngeal abscess

\# Animal 1035♂ killed for humane reasons on Day 6 of Week 5

APPENDIX 1b

Bodyweights - group mean values (kg)

Week	Diet concentration ppm							
	Control		100		300		1000	
	♂	♀	♂	♀	♂	♀	♂	♀
Pre-dose								
-4	7.2	6.6	7.0	6.6	7.2	6.5	7.2	6.6
-3	7.5	7.0	7.4	7.0	7.5	6.9	7.5	6.9
-2	7.7	7.3	7.7	7.3	7.7	7.2	7.6	7.2
-1	8.1	7.4	7.8	7.6	8.0	7.3	7.9	7.5
Dosing								
0	8.6	8.0	8.2	8.0	8.4	7.8	8.3	8.0
1	8.8	8.1	8.4	8.0	8.6	8.0	8.3	8.1
2	9.2	8.4	8.9	8.5	8.9	8.3	8.4	8.4
3	9.4	8.7	9.1	8.7	9.2	8.5	9.1	8.6
4	9.6	8.8	9.5	9.0	9.5	8.9	9.3	8.6
5	9.8	9.2	9.8	9.2	9.8	9.0	8.7	8.7
6	10.0	9.0	9.9	9.3	9.9	9.3	8.9	8.8
7	10.2	9.3	10.1	9.5	10.1	9.4	9.0	9.1
8	10.4	9.4	10.2	9.6	10.3	9.7	9.2	9.2
9	10.5	9.6	10.5	9.7	10.5	9.8	9.4	9.5
10	10.8	9.8	10.7	9.9	10.7	10.0	9.6	9.7
11	11.0	9.9	11.0	9.9	11.0	10.2	9.7	9.9
12	11.2	10.1	11.2	10.1	11.1	10.2	9.8	10.1
13	11.2	9.9	11.2	10.2	11.2	10.3	9.9	10.1

APPENDIX 2a

Food consumption - individual values (g)

Week	Control							
	1009♂	1011♂	1013♂	1015♂	1010♀	1012♀	1014♀	1016♀
Pre-dose								
-4	2440	2800	2800	2800	2770	2800	2630	2490
-3	2650	2800	2800	2800	2800	2750	2750	2750
-2	2750	2800	2800	2800	2800	2800	2800	2800
-1	2800	2800	2800	2800	2800	2800	2800	2800
-4 to -1	2660	2800	2800	2800	2793	2788	2745	2710
Dosing								
1	2800	2800	2800	2800	2800	2800	2800	2800
2	2800	2800	2800	2800	2800	2800	2800	2800
3	2800	2800	2800	2800	2800	2800	2800	2800
4	2800	2800	2800	2800	2800	2800	2750	2800
5	2800	2800	2800	2800	2800	2800	2760	2800
6	2800	2800	2800	2800	2800	2800	2800	2800
7	2800	2800	2800	2800	2800	2800	2800	2800
8	2800	2800	2800	2800	2800	2800	2800	2800
9	2800	2800	2800	2800	2800	2800	2800	2800
10	2800	2800	2800	2800	2800	2800	2800	2800
11	2800	2800	2800	2800	2800	2800	2800	2800
12	2800	2800	2800	2800	2800	2800	2800	2800
13	2800	2800	2800	2800	2800	2800	2800	2800
1 to 13	2800	2800	2800	2800	2800	2800	2793	2800

APPENDIX 2a

(Food consumption - continued)

Week	100 ppm							
	1017♂	1019♂	1021♂	1023♂	1018♀	1020♀	1022♀	1024♀
Pre-dose								
-4	2800	2800	2800	2800	2320	2790	2730	2800
-3	2800	2800	2800	2800	2200	2800	2800	2800
-2	2800	2800	2800	2800	2360	2800	2800	2800
-1	2800	2800	2800	2800	2340	2750	2800	2800
-4 to -1	2800	2800	2800	2800	2305	2785	2783	2800
Dosing								
1	2800	2800	2800	2800	2450	2800	2800	2800
2	2800	2800	2800	2800	2800	2800	2800	2800
3	2800	2800	2800	2800	2800	2800	2800	2800
4	2800	2800	2800	2800	2800	2800	2800	2800
5	2800	2800	2800	2800	2800	2800	2800	2800
6	2800	2800	2800	2800	2720	2800	2800	2800
7	2800	2800	2800	2800	2800	2800	2800	2800
8	2800	2800	2800	2800	2770	2800	2800	2800
9	2800	2800	2800	2800	2480	2800	2800	2800
10	2800	2800	2800	2800	2660	2800	2800	2800
11	2800	2800	2800	2800	2800	2800	2800	2800
12	2800	2800	2800	2800	2690	2800	2800	2800
13	2800	2800	2800	2800	2720	2800	2800	2800
1 to 13	2800	2800	2800	2800	2715	2800	2800	2800

APPENDIX 2a

(Food consumption - continued)

Week	300 ppm							
	1025♂	1027♂	1029♂	1031♂	1026♀	1028♀	1030♀	1032♀
Pre-dose								
-4	2740	2800	2800	2800	2700	2800	2750	2800
-3	2790	2800	2800	2800	2750	2800	2800	2800
-2	2800	2800	2800	2800	2800	2800	2800	2800
-1	2800	2800	2800	2800	2800	2800	2800	2800
-4 to -1	2783	2800	2800	2800	2763	2800	2788	2800
Dosing								
1	2730	2800	2800	2800	2730	2800	2800	2800
2	2800	2800	2800	2800	2800	2800	2800	2800
3	2800	2800	2800	2800	2800	2800	2800	2800
4	2800	2800	2800	2800	2800	2800	2800	2800
5	2800	2800	2800	2800	2800	2800	2800	2800
6	2800	2800	2800	2800	2590	2800	2800	2800
7	2800	2800	2800	2800	2800	2800	2800	2800
8	2800	2800	2800	2800	2800	2800	2800	2800
9	2800	2800	2800	2800	2800	2800	2800	2800
10	2800	2800	2800	2800	2800	2800	2800	2800
11	2800	2800	2800	2800	2800	2800	2800	2800
12	2800	2800	2800	2800	2800	2800	2800	2800
13	2800	2800	2800	2800	2800	2800	2800	2800
1 to 13	2795	2800	2800	2800	2778	2800	2800	2800

APPENDIX 3

(Ophthalmoscopy findings - continued)

Week 13 (16 August 1990)

Diet concentration ppm	Dog no./sex	Comments*
Control	1009♂	Retina: B/E Slight deficiency of pigment
	1013♂	Vitreous: B/E Hyaloid remnants
	1010♀	Vitreous: B/E Hyaloid remnants
	1014♀	Conjunctiva: R/E Slight conjunctivitis
100	1019♂	Vitreous: B/E Hyaloid remnants
	1018♀	Lens: L/E Faint posterior capsular scarring
300	1028♀	Vitreous: B/E Hyaloid remnants
	1030♀	Vitreous: B/E Hyaloid remnants
1000	1039♂	Vitreous: B/E Prominent hyaloid remnants
	1038♀	Lens: R/E Small posterior capsular opacity around attachments of hyaloid remnants
		Vitreous: B/E Hyaloid remnants

* Only animals with comments listed (ophthalmoscopy was not performed on Dog 1035♂ prior to its being killed for humane reasons in Week 5)

B/E Both eyes

R/E Right eye

L/E Left eye

APPENDIX 4a

(Haematology - continued)

Before dosing commenced (3 May 1990)

Diet concn. ppm	Dog no.	PCV %	Hb g/dl	RBC x10⁶/mm³	MCHC %	MCV fl	Retic %	WBC + Diff x10³/mm³ Total	N	L	E	B	M	Plts x10³/mm³	PT s	APTT s
300	1025♂	41	12.2	5.4	29.8	76	<2.0	14.8	8.73	5.18	0.59	0.00	0.30	362	5.7	12.8
	1027♂	38	11.8	5.0	31.1	76	<2.0	16.3	11.41	4.08	0.16	0.00	0.65	451	8.9	14.0
	1029♂	36	10.6	4.8	29.4	75	<2.0	11.5	7.25	3.91	0.12	0.00	0.23	487	5.7	11.1
	1031♂	37	11.3	4.9	30.5	76	<2.0	21.5	13.55	6.24	0.22	0.00	1.51	429	5.7	13.3
	1026♀	32	9.3	4.2	29.1	76	<2.0	17.5	14.70	2.45	0.18	0.00	0.18	484	5.6	13.9
	1028♀	41	12.4	5.2	30.2	79	<2.0	20.7	14.08	5.80	0.41	0.00	0.41	485	6.0	13.3
	1030♀	37	11.0	5.0	29.7	74	<2.0	21.1	17.72	1.69	0.21	0.00	1.48	612	5.5	12.6
	1032♀	38	11.0	5.1	28.9	75	<2.0	9.0	5.13	3.06	0.27	0.00	0.54	446	6.1	13.6
	Mean ♂	38	11.5	5.0	30.2	76		16.0	10.24	4.85	0.27	0.00	0.67	432	6.5	12.8
	Mean ♀	37	10.9	4.9	29.5	76		17.1	12.91	3.25	0.27	0.00	0.65	507	5.8	13.4
	Mean	38	11.2	5.0	29.8	76		16.6	11.57	4.05	0.27	0.00	0.66	470	6.2	13.1
	SD ♂	2.2	0.69	0.26	0.75	0.5		4.16	2.801	1.083	0.216	0.000	0.588	52.6	1.60	1.24
	SD ♀	3.7	1.27	0.46	0.59	2.2		5.62	5.423	1.790	0.102	0.000	0.571	72.5	0.29	0.56
	SD	2.9	0.99	0.35	0.74	1.5		4.61	4.244	1.615	0.156	0.000	0.537	70.9	1.13	0.93
1000	1033♂	37	11.0	5.1	29.7	73	<2.0	13.6	9.93	3.26	0.27	0.00	0.14	486	5.6	13.0
	1035♂	37	10.8	4.8	29.2	77	<2.0	14.7	10.58	2.79	0.74	0.00	0.59	360	5.6	13.1
	1037♂	40	12.2	5.3	30.5	75	<2.0	27.1	21.95	4.88	0.00	0.00	0.27	524	5.6	12.4
	1039♂	37	11.0	4.7	29.7	79	<2.0	10.2	6.73	3.47	0.00	0.00	0.00	384	5.6	13.1
	1034♀	42	12.7	5.4	30.2	78	<2.0	13.8	10.07	2.90	0.41	0.00	0.41	345	5.6	13.2
	1036♀	40	12.3	5.5	30.8	73	<2.0	14.0	7.42	5.88	0.28	0.00	0.42	368	6.1	15.5
	1038♀	38	11.8	5.0	31.1	76	<2.0	16.1	9.50	5.47	0.16	0.00	0.97	404	5.6	13.0
	1040♀	40	12.3	5.4	30.8	74	<2.0	16.0	12.00	3.36	0.00	0.00	0.64	397	6.0	13.0
	Mean ♂	38	11.3	5.0	29.8	76		16.4	12.30	3.60	0.25	0.00	0.25	439	5.6	12.9
	Mean ♀	40	12.3	5.3	30.7	75		15.0	9.75	4.40	0.21	0.00	0.61	379	5.8	13.7
	Mean	39	11.8	5.2	30.3	76		15.7	11.02	4.00	0.23	0.00	0.43	409	5.7	13.3
	SD ♂	1.5	0.64	0.28	0.54	2.6		7.39	6.651	0.899	0.349	0.000	0.252	78.9	0.00	0.34
	SD ♀	1.6	0.37	0.22	0.38	2.2		1.24	1.885	1.491	0.175	0.000	0.262	27.2	0.26	1.22
	SD	1.9	0.73	0.30	0.67	2.3		4.96	4.727	1.218	0.256	0.000	0.306	63.4	0.21	0.93
Repeat investigation (11 May 1990)																
300	1030♀													602		

SD Standard deviation

APPENDIX 4b

Haematology - pre-terminal data for Dog 1035♂ (Day 6 of Week 5; 25 June 1990)

Diet concn. ppm	PCV %	Hb g/dl	RBC x10⁶/mm³	MCHC %	MCV fl	Retic %	WBC + Diff x10³/mm³ Total	N	L	E	B	M	Plts x10³/mm³	PT s	APTT s
1000	CID	CID	CID	-	-	CID	CID	CID	CID	CID	CID	CID	CID	5.1	12.2

CID Sample clotted

APPENDIX 4c

Haematology - individual values

Week 6 (27 June 1990)

Diet concn. ppm	Dog no.	PCV %	Hb g/dl	RBC x10⁶/mm³	MCHC %	MCV fl	Retic %	WBC + Diff x10³/mm³ Total	N	L	E	B	M	Plts x10³/mm³	PT s	APTT s
Control	1009♂	36	11.3	4.8	31.4	75	<2.0	12.9	9.16	3.48	0.13	0.00	0.13	500	5.9	13.2
	1011♂	41	12.8	5.4	31.2	76	<2.0	13.0	7.93	4.55	0.39	0.00	0.13	327	5.8	14.2
	1013♂	43	13.3	6.0	30.9	72	<2.0	13.1	8.91	3.67	0.13	0.00	0.39	487	5.8	12.5
	1015♂	44	13.9	5.9	31.6	75	<2.0	11.8	7.20	4.01	0.59	0.00	0.00	521	6.2	12.7
	1010♀	47	14.5	6.5	30.9	72	<2.0	12.6	7.18	5.04	0.13	0.00	0.25	373	5.6	12.4
	1012♀	45	13.7	6.0	30.4	75	<2.0	13.2	8.45	4.36	0.13	0.00	0.26	345	9.4	12.2
	1014♀	40	12.7	5.6	31.8	71	<2.0	9.1	5.92	2.73	0.18	0.00	0.27	318	5.6	13.3
	1016♀	44	13.8	5.9	31.4	75	<2.0	11.0	5.50	5.06	0.22	0.00	0.22	403	5.6	14.9
	Mean ♂	41	12.8	5.5	31.3	75		12.7	8.30	3.93	0.31	0.00	0.16	459	5.9	13.2
	Mean ♀	44	13.7	6.0	31.1	73		11.5	6.76	4.30	0.17	0.00	0.25	360	6.6	13.2
	Mean	43	13.3	5.8	31.2	74		12.1	7.53	4.11	0.24	0.00	0.21	409	6.2	13.2
	SD ♂	3.6	1.11	0.55	0.30	1.7		0.61	0.905	0.469	0.223	0.000	0.164	88.9	0.19	0.76
	SD ♀	2.9	0.74	0.37	0.61	2.1		1.84	1.332	1.094	0.044	0.000	0.022	36.5	1.90	1.23
	SD	3.4	0.99	0.50	0.45	1.9		1.42	1.337	0.804	0.168	0.000	0.118	82.2	1.29	0.95
100	1017♂	41	12.4	5.3	30.2	77	<2.0	12.4	5.70	5.08	0.87	0.00	0.74	519	6.2	13.5
	1019♂	39	12.2	5.2	31.3	75	<2.0	10.1	5.56	4.24	0.20	0.00	0.10	544	6.5	13.7
	1021♂	41	13.0	5.5	31.7	75	<2.0	11.7	7.37	3.86	0.35	0.00	0.12	383	6.4	14.3
	1023♂	43	13.4	5.7	31.2	75	<2.0	10.3	5.05	5.05	0.21	0.00	0.00	432	6.0	14.1
	1018♀	36	11.2	5.0	31.1	72	<2.0	15.6	10.92	4.21	0.31	0.00	0.16	429	5.9	13.3
	1020♀	38	11.6	4.9	30.5	78	<2.0	12.4	6.20	5.70	0.50	0.00	0.00	430	5.6	16.2
	1022♀	46	14.2	6.3	30.9	73	<2.0	11.1	6.66	4.11	0.11	0.00	0.22	338	6.0	15.1
	1024♀	41	13.2	5.7	32.2	72	<2.0	13.3	7.05	5.59	0.53	0.00	0.13	476	6.1	13.6
	Mean ♂	41	12.8	5.4	31.1	76		11.1	5.92	4.56	0.41	0.00	0.24	470	6.3	13.9
	Mean ♀	40	12.6	5.5	31.2	74		13.1	7.71	4.90	0.36	0.00	0.13	418	5.9	14.6
	Mean	41	12.7	5.5	31.1	75		12.1	6.81	4.73	0.39	0.00	0.18	444	6.1	14.2
	SD ♂	1.6	0.55	0.22	0.64	1.0		1.11	1.006	0.606	0.316	0.000	0.337	75.0	0.22	0.37
	SD ♀	4.3	1.40	0.66	0.73	2.9		1.90	2.170	0.860	0.194	0.000	0.093	57.8	0.22	1.35
	SD	3.1	0.99	0.45	0.63	2.2		1.78	1.834	0.713	0.244	0.000	0.237	67.8	0.29	0.98

SD Standard deviation

APPENDIX 4c

(Haematology - continued)

Week 6 (27 June 1990)

Diet concn. ppm	Dog no.	PCV %	Hb g/dl	RBC x10⁶/mm³	MCHC %	MCV fl	Retic %	WBC + Diff x10³/mm³ Total	N	L	E	B	M	Plts x10³/mm³	PT s	APTT s
300	1025♂	47	14.7	6.3	31.3	75	<2.0	12.6	7.56	4.28	0.25	0.00	0.50	243	6.0	11.5
	1027♂	39	12.3	5.0	31.5	78	<2.0	14.5	10.73	3.05	0.29	0.00	0.44	373	9.1	13.8
	1029♂	37	11.1	4.9	30.0	76	<2.0	10.9	6.76	3.82	0.11	0.00	0.22	426	5.6	13.0
	1031♂	42	13.1	5.6	31.2	75	<2.0	10.6	5.72	4.55	0.21	0.00	0.11	338	5.6	14.9
	1026♀	41	12.1	5.6	29.5	73	<2.0	11.6	8.00	3.48	0.12	0.00	0.00	465	5.7	13.5
	1028♀	43	13.7	5.5	31.9	78	<2.0	15.6	10.30	5.30	0.00	0.00	0.00	444	6.2	14.0
	1030♀	40	12.4	5.4	31.0	74	<2.0	16.0	9.28	6.56	0.00	0.00	0.16	445	5.8	13.3
	1032♀	45	14.4	5.9	32.0	76	<2.0	12.2	6.34	5.49	0.37	0.00	0.00	461	5.9	12.9
	Mean ♂	41	12.8	5.5	31.0	76		12.2	7.69	3.93	0.22	0.00	0.32	345	6.6	13.3
	Mean ♀	42	13.2	5.6	31.1	75		13.9	8.48	5.21	0.12	0.00	0.04	454	5.9	13.4
	Mean	42	13.0	5.5	31.1	76		13.0	8.09	4.57	0.17	0.00	0.18	399	6.2	13.4
	SD ♂	4.3	1.51	0.65	0.68	1.4		1.80	2.161	0.660	0.077	0.000	0.183	77.0	1.69	1.43
	SD ♀	2.2	1.08	0.22	1.16	2.2		2.27	1.709	1.278	0.174	0.000	0.080	10.8	0.22	0.46
	SD	3.2	1.23	0.45	0.88	1.8		2.10	1.852	1.164	0.134	0.000	0.198	77.3	1.17	0.99
1000	1033♂	35	10.5	4.8	30.0	73	2.9	11.2	5.60	5.04	0.34	0.00	0.22	573	5.5	15.0
	1037♂	39	12.4	5.1	31.8	76	<2.0	13.4	8.98	4.29	0.13	0.00	0.00	463	5.6	15.1
	1039♂	35	10.9	4.6	31.1	76	<2.0	8.8	5.46	3.26	0.00	0.00	0.09	361	5.9	15.1
	1034♀	43	13.8	5.5	32.1	78	<2.0	14.8	8.58	5.48	0.30	0.00	0.44	546	5.6	17.6
	1036♀	43	13.6	5.5	31.6	78	3.5	16.2	11.02	4.70	0.00	0.00	0.49	566	6.4	12.0
	1038♀	35	10.8	4.6	30.9	76	<2.0	14.0	7.84	6.16	0.00	0.00	0.00	331	5.5	12.4
	1040♀	39	12.3	5.0	31.5	78	<2.0	12.6	7.94	4.41	0.13	0.00	0.13	447	5.9	15.2
	Mean ♂	36	11.3	4.8	31.0	75		11.1	6.68	4.20	0.16	0.00	0.10	466	5.7	15.1
	Mean ♀	40	12.6	5.2	31.5	78		14.4	8.85	5.19	0.11	0.00	0.27	473	5.9	14.3
	Mean	38	12.0	5.0	31.3	76		13.0	7.92	4.76	0.13	0.00	0.20	470	5.8	14.6
	SD ♂	2.3	1.00	0.25	0.91	1.7		2.30	1.993	0.894	0.172	0.000	0.111	106.0	0.21	0.06
	SD ♀	3.8	1.39	0.44	0.49	1.0		1.51	1.487	0.790	0.142	0.000	0.238	107.7	0.40	2.62
	SD	3.6	1.35	0.38	0.70	1.8		2.44	1.941	0.927	0.144	0.000	0.200	97.8	0.33	1.90

SD Standard deviation
Film comments:

1033♂ - Slight polychromasia, slight hypochromasia, slight anisocytosis, slight rouleaux,
1036♀ - Slight polychromasia, slight anisocytosis

APPENDIX 4d

Haematology - individual values

Week 13 (15 August 1990)

Diet concn. ppm	Dog no.	PCV %	Hb g/dl	RBC x10⁶/mm³	MCHC %	MCV fl	Retic %	WBC + Diff x10³/mm³ Total	N	L	E	B	M	Plts x10³/mm³	PT s	APTT s
Control	1009♂	39	11.3	5.1	29.0	76	<2.0	8.3	4.32	3.49	0.50	0.00	0.00	525	5.8	12.2
	1011♂	45	13.3	5.8	29.6	78	<2.0	11.6	6.26	4.41	0.70	0.00	0.23	291	5.8	12.8
	1013♂	44	13.0	6.0	29.5	73	<2.0	12.0	7.80	3.48	0.36	0.00	0.36	446	5.9	12.1
	1015♂	47	14.1	6.1	30.0	77	<2.0	11.5	7.02	3.91	0.35	0.00	0.23	512	6.0	12.3
	1010♀	47	13.8	6.4	29.4	73	<2.0	12.9	6.45	6.19	0.26	0.00	0.00	322	5.6	12.0
	1012♀	50	14.6	6.5	29.2	77	<2.0	11.8	7.55	3.78	0.24	0.00	0.24	331	9.2	11.1
	1014♀	46	13.5	6.1	29.3	75	<2.0	9.4	4.89	4.32	0.09	0.00	0.09	358	5.7	15.0
	1016♀	CID	CID	CID	NR	NR	CID	CID	CID	CID	CID	CID	CID	CID	6.4	13.2
	Mean ♂	44	12.9	5.8	29.5	76		10.9	6.35	3.82	0.48	0.00	0.21	444	5.9	12.4
	Mean ♀	48	14.0	6.3	29.3	75		11.4	6.30	4.76	0.20	0.00	0.11	337	6.7	12.8
	Mean	45	13.4	6.0	29.4	76		11.1	6.33	4.23	0.36	0.00	0.16	398	6.3	12.6
	SD ♂	3.4	1.18	0.45	0.41	2.2		1.71	1.492	0.440	0.163	0.000	0.150	107.4	0.10	0.31
	SD ♀	2.1	0.57	0.21	0.10	2.0		1.79	1.337	1.265	0.093	0.000	0.121	18.7	1.69	1.69
	SD	3.4	1.05	0.46	0.32	2.0		1.62	1.308	0.940	0.197	0.000	0.137	95.5	1.20	1.15
100	1017♂	42	12.3	5.4	29.3	78	<2.0	10.9	6.00	4.47	0.22	0.00	0.22	515	5.9	11.7
	1019♂	37	10.9	4.9	29.5	76	<2.0	8.4	4.45	3.53	0.34	0.00	0.08	533	6.5	12.8
	1021♂	42	12.6	5.4	30.0	78	<2.0	11.6	8.00	3.25	0.35	0.00	0.00	349	5.9	12.2
	1023♂	44	13.0	5.5	29.5	80	<2.0	10.1	5.66	4.14	0.00	0.00	0.30	454	6.1	13.3
	1018♀	39	11.7	5.2	30.0	75	<2.0	13.4	8.58	4.69	0.13	0.00	0.00	411	5.7	13.2
	1020♀	39	11.4	5.0	29.2	78	<2.0	12.8	7.68	4.86	0.26	0.00	0.00	441	5.9	15.2
	1022♀	46	13.7	6.0	29.8	77	<2.0	11.9	7.62	3.93	0.12	0.00	0.24	325	6.1	14.3
	1024♀	42	12.8	5.6	30.5	75	<2.0	12.1	7.62	4.24	0.12	0.00	0.12	425	6.0	13.5
	Mean ♂	41	12.2	5.3	29.6	78		10.3	6.03	3.85	0.23	0.00	0.15	463	6.1	12.5
	Mean ♀	42	12.4	5.5	29.9	76		12.6	7.88	4.43	0.16	0.00	0.09	401	5.9	14.1
	Mean	41	12.3	5.4	29.7	77		11.4	6.95	4.14	0.19	0.00	0.12	432	6.0	13.3
	SD ♂	3.0	0.91	0.27	0.30	1.6		1.38	1.474	0.557	0.163	0.000	0.135	83.0	0.28	0.70
	SD ♀	3.3	1.06	0.44	0.54	1.5		0.69	0.471	0.424	0.068	0.000	0.115	51.8	0.17	0.90
	SD	2.9	0.92	0.35	0.43	1.7		1.59	1.415	0.554	0.122	0.000	0.120	72.2	0.24	1.11
Repeat investigation (17 August 1990)																
Control	1016♀	45	13.9	5.8	30.9	78	<2.0	9.5	5.42	3.61	0.29	0.00	0.19	410		

SD Standard deviation
CID Sample clotted
NR Not recorded

APPENDIX 4d

(Haematology - continued)

Week 13 (15 August 1990)

Diet concn. ppm	Dog no.	PCV %	Hb g/dl	RBC x10⁶/mm³	MCHC %	MCV fl	Retic %	WBC + Diff x10³/mm³ Total	N	L	E	B	M	Plts x10³/mm³	PT s	APTT s
300	1025♂	44	13.0	5.6	29.5	79	<2.0	11.7	6.90	4.45	0.12	0.00	0.23	377	5.6	13.5
	1027♂	38	11.1	4.7	29.2	81	<2.0	13.8	8.97	4.69	0.00	0.00	0.14	376	9.1	13.7
	1029♂	40	11.8	5.3	29.5	75	<2.0	12.1	6.78	5.08	0.12	0.00	0.12	443	5.6	12.4
	1031♂	45	13.5	5.9	30.0	76	<2.0	13.2	7.26	5.54	0.13	0.00	0.26	363	5.4	13.8
	1026♀	45	13.1	5.6	29.1	80	<2.0	12.1	8.47	3.27	0.12	0.00	0.24	548	5.6	13.2
	1028♀	42	12.7	5.3	30.2	79	<2.0	13.2	7.66	5.54	0.00	0.00	0.00	443	6.1	15.3
	1030♀	41	12.3	5.5	30.0	75	<2.0	12.1	8.11	3.99	0.00	0.00	0.00	455	5.9	13.1
	1032♀	48	14.2	6.1	29.6	79	<2.0	9.6	5.38	4.22	0.00	0.00	0.00	428	5.6	12.2
	Mean ♂	42	12.4	5.4	29.6	78		12.7	7.48	4.94	0.09	0.00	0.19	390	6.4	13.4
	Mean ♀	44	13.1	5.6	29.7	78		11.8	7.41	4.26	0.03	0.00	0.06	469	5.8	13.5
	Mean	43	12.7	5.5	29.6	78		12.2	7.44	4.60	0.06	0.00	0.12	429	6.1	13.4
	SD ♂	3.3	1.10	0.51	0.33	2.8		0.97	1.016	0.477	0.062	0.000	0.068	36.1	1.79	0.65
	SD ♀	3.2	0.82	0.34	0.49	2.2		1.52	1.390	0.947	0.060	0.000	0.120	54.1	0.24	1.31
	SD	3.2	0.98	0.42	0.40	2.3		1.29	1.128	0.785	0.066	0.000	0.113	59.9	1.23	0.96
1000	1033♂	34	9.6	4.5	28.2	76	<2.0	15.2	11.40	3.19	0.00	0.00	0.61	485	5.1	13.8
	1037♂	42	12.8	5.4	30.5	78	<2.0	13.5	9.45	4.05	0.00	0.00	0.00	497	5.7	14.9
	1039♂	39	11.6	5.0	29.7	78	<2.0	10.3	6.80	2.88	0.31	0.00	0.31	402	5.6	15.3
	1034♀	54	15.9	6.8	29.4	79	2.5	17.8	10.32	6.41	0.18	0.00	0.89	544	5.6	15.5
	1036♀	CID	CID	CID	NR	NR	CID	CID	CID	CID	CID	CID	CID	CID	5.8	15.0
	1038♀	43	13.0	5.6	30.2	77	<2.0	13.7	8.08	5.21	0.41	0.00	0.00	341	5.5	13.7
	1040♀	41	12.0	5.0	29.3	82	<2.0	13.2	9.90	2.90	0.13	0.00	0.26	466	6.1	14.8
	Mean ♂	38	11.3	5.0	29.5	77		13.0	9.22	3.37	0.10	0.00	0.31	461	5.5	14.7
	Mean ♀	46	13.6	5.8	29.6	79		14.9	9.43	4.84	0.24	0.00	0.38	450	5.8	14.8
	Mean	42	12.5	5.4	29.6	78		14.0	9.33	4.11	0.17	0.00	0.35	456	5.6	14.7
	SD ♂	4.0	1.62	0.45	1.17	1.2		2.49	2.309	0.606	0.179	0.000	0.305	51.7	0.32	0.78
	SD ♀	7.0	2.03	0.92	0.49	2.5		2.52	1.191	1.784	0.149	0.000	0.458	102.4	0.26	0.76
	SD	6.6	2.07	0.79	0.81	2.1		2.47	1.647	1.437	0.165	0.000	0.350	72.8	0.30	0.70
Repeat investigations (17 August 1990)																
1000	1033♂	39	11.7	5.1												
	1036♀	41	12.7	5.4	31.0	76	<2.0	14.5	8.70	5.08	0.15	0.00	0.58	176		

SD Standard deviation
CID Sample clotted
NR Not recorded

APPENDIX 5a

Biochemistry - individual values

Before dosing commenced (3 May 1990)

Diet concn. ppm	Dog no.	Glucose mg/dl	Protein g/dl Total	Alb	Glob	Urea mg/dl	Creatinine mg/dl	AP mU/ml	GPT mU/ml	GOT mU/ml	γGT mU/ml	OCT mU/ml	CPK mU/ml	Bilirubin mg/dl	Na mEq/l	K mEq/l	Ca mEq/l	P mEq/l	Cl mEq/l	Chol mg/dl
Control	1009♂	101	4.8	2.1	2.7	17	0.5	312	19	16	3	2.9	142	0.1	145	5.1	5.6	4.9	107	97
	1011♂	106	4.8	2.3	2.5	24	0.5	298	23	16	2	6.4	139	0.2	144	4.9	5.5	5.2	106	163
	1013♂	109	4.9	2.4	2.5	28	0.6	287	15	13	3	6.1	95	0.1	146	4.8	5.7	5.2	108	129
	1015♂	100	5.1	2.5	2.6	26	0.6	292	20	15	3	1.4	80	0.2	146	4.7	5.7	4.9	107	131
	1010♀	107	4.7	2.3	2.4	24	0.5	311	18	17	3	6.9	109	0.3	145	4.2	5.5	4.9	106	133
	1012♀	99	4.8	2.2	2.6	24	0.6	255	19	12	2	1.5	92	0.2	145	4.5	5.4	5.1	110	118
	1014♀	104	5.4	2.5	2.9	36	0.7	363	12	13	2	7.3	111	0.2	144	5.2	6.0	5.3	106	146
	1016♀	109	4.9	2.3	2.6	17	0.6	361	27	17	2	8.0	98	0.1	147	4.4	5.8	5.7	110	133
	Mean ♂	104	4.9	2.3	2.6	24	0.6	297	19	15	3	4.2	114	0.2	145	4.9	5.6	5.1	107	130
	Mean ♀	105	5.0	2.3	2.6	25	0.6	323	19	15	2	5.9	103	0.2	145	4.6	5.7	5.3	108	133
	Mean	104	4.9	2.3	2.6	25	0.6	310	19	15	3	5.1	108	0.2	145	4.7	5.7	5.2	108	131
	SD ♂	4.2	0.14	0.17	0.10	4.8	0.06	10.8	3.3	1.4	0.5	2.45	31.2	0.06	1.0	0.17	0.10	0.17	0.8	27.0
	SD ♀	4.3	0.31	0.13	0.21	7.9	0.08	51.0	6.2	2.6	0.5	2.98	9.0	0.08	1.3	0.43	0.28	0.34	2.3	11.4
	SD	4.0	0.23	0.14	0.15	6.1	0.07	36.7	4.6	2.0	0.5	2.69	22.2	0.07	1.0	0.35	0.19	0.27	1.7	19.2
100	1017♂	105	5.0	2.2	2.8	24	0.6	246	18	21	2	6.3	151	0.1	145	5.0	5.5	5.4	108	145
	1019♂	107	4.8	2.5	2.3	21	0.6	331	19	14	2	7.8	125	0.2	144	4.7	5.6	5.0	107	156
	1021♂	106	5.1	2.4	2.7	28	0.7	348	25	12	3	6.6	86	0.2	145	4.4	6.0	5.5	105	119
	1023♂	101	4.7	2.3	2.4	30	0.7	304	25	14	2	5.6	108	0.2	145	5.0	5.5	5.8	107	146
	1018♀	106	4.5	2.3	2.2	21	0.6	348	20	14	2	1.9	125	0.1	144	4.5	5.5	4.7	107	92
	1020♀	102	4.9	2.3	2.6	19	0.6	250	17	12	2	1.7	103	0.1	144	4.6	5.7	4.2	107	174
	1022♀	103	5.5	2.5	3.0	19	0.7	362	17	15	2	1.7	152	0.1	145	4.8	5.6	4.9	105	120
	1024♀	101	5.0	2.1	2.9	28	0.6	269	16	20	2	6.1	135	0.2	146	4.5	5.5	5.6	107	119
	Mean ♂	105	4.9	2.4	2.6	26	0.7	307	22	15	2	6.6	118	0.2	145	4.8	5.7	5.4	107	142
	Mean ♀	103	5.0	2.3	2.7	22	0.6	307	18	15	2	2.9	129	0.1	145	4.6	5.6	4.9	107	126
	Mean	104	4.9	2.3	2.6	24	0.6	307	20	15	2	4.7	123	0.2	145	4.7	5.6	5.1	107	134
	SD ♂	2.6	0.18	0.13	0.24	4.0	0.06	44.7	3.8	3.9	0.5	0.92	27.5	0.05	0.5	0.29	0.24	0.33	1.3	15.8
	SD ♀	2.2	0.41	0.16	0.36	4.3	0.05	56.0	1.7	3.4	0.0	2.17	20.5	0.05	1.0	0.14	0.10	0.58	1.0	34.4
	SD	2.4	0.30	0.14	0.29	4.4	0.05	46.9	3.5	3.4	0.4	2.52	23.2	0.05	0.7	0.23	0.17	0.53	1.1	26.1

SD Standard deviation

APPENDIX 5a

(Biochemistry - continued)

Before dosing commenced (3 May 1990)

Diet concn. ppm	Dog no.	Glucose mg/dl	Protein g/dl Total	Alb	Glob	Urea mg/dl	Creatinine mg/dl	AP mU/ml	GPT mU/ml	GOT mU/ml	γGT mU/ml	OCT mU/ml	CPK mU/ml	Bilirubin mg/dl	Na mEq/l	K mEq/l	Ca mEq/l	P mEq/l	Cl mEq/l	Chol mg/dl
300	1025♂	115	4.9	2.4	2.5	24	0.4	367	24	20	2	6.8	177	0.2	144	5.1	5.4	5.7	106	128
	1027♂	102	5.1	2.3	2.8	24	0.6	251	18	11	3	6.8	82	0.2	145	4.5	5.5	5.3	106	123
	1029♂	106	4.7	2.3	2.4	15	0.6	335	19	14	2	4.2	132	0.1	145	4.8	5.5	5.0	108	118
	1031♂	103	4.9	2.3	2.6	19	0.5	363	21	16	2	5.9	101	0.2	145	4.7	5.6	4.8	107	157
	1026♀	103	4.7	2.0	2.7	15	0.6	283	13	14	2	1.7	109	0.1	145	4.9	5.6	4.8	107	96
	1028♀	94	5.0	2.4	2.6	30	0.5	277	15	11	3	2.4	76	0.3	144	4.5	5.6	5.2	107	155
	1030♀	91	5.1	2.3	2.8	28	0.5	216	17	12	2	1.4	86	0.3	144	4.5	5.6	5.5	109	134
	1032♀	96	4.7	2.2	2.5	17	0.6	274	19	15	2	3.6	95	0.2	145	4.5	5.4	4.9	110	128
	Mean ♂	107	4.9	2.3	2.6	21	0.5	329	21	15	2	5.9	123	0.2	145	4.8	5.5	5.2	107	132
	Mean ♀	96	4.9	2.2	2.7	23	0.6	263	16	13	2	2.3	92	0.2	145	4.6	5.6	5.1	108	128
	Mean	101	4.9	2.3	2.6	22	0.5	296	18	14	2	4.1	107	0.2	145	4.7	5.5	5.2	108	130
	SD ♂	5.9	0.16	0.05	0.17	4.4	0.10	53.9	2.6	3.8	0.5	1.23	41.5	0.05	0.5	0.25	0.08	0.39	1.0	17.5
	SD ♀	5.1	0.21	0.17	0.13	7.6	0.06	31.2	2.6	1.8	0.5	0.98	14.0	0.10	0.6	0.20	0.10	0.32	1.5	24.4
	SD	7.6	0.17	0.13	0.15	5.8	0.07	54.1	3.4	3.0	0.5	2.20	33.2	0.08	0.5	0.23	0.09	0.33	1.4	19.7
1000	1033♂	103	5.0	2.4	2.6	21	0.5	260	25	18	3	5.9	104	0.1	146	4.9	5.7	4.3	110	122
	1035♂	103	4.7	2.3	2.4	21	0.6	254	18	15	2	5.6	90	0.1	146	5.1	5.6	5.0	108	87
	1037♂	97	4.8	2.3	2.5	43	0.6	224	17	11	3	3.9	69	0.3	145	4.9	5.4	5.5	108	152
	1039♂	109	4.7	2.4	2.3	21	0.5	301	20	18	2	8.6	188	0.2	144	5.3	5.5	5.0	107	152
	1034♀	103	4.5	2.2	2.3	21	0.5	319	24	19	3	7.1	156	0.1	145	4.7	5.4	5.0	109	137
	1036♀	100	5.3	2.4	2.9	26	0.7	368	19	17	2	2.0	109	0.1	144	4.5	5.6	5.8	107	124
	1038♀	92	4.7	2.2	2.5	30	0.6	176	14	15	3	3.6	115	0.3	147	4.4	5.5	5.2	109	138
	1040♀	102	4.9	2.3	2.6	28	0.5	265	37	17	3	6.8	87	0.3	145	4.9	5.7	5.8	107	139
	Mean ♂	103	4.8	2.4	2.5	27	0.6	260	20	16	3	6.0	113	0.2	145	5.1	5.6	5.0	108	128
	Mean ♀	99	4.9	2.3	2.6	26	0.6	282	24	17	3	4.9	117	0.2	145	4.6	5.6	5.5	108	135
	Mean	101	4.8	2.3	2.5	26	0.6	271	22	16	3	5.4	115	0.2	145	4.8	5.6	5.2	108	131
	SD ♂	4.9	0.14	0.06	0.13	11.0	0.06	31.7	3.6	3.3	0.6	1.94	52.2	0.10	1.0	0.19	0.13	0.49	1.3	30.9
	SD ♀	5.0	0.34	0.10	0.25	3.9	0.10	82.2	9.9	1.6	0.5	2.49	28.8	0.12	1.3	0.22	0.13	0.41	1.2	7.0
	SD	5.0	0.24	0.08	0.20	7.6	0.07	58.9	7.1	2.5	0.5	2.15	39.1	0.10	1.0	0.30	0.12	0.50	1.1	21.0

SD Standard deviation

APPENDIX 5b

Biochemistry - pre-terminal data for Dog 1035♂ (Day 6 of Week 5; 25 June 1990)

Diet concn. ppm	Glucose mg/dl	Protein g/dl Total	Protein g/dl Alb	Protein g/dl Glob	Urea mg/dl	Creatinine mg/dl	AP mU/ml	GPT mU/ml	GOT mU/ml	γGT mU/ml	OCT mU/ml	CPK mU/ml	Bilirubin mg/dl	Na mEq/l	K mEq/l	Ca mEq/l	P mEq/l	Cl mEq/l	Chol mg/dl
1000	118	6.1	2.8	3.3	30	0.7	691	45	84	<1	4.1	1251	0.1	154	4.6	5.2	3.8	116	218

APPENDIX 5c

Biochemistry - individual values

Week 6 (27 June 1990)

Diet concn. ppm	Dog no.	Glucose mg/dl	Protein g/dl Total	Protein g/dl Alb	Protein g/dl Glob	Urea mg/dl	Creatinine mg/dl	AP mU/ml	GPT mU/ml	GOT mU/ml	γGT mU/ml	OCT mU/ml	CPK mU/ml	Bilirubin mg/dl	Na mEq/l	K mEq/l	Ca mEq/l	P mEq/l	Cl mEq/l	Chol mg/dl
Control	1009♂	93	4.8	2.8	2.0	34	0.7	228	24	20	2	2.8	110	0.2	148	5.1	5.5	4.3	108	89
	1011♂	93	4.9	2.6	2.3	26	0.6	254	24	20	2	3.4	122	0.1	146	4.5	5.3	4.2	106	147
	1013♂	91	5.1	2.7	2.4	30	0.7	238	17	17	2	5.6	138	0.1	147	4.8	5.4	3.8	107	134
	1015♂	94	5.2	2.9	2.3	24	0.7	222	23	19	1	2.1	331	0.1	148	4.6	5.3	3.6	108	142
	1010♀	96	5.3	3.1	2.2	26	0.7	297	27	25	2	6.9	191	0.2	148	4.6	5.7	4.3	104	142
	1012♀	96	5.1	2.8	2.3	36	0.6	224	27	24	2	1.6	137	0.2	147	5.0	5.4	4.8	112	115
	1014♀	90	5.3	2.8	2.5	26	0.6	228	16	22	<1	2.8	547	0.1	146	4.7	5.5	4.1	108	163
	1016♀	93	5.1	2.8	2.3	21	0.6	289	29	14	2	1.8	96	0.2	148	4.8	5.4	4.8	110	133
	Mean ♂	93	5.0	2.8	2.3	29	0.7	236	22	19	2	3.5	175	0.1	147	4.8	5.4	4.0	107	128
	Mean ♀	94	5.2	2.9	2.3	27	0.6	260	25	21	<2	3.3	243	0.2	147	4.8	5.5	4.5	109	138
	Mean	93	5.1	2.8	2.3	28	0.7	248	23	20	<2	3.4	209	0.2	147	4.8	5.4	4.2	108	133
	SD ♂	1.3	0.18	0.13	0.17	4.4	0.05	14.0	3.4	1.4	0.5	1.51	104.5	0.05	1.0	0.26	0.10	0.33	1.0	26.5
	SD ♀	2.9	0.12	0.15	0.13	6.3	0.05	38.9	5.9	5.0		2.47	206.5	0.05	1.0	0.17	0.14	0.36	3.4	20.0
	SD	2.1	0.18	0.15	0.15	5.1	0.05	29.9	4.7	3.6		1.90	155.8	0.05	0.9	0.21	0.13	0.42	2.4	22.4
100	1017♂	95	5.4	2.7	2.7	24	0.7	200	20	21	2	1.6	104	0.1	148	4.6	5.4	4.0	109	134
	1019♂	91	4.7	2.7	2.0	34	0.7	249	23	35	2	2.7	103	0.2	147	5.0	5.4	4.7	108	169
	1021♂	94	5.2	2.6	2.6	26	0.7	269	30	11	2	4.6	168	0.1	149	4.9	5.6	3.8	109	153
	1023♂	89	5.0	2.6	2.4	30	0.7	324	36	18	3	5.3	104	0.1	149	4.7	5.3	4.6	111	183
	1018♀	89	4.7	2.7	2.0	26	0.6	283	25	19	2	3.6	119	0.1	148	4.6	5.4	4.2	109	106
	1020♀	88	5.3	2.6	2.7	24	0.6	210	24	17	2	2.8	126	0.1	147	4.3	5.5	3.7	107	190
	1022♀	89	5.7	2.9	2.8	30	0.7	299	32	15	2	4.1	95	0.1	147	4.8	5.5	4.4	107	155
	1024♀	90	5.2	2.7	2.5	39	0.7	235	21	19	2	3.1	114	0.2	150	4.5	5.6	4.1	111	125
	Mean ♂	92	5.1	2.7	2.4	29	0.7	261	27	21	2	3.6	120	0.1	148	4.8	5.4	4.3	109	160
	Mean ♀	89	5.2	2.7	2.5	30	0.7	257	26	18	2	3.4	114	0.1	148	4.6	5.5	4.1	109	147
	Mean	91	5.2	2.7	2.5	29	0.7	259	26	19	2	3.5	117	0.1	148	4.7	5.5	4.2	109	153
	SD ♂	2.8	0.30	0.06	0.31	4.4	0.00	51.3	7.2	10.1	0.5	1.70	32.2	0.05	1.0	0.18	0.13	0.44	1.3	21.1
	SD ♀	0.8	0.41	0.13	0.36	6.7	0.06	41.4	4.7	1.9	0.0	0.57	13.3	0.05	1.4	0.21	0.08	0.29	1.9	38.0
	SD	2.6	0.34	0.10	0.31	5.3	0.05	43.2	5.7	7.0	0.4	1.18	23.0	0.05	1.1	0.23	0.11	0.36	1.6	29.3
Repeat investigations (3 July 1990)																				
Control	1015♂												206							
	1014♀												103							
100	1019♂									14										

SD Standard deviation

APPENDIX 5c

(Biochemistry - continued)

Week 6 (27 June 1990)

Diet concn. ppm	Dog no.	Glucose mg/dl	Protein g/dl Total	Alb	Glob	Urea mg/dl	Creatinine mg/dl	AP mU/ml	GPT mU/ml	GOT mU/ml	γGT mU/ml	OCT mU/ml	CPK mU/ml	Bilirubin mg/dl	Na mEq/l	K mEq/l	Ca mEq/l	P mEq/l	Cl mEq/l	Chol mg/dl
300	1025♂	102	5.3	2.8	2.5	24	0.6	339	25	21	2	5.3	202	0.2	149	5.4	5.4	4.7	108	177
	1027♂	93	5.4	2.7	2.7	26	0.7	242	24	12	2	2.1	92	0.2	149	4.6	5.5	4.2	111	169
	1029♂	93	4.8	2.5	2.3	24	0.6	302	20	15	2	3.4	109	0.1	148	4.6	5.2	3.9	110	132
	1031♂	96	4.9	2.6	2.3	26	0.6	294	21	13	2	2.6	83	0.1	147	4.6	5.3	3.9	108	204
	1026♀	96	4.6	2.4	2.2	24	0.7	231	18	15	2	1.1	100	0.1	148	4.7	5.3	4.4	111	116
	1028♀	91	5.3	2.9	2.4	36	0.6	324	22	14	2	3.1	75	0.3	150	5.2	5.7	4.9	108	175
	1030♀	87	5.2	2.6	2.6	28	0.6	196	17	18	2	2.8	103	0.2	148	4.3	5.4	4.0	109	151
	1032♀	96	5.5	2.9	2.6	24	0.6	328	15	15	2	8.7	91	0.2	150	5.1	5.6	4.1	108	181
	Mean ♂	96	5.1	2.7	2.5	25	0.6	294	23	15	2	3.4	122	0.2	148	4.8	5.4	4.2	109	171
	Mean ♀	93	5.2	2.7	2.5	28	0.6	270	18	16	2	3.9	92	0.2	149	4.8	5.5	4.4	109	156
	Mean	94	5.1	2.7	2.5	27	0.6	282	20	15	2	3.6	107	0.2	149	4.8	5.4	4.3	109	163
	SD ♂	4.2	0.29	0.13	0.19	1.2	0.05	40.0	2.4	4.0	0.0	1.41	54.7	0.06	1.0	0.40	0.13	0.38	1.5	29.7
	SD ♀	4.4	0.39	0.24	0.19	5.7	0.05	66.5	2.9	1.7	0.0	3.30	12.6	0.08	1.2	0.41	0.18	0.40	1.4	29.5
	SD	4.4	0.32	0.18	0.18	4.1	0.05	52.5	3.5	2.9	0.0	2.37	40.0	0.07	1.1	0.38	0.17	0.37	1.4	28.5
1000	1033♂	88	5.1	2.1	3.0	30	0.4	350	14	18	2	1.0	65	0.1	147	4.8	5.1	4.2	107	149
	1037♂	88	5.3	2.6	2.7	43	0.7	194	19	14	2	5.1	76	0.2	149	4.5	5.5	4.0	110	187
	1039♂	99	4.7	2.4	2.3	28	0.6	254	15	13	2	4.1	82	0.1	147	4.8	5.2	3.9	108	174
	1034♀	94	5.1	2.5	2.6	24	0.7	470	15	21	1	3.9	143	0.1	150	4.1	5.3	4.1	110	208
	1036♀	93	5.8	2.8	3.0	21	0.7	1229	235	47	4	37.7	158	0.5	149	5.3	5.6	4.4	107	231
	1038♀	88	5.1	2.6	2.5	26	0.7	272	13	13	1	4.6	75	0.1	149	4.8	5.5	4.4	109	167
	1040♀	93	5.1	2.6	2.5	28	0.6	287	26	16	1	3.1	75	0.2	148	4.7	5.4	4.8	109	233
	Mean ♂	92	5.0	2.4	2.7	34	0.6	266	16	15	2	3.4	74	0.1	148	4.7	5.3	4.0	108	170
	Mean ♀	92	5.3	2.6	2.7	25	0.7	565	72	24	2	12.3	113	0.2	149	4.7	5.5	4.4	109	210
	Mean	92	5.2	2.5	2.7	29	0.6	437	48	20	2	8.5	96	0.2	148	4.7	5.4	4.3	109	193
	SD ♂	6.4	0.31	0.25	0.35	8.1	0.15	78.7	2.6	2.6	0.0	2.14	8.6	0.06	1.2	0.17	0.21	0.15	1.5	19.3
	SD ♀	2.7	0.35	0.13	0.24	3.0	0.05	452.1	108.7	15.5	1.5	16.93	44.0	0.19	0.8	0.49	0.13	0.29	1.3	30.7
	SD	4.1	0.33	0.22	0.26	7.0	0.11	360.1	82.5	12.1	1.1	12.94	37.6	0.15	1.1	0.36	0.18	0.30	1.3	32.3
Repeat investigations (3 July 1990)																				
1000	1037♂					41														
	1034♀							357												
	1036♀							875	125	26		18.5		0.1						

SD Standard deviation

APPENDIX 5d

Biochemistry - individual values

Week 13 (15 August 1990)

Diet concn. ppm	Dog no.	Glucose mg/dl	Protein g/dl Total	Protein g/dl Alb	Protein g/dl Glob	Urea mg/dl	Creatinine mg/dl	AP mU/ml	GPT mU/ml	GOT mU/ml	γGT mU/ml	OCT mU/ml	CPK mU/ml	Bilirubin mg/dl	Na mEq/l	K mEq/l	Ca mEq/l	P mEq/l	Cl mEq/l	Chol mg/dl
Control	1009♂	87	5.0	2.7	2.3	28	0.8	153	22	18	2	2.0	118	0.1	147	4.8	5.5	3.5	109	104
	1011♂	102	5.1	2.9	2.2	28	0.7	166	26	23	2	4.7	113	0.1	145	4.4	5.2	3.8	107	148
	1013♂	94	5.0	2.7	2.3	26	0.7	164	18	18	2	8.9	129	0.1	146	4.5	5.3	3.2	109	108
	1015♂	96	5.3	3.0	2.3	30	0.7	190	26	20	2	4.6	90	0.1	146	4.8	5.2	3.3	108	127
	1010♀	101	4.7	2.8	1.9	26	0.8	228	29	24	2	2.8	172	0.2	144	4.3	5.1	3.2	106	119
	1012♀	100	5.3	2.8	2.5	36	0.8	164	35	24	2	9.4	178	0.2	146	4.6	5.2	4.4	109	95
	1014♀	92	5.2	2.8	2.4	32	0.8	197	16	12	2	2.9	82	0.2	145	4.5	5.4	3.5	109	152
	1016♀	96	5.3	3.0	2.3	28	0.7	235	33	23	2	5.9	120	0.2	145	5.0	5.1	4.2	110	129
	Mean ♂	95	5.1	2.8	2.3	28	0.7	168	23	20	2	5.1	113	0.1	146	4.6	5.3	3.5	108	122
	Mean ♀	97	5.1	2.9	2.3	31	0.8	206	28	21	2	5.3	138	0.2	145	4.6	5.2	3.8	109	124
	Mean	96	5.1	2.8	2.3	29	0.8	187	26	20	2	5.2	125	0.2	146	4.6	5.3	3.6	108	123
	SD ♂	6.2	0.14	0.15	0.05	1.6	0.05	15.6	3.8	2.4	0.0	2.85	16.4	0.00	0.8	0.21	0.14	0.26	1.0	20.2
	SD ♀	4.1	0.29	0.10	0.26	4.4	0.05	32.5	8.5	5.9	0.0	3.12	45.5	0.00	0.8	0.29	0.14	0.57	1.7	23.6
	SD	5.0	0.21	0.12	0.18	3.4	0.05	31.1	6.7	4.2	0.0	2.77	34.5	0.05	0.9	0.24	0.14	0.46	1.3	20.4
100	1017♂	97	5.1	2.7	2.4	28	0.8	139	23	21	2	9.8	131	0.1	145	4.2	5.2	3.6	109	140
	1019♂	94	4.7	2.4	2.3	30	0.7	172	33	21	2	11.4	112	0.2	145	4.5	5.1	4.1	111	163
	1021♂	90	5.5	2.5	3.0	26	0.7	239	25	14	2	2.3	100	0.1	145	4.7	5.2	3.8	108	167
	1023♂	90	4.8	2.5	2.3	32	0.7	233	35	17	2	5.0	96	0.1	146	4.4	5.1	4.2	110	163
	1018♀	91	4.8	2.4	2.4	32	0.8	215	24	18	2	2.8	105	0.1	145	4.3	5.2	3.3	110	103
	1020♀	94	5.4	2.7	2.7	26	0.7	176	28	15	2	6.0	71	0.1	145	4.1	5.3	3.6	108	166
	1022♀	91	5.2	2.5	2.7	24	0.7	204	21	18	2	10.1	99	0.1	145	4.5	5.2	2.9	109	156
	1024♀	92	4.9	2.5	2.4	32	0.8	150	24	22	2	6.0	103	0.2	148	4.5	5.3	3.6	112	124
	Mean ♂	93	5.0	2.5	2.5	29	0.7	196	29	18	2	7.1	110	0.1	145	4.5	5.2	3.9	110	158
	Mean ♀	92	5.1	2.5	2.6	29	0.8	186	24	18	2	6.2	95	0.1	146	4.4	5.3	3.4	110	137
	Mean	92	5.1	2.5	2.5	29	0.7	191	27	18	2	6.7	102	0.1	146	4.4	5.2	3.6	110	148
	SD ♂	3.4	0.36	0.13	0.34	2.6	0.05	48.5	5.9	3.4	0.0	4.21	15.7	0.05	0.5	0.21	0.06	0.28	1.3	12.3
	SD ♀	1.4	0.28	0.13	0.17	4.1	0.06	29.2	2.9	2.9	0.0	2.99	15.9	0.05	1.5	0.19	0.06	0.33	1.7	29.0
	SD	2.4	0.30	0.12	0.25	3.2	0.05	37.4	5.0	2.9	0.0	3.42	16.7	0.05	1.1	0.19	0.08	0.42	1.4	23.5

SD Standard deviation

APPENDIX 5d

(Biochemistry - continued)

Week 13 (15 August 1990)

Diet concn. ppm	Dog no.	Glucose mg/dl	Protein g/dl Total	Alb	Glob	Urea mg/dl	Creatinine mg/dl	AP mU/ml	GPT mU/ml	GOT mU/ml	γGT mU/ml	OCT mU/ml	CPK mU/ml	Bilirubin mg/dl	Na mEq/l	K mEq/l	Ca mEq/l	P mEq/l	Cl mEq/l	Chol mg/dl
300	1025♂	103	5.0	2.5	2.5	19	0.7	221	27	24	2	3.6	153	0.1	147	4.3	5.1	3.5	108	174
	1027♂	95	5.1	2.3	2.8	19	0.6	192	30	16	2	9.4	100	0.1	146	4.4	5.0	3.2	113	174
	1029♂	94	4.8	2.4	2.4	26	0.6	229	22	23	2	3.1	101	0.1	146	4.5	4.9	3.8	110	124
	1031♂	93	4.8	2.4	2.4	28	0.6	202	19	17	2	7.0	87	0.1	146	4.5	5.2	4.0	109	189
	1026♀	91	4.5	2.2	2.3	30	0.9	177	19	18	2	9.1	114	0.1	148	4.8	5.2	3.7	111	122
	1028♀	86	5.1	2.5	2.6	30	0.6	239	21	17	2	8.3	87	0.2	145	4.4	5.1	4.2	110	158
	1030♀	91	5.1	2.5	2.6	26	0.7	158	18	20	2	4.2	100	0.2	146	4.2	5.2	3.5	110	154
	1032♀	91	5.5	2.9	2.6	26	0.8	242	18	34	2	5.7	774	0.2	147	4.6	5.2	3.1	108	164
	Mean ♂	96	4.9	2.4	2.5	23	0.6	211	25	20	2	5.8	110	0.1	146	4.4	5.1	3.6	110	165
	Mean ♀	90	5.1	2.5	2.5	28	0.8	204	19	22	2	6.8	269	0.2	147	4.5	5.2	3.6	110	150
	Mean	93	5.0	2.5	2.5	26	0.7	208	22	21	2	6.3	190	0.1	146	4.5	5.1	3.6	110	157
	SD ♂	4.6	0.15	0.08	0.19	4.7	0.05	17.0	4.9	4.1	0.0	2.97	29.2	0.00	0.5	0.10	0.13	0.35	2.2	28.4
	SD ♀	2.5	0.41	0.29	0.15	2.3	0.13	42.9	1.4	7.9	0.0	2.27	337.0	0.05	1.3	0.26	0.05	0.46	1.3	18.8
	SD	4.9	0.29	0.21	0.16	4.3	0.11	30.4	4.5	6.0	0.0	2.51	237.1	0.05	0.9	0.18	0.11	0.38	1.6	23.8
1000	1033♂	99	4.7	2.1	2.6	30	0.5	253	15	22	2	5.9	91	0.1	145	4.1	5.0	3.7	107	152
	1037♂	85	5.1	2.4	2.7	36	0.8	142	13	12	1	4.7	66	0.1	148	4.8	5.2	3.5	111	196
	1039♂	97	4.9	2.4	2.5	26	0.5	217	13	10	2	5.7	83	0.1	145	4.4	5.2	4.0	107	244
	1034♀	98	5.2	2.4	2.8	24	0.7	393	19	27	1	6.3	135	0.1	149	5.1	5.2	4.3	107	215
	1036♀	99	5.3	2.6	2.7	24	0.7	300	45	18	1	10.2	82	0.2	145	4.7	5.2	4.1	108	189
	1038♀	85	5.7	2.7	3.0	26	0.7	254	16	16	2	2.4	63	0.2	146	4.1	5.3	4.7	108	177
	1040♀	98	4.7	2.4	2.3	30	0.6	229	28	18	1	2.0	73	0.2	147	4.3	5.1	4.1	110	217
	Mean ♂	94	4.9	2.3	2.6	31	0.6	204	14	15	2	5.4	80	0.1	146	4.4	5.1	3.7	108	197
	Mean ♀	95	5.2	2.5	2.7	26	0.7	294	27	20	1	5.2	88	0.2	147	4.6	5.2	4.3	108	200
	Mean	94	5.1	2.4	2.7	28	0.6	255	21	18	1	5.3	85	0.1	146	4.5	5.2	4.1	108	199
	SD ♂	7.6	0.20	0.17	0.10	5.0	0.17	56.6	1.2	6.4	0.6	0.64	12.8	0.00	1.7	0.35	0.12	0.25	2.3	46.0
	SD ♀	6.7	0.41	0.15	0.29	2.8	0.05	72.3	13.0	4.9	0.5	3.84	32.1	0.05	1.7	0.44	0.08	0.28	1.3	19.7
	SD	6.5	0.36	0.19	0.22	4.3	0.11	77.4	11.7	5.8	0.5	2.74	24.3	0.05	1.6	0.38	0.10	0.39	1.6	30.0
Repeat investigation (17 August 1990)																				
300	1032♀									27			222							

SD Standard deviation

APPENDIX 6a

Urinalysis - individual values

Before dosing commenced (4 May 1990)

Diet concn. ppm	Dog no.	Volume ml	pH	SG	Protein mg/dl	TRS	Glucose	Ketones	Bile pigments	Urobilinogen	Haem pigments	Microscopy E	P	M	R	O	C	A
Control	1009♂	34	5.6	1042	58	0	0	0	0	0	+	1	1	0	1	1	0	0
	1011♂	96	6.3	1039	33	0	0	0	0	0	+	1	1	0	1	1	0	0
	1013♂	78	5.4	1040	32	0	0	0	0	0	+	1	1	0	1	1	0	0
	1015♂	25	5.8	1035	26	0	0	0	0	0	+	0	0	0	1	1	0	0
	1010♀	302	5.6	1022	20	0	0	0	0	0	0	1	1	0	0	1	0	0
	1012♀	200	5.8	1026	21	0	0	0	0	0	0	1	1	0	0	1	0	0
	1014♀	340	5.6	1019	10	0	0	0	0	0	0	1	1	0	0	1	0	0
	1016♀	81	5.9	1033	38	0	0	0	0	0	+	1	1	0	1	1	0	0
	Mean ♂	58	5.8	1039	37													
	Mean ♀	231	5.7	1025	22													
	Mean	145	5.8	1032	30													
	SD ♂	34.2	0.39	2.9	14.2													
	SD ♀	116.0	0.15	6.1	11.6													
	SD	121.5	0.27	8.7	14.4													
100	1017♂	60	5.5	1040	40	0	0	0	0	0	0	1	1	0	0	1	0	0
	1019♂	26	5.9	1041	46	0	0	0	0	0	+	1	1	0	1	1	0	0
	1021♂	106	5.5	1037	70	0	0	0	0	0	+	1	1	0	1	2	0	0
	1023♂	88	5.3	1038	39	0	0	0	0	0	0	1	1	0	0	1	0	0
	1018♀	16	6.3	1047	37	0	0	0	0	0	+	1	1	0	2	1	0	0
	1020♀	230	5.6	1023	31	0	0	0	0	0	0	1	1	0	0	2	0	0
	1022♀	150	5.8	1031	19	0	0	0	0	0	0	1	1	0	0	2	0	0
	1024♀	76	5.5	1046	32	0	0	0	0	0	0	1	1	0	0	1	0	0
	Mean ♂	70	5.6	1039	49													
	Mean ♀	118	5.8	1037	30													
	Mean	94	5.7	1038	39													
	SD ♂	34.9	0.25	1.8	14.5													
	SD ♀	92.6	0.36	11.7	7.6													
	SD	69.7	0.32	7.9	14.8													
Repeat investigations (15 May 1990)																		
Control	1009♂				47													
100	1021♂				32													

SD Standard deviation

APPENDIX 6a

(Urinalysis - continued)

Before dosing commenced (4 May 1990)

Diet concn. ppm	Dog no.	Volume ml	pH	SG	Protein mg/dl	TRS	Glucose	Ketones	Bile pigments	Urobilinogen	Haem pigments	Microscopy E	P	M	R	O	C	A
300	1025♂	118	5.8	1037	39	0	0	0	0	0	+	1	1	0	1	2	0	0
	1027♂	78	6.0	1038	40	0	0	0	0	0	+	1	1	0	1	1	0	0
	1029♂	100	5.8	1038	30	0	0	0	0	0	0	1	1	0	0	1	0	0
	1031♂	126	5.9	1039	26	0	0	0	0	0	0	1	1	0	0	1	0	0
	1026♀	190	5.4	1031	56	0	0	0	0	0	+	1	1	0	1	2	0	0
	1028♀	160	5.3	1024	28	0	0	0	0	0	+	1	1	0	1	1	0	0
	1030♀	128	5.7	1037	85	0	0	0	0	0	+	1	1	0	1	2	0	0
	1032♀	360	6.0	1018	15	0	0	0	0	0	0	1	1	0	0	1	0	0
	Mean ♂	106	5.9	1038	34													
	Mean ♀	210	5.6	1028	46													
	Mean	158	5.7	1033	40													
	SD ♂	21.3	0.10	0.8	6.8													
	SD ♀	103.5	0.32	8.3	31.1													
	SD	88.7	0.26	7.8	21.9													
1000	1033♂	60	5.1	1041	44	0	0	0	0	0	+	1	1	0	1	2	0	0
	1035♂	102	5.2	1036	43	0	0	0	0	0	+	1	1	0	1	1	0	0
	1037♂	108	5.3	1035	35	0	0	0	0	0	0	1	1	0	0	2	0	0
	1039♂	74	5.6	1042	33	0	0	0	0	0	0	1	1	0	0	1	0	0
	1034♀	120	5.7	1020	25	0	0	0	0	0	+	1	1	0	1	1	0	0
	1036♀	400	6.2	1015	15	0	0	0	0	0	0	1	1	0	0	1	0	0
	1038♀	98	5.2	1042	37	0	0	0	0	0	+	1	1	0	1	1	0	0
	1040♀	170	5.5	1033	27	0	0	0	0	0	0	1	1	0	0	1	0	0
	Mean ♂	86	5.3	1039	39													
	Mean ♀	197	5.7	1028	26													
	Mean	142	5.5	1033	32													
	SD ♂	22.8	0.22	3.5	5.6													
	SD ♀	138.6	0.42	12.3	9.0													
	SD	109.5	0.36	10.2	9.7													
Repeat investigations (15 May 1990)																		
300	1026♀				25													
	1030♀				39													

SD Standard deviation

APPENDIX 6b

Urinalysis - pre-terminal data for Dog 1035♂ (Day 6 of Week 5; 25 June 1990)

Diet concn. ppm	Volume ml	pH	SG	Protein mg/dl	TRS	Glucose	Ketones	Bile pigments	Urobilinogen	Haem pigments	Microscopy						
											E	P	M	R	O	C	A
1000	* 10	5.7	1074	116	TR	0	0	0	0	0	0	1	0	0	1	0	0

TR Trace
* Sample taken from bladder at post mortem

APPENDIX 6c

Urinalysis - individual values

Week 6 (28 June 1990)

Diet concn. ppm	Dog no.	Volume ml	pH	SG	Protein mg/dl	TRS	Glucose	Ketones	Bile pigments	Urobilinogen	Haem pigments	Microscopy E	P	M	R	O	C	A
Control	1009♂	172	5.9	1034	32	0	0	0	0	0	0	1	1	0	0	1	0	0
	1011♂	100	7.9	1041	30	0	0	0	0	0	0	1	1	0	0	1	0	0
	1013♂	52	5.5	1042	31	0	0	0	0	0	0	1	1	0	0	1	0	0
	1015♂	124	6.7	1034	23	0	0	0	0	0	0	1	1	0	0	1	0	0
	1010♀	144	5.9	1037	36	0	0	0	0	0	0	1	1	0	0	1	0	0
	1012♀	320	6.3	1024	18	0	0	0	0	0	0	1	1	0	0	1	0	0
	1014♀	224	6.2	1033	27	0	0	0	0	0	0	1	1	0	0	1	0	0
	1016♀	310	6.0	1024	17	0	0	0	0	0	0	1	1	0	0	2	0	0
	Mean ♂	112	6.5	1038	29													
	Mean ♀	250	6.1	1030	25													
	Mean	181	6.3	1034	27													
	SD ♂	50.0	1.06	4.3	4.1													
	SD ♀	82.5	0.18	6.6	8.9													
	SD	96.9	0.73	6.8	6.8													
100	1017♂	124	5.7	1037	22	0	0	0	0	0	+	1	1	0	0	1	0	0
	1019♂	72	6.3	1031	24	0	0	0	0	0	+	1	1	0	1	2	0	0
	1021♂	64	5.8	1019	22	0	0	0	0	0	+	1	1	0	1	1	0	0
	1023♂	390	5.1	1016	12	0	0	0	0	0	0	1	1	0	0	1	0	0
	1018♀	176	5.7	1036	28	0	0	0	0	0	0	1	1	0	0	1	0	0
	1020♀	158	6.1	1033	21	0	0	0	0	0	0	1	1	0	0	1	0	0
	1022♀	480	6.5	1017	12	0	0	0	0	0	0	1	1	0	0	1	0	0
	1024♀	220	5.2	1024	18	0	0	0	0	0	0	1	1	0	0	1	0	0
	Mean ♂	163	5.7	1026	20													
	Mean ♀	259	5.9	1028	20													
	Mean	211	5.8	1027	20													
	SD ♂	154.0	0.49	9.9	5.4													
	SD ♀	149.9	0.56	8.7	6.7													
	SD	149.8	0.49	8.7	5.6													

SD Standard deviation

APPENDIX 6c

(Urinalysis - continued)

Week 6 (28 June 1990)

Diet concn. ppm	Dog no.	Volume ml	pH	SG	Protein mg/dl	TRS	Glucose	Ketones	Bile pigments	Urobilinogen	Haem pigments	Microscopy E	P	M	R	O	C	A
300	1025♂	204	6.3	1031	20	0	0	0	0	0	0	1	1	0	0	1	0	0
	1027♂	470	5.0	1013	8	0	0	0	0	0	0	1	1	0	0	1	0	0
	1029♂	232	5.8	1019	20	0	0	0	0	0	0	1	1	0	0	1	0	0
	1031♂	380	5.7	1017	11	0	0	0	0	0	0	1	1	0	0	1	0	0
	1026♀	158	5.3	1031	23	0	0	0	0	0	0	1	1	0	0	1	0	0
	1028♀	200	6.0	1019	25	0	0	0	0	0	+	1	1	0	1	1	0	0
	1030♀	176	5.2	1030	21	0	0	0	0	0	0	1	1	0	0	1	0	0
	1032♀	240	6.8	1026	17	0	0	0	0	0	0	1	1	0	0	1	0	0
	Mean ♂	322	5.7	1020	15													
	Mean ♀	194	5.8	1027	22													
	Mean	258	5.8	1023	18													
	SD ♂	125.6	0.54	7.7	6.2													
	SD ♀	35.5	0.74	5.4	3.4													
	SD	109.4	0.60	7.1	5.9													
1000	1033♂	218	6.0	1023	17	0	0	0	0	0	0	1	1	0	1	1	0	0
	1037♂	320	5.4	1019	13	0	0	0	0	0	0	1	1	0	0	1	0	0
	1039♂	38	5.7	1048	34	0	0	0	0	0	0	1	1	0	1	1	0	0
	1034♀	320	6.4	1023	19	0	0	0	0	0	0	1	1	0	0	1	0	0
	1036♀	42	6.3	1042	42	0	0	0	0	0	+	1	1	0	1	1	0	0
	1038♀	104	5.7	1038	22	0	0	0	0	0	0	1	1	0	0	1	0	0
	1040♀	172	5.7	1035	23	0	0	0	0	0	0	1	1	0	0	1	0	0
	Mean ♂	192	5.7	1030	21													
	Mean ♀	160	6.0	1035	27													
	Mean	173	5.9	1033	24													
	SD ♂	142.8	0.30	15.7	11.2													
	SD ♀	119.4	0.38	8.2	10.5													
	SD	119.3	0.36	11.0	10.2													

SD Standard deviation

APPENDIX 6d

Urinalysis - individual values

Week 13 (16 August 1990)

Diet concn. ppm	Dog no.	Volume ml	pH	SG	Protein mg/dl	TRS	Glucose	Ketones	Bile pigments	Urobilinogen	Haem pigments	Microscopy E	P	M	R	O	C	A
Control	1009♂	186	6.6	1032	45	0	0	0	0	0	0	0	0	0	0	1	0	0
	1011♂	86	6.4	1037	19	0	0	0	0	0	0	1	0	0	0	1	0	0
	1013♂	74	5.7	1038	30	0	0	0	0	0	0	0	1	0	0	1	0	0
	1015♂	212	6.5	1039	31	0	0	0	0	0	0	1	0	0	0	1	0	0
	1010♀	122	6.7	1039	19	0	0	0	0	0	0	1	1	0	0	1	0	0
	1012♀	174	5.9	1035	27	0	0	0	0	0	0	1	1	0	0	1	0	0
	1014♀	178	6.1	1041	31	0	0	0	0	0	0	0	0	0	0	1	0	0
	1016♀	140	6.6	1038	34	0	0	0	0	0	0	0	0	0	0	1	0	0
	Mean ♂	140	6.3	1037	31													
	Mean ♀	154	6.3	1038	28													
	Mean	147	6.3	1037	30													
	SD ♂	69.7	0.41	3.1	10.7													
	SD ♀	27.0	0.39	2.5	6.5													
	SD	49.5	0.37	2.8	8.4													
100	1017♂	118	6.6	1044	51	0	0	0	0	0	0	1	1	0	0	1	0	0
	1019♂	130	6.0	1035	24	0	0	0	0	0	0	0	0	0	0	1	0	0
	1021♂	400	5.8	1022	25	0	0	0	0	0	0	0	2	0	0	1	0	0
	1023♂	100	5.9	1037	47	0	0	0	0	0	0	1	1	0	0	1	0	0
	1018♀	124	6.0	1035	30	0	0	0	0	0	0	0	1	0	0	1	0	0
	1020♀	234	6.3	1031	20	0	0	0	0	0	0	1	0	0	0	1	0	0
	1022♀	340	6.1	1025	17	0	0	0	0	0	0	0	0	0	0	1	0	0
	1024♀	194	6.1	1040	60	0	0	0	0	0	+	1	0	0	1	1	0	0
	Mean ♂	187	6.1	1035	37													
	Mean ♀	223	6.1	1033	32													
	Mean	205	6.1	1034	34													
	SD ♂	142.5	0.36	9.2	14.2													
	SD ♀	90.3	0.13	6.3	19.6													
	SD	112.1	0.25	7.4	16.1													

SD Standard deviation

APPENDIX 6d

(Urinalysis - continued)

Week 13 (16 August 1990)

Diet concn. ppm	Dog no.	Volume ml	pH	SG	Protein mg/dl	TRS	Glucose	Ketones	Bile pigments	Urobilinogen	Haem pigments	Microscopy E	P	M	R	O	C	A
300	1025♂	58	5.2	1013	135	0	0	0	0	0	+	1	0	0	1	1	0	0
	1027♂	100	5.9	1037	29	0	0	0	0	0	0	1	1	0	0	1	0	0
	1029♂	30	6.8	1040	32	0	0	0	0	0	0	2	0	0	0	1	0	0
	1031♂	138	6.8	1036	31	0	0	0	0	0	0	1	1	0	0	1	0	0
	1026♀	116	6.1	1041	33	0	0	0	0	0	0	0	1	0	0	1	0	0
	1028♀	226	5.8	1028	21	0	0	0	0	0	0	1	2	0	0	1	0	0
	1030♀	200	7.0	1032	118	0	0	0	0	0	+	0	0	0	1	1	0	0
	1032♀	242	6.4	1030	23	0	0	0	0	0	0	0	0	0	0	1	0	0
	Mean ♂	82	6.2	1032	57													
	Mean ♀	196	6.3	1033	49													
	Mean	139	6.3	1032	53													
	SD ♂	47.4	0.78	12.4	52.2													
	SD ♀	56.1	0.51	5.7	46.5													
	SD	77.8	0.61	9.0	45.9													
1000	1033♂	26	6.9	1036	27	0	0	0	0	0	0	1	0	0	0	1	0	0
	1037♂	218	6.5	1026	63	0	0	0	0	0	+	0	0	0	1	1	0	0
	1039♂	172	6.4	1036	29	0	0	0	0	0	0	1	1	0	0	1	0	0
	1034♀	176	6.9	1028	19	0	0	0	0	0	0	1	0	0	0	1	0	0
	1036♀	100	6.4	1039	24	0	0	0	0	0	0	0	1	0	0	1	0	0
	1038♀	144	5.8	1037	27	0	0	0	0	0	0	1	0	0	0	1	0	0
	1040♀	136	6.4	1036	18	0	0	0	0	0	0	0	1	0	0	1	0	0
	Mean ♂	139	6.6	1033	40													
	Mean ♀	139	6.4	1035	22													
	Mean	139	6.5	1034	30													
	SD ♂	100.2	0.26	5.8	20.2													
	SD ♀	31.2	0.45	4.8	4.2													
	SD	61.9	0.37	4.9	15.3													

SD Standard deviation

APPENDIX 7

Absolute organ weights - individual values

Diet concn. ppm	Dog no.	Body wt. kg	Brain g	Pitu-itary mg	Heart g	Lungs g	Liver g	Spleen g	Panc-reas g	Thymus g
Control	1009♂	10.8	70.5	67	79.9	83.9	374.8	55.7	15.8	18.2
	1011♂	11.3	90.1	52	79.2	86.6	345.2	66.3	24.7	13.3
	1013♂	11.2	85.2	75	70.3	96.8	352.9	67.1	24.3	13.4
	1015♂	11.2	81.1	79	80.4	96.0	424.4	63.4	16.9	13.7
	1010♀	10.7	76.7	76	81.9	94.1	328.5	93.3	26.3	22.3
	1012♀	8.9	71.3	74	68.8	89.8	307.2	43.0	19.9	9.2
	1014♀	10.0	77.6	76	74.0	91.2	355.7	61.5	20.2	12.9
	1016♀	10.0	73.7	65	69.6	106.9	333.5	76.7	18.3	8.4
	Mean ♂	11.1	81.7	68	77.5	90.8	374.3	63.1	20.4	14.7
	Mean ♀	9.9	74.8	73	73.6	95.5	331.2	68.6	21.2	13.2
	Mean	10.5	78.3	71	75.5	93.2	352.8	65.9	20.8	13.9
100	1017♂	11.2	88.1	69	80.9	98.1	328.6	58.6	22.9	22.3
	1019♂	11.3	79.3	71	78.9	102.7	404.6	51.3	27.1	19.5
	1021♂	10.7	79.9	67	80.3	94.9	389.5	45.1	21.8	11.5
	1023♂	11.2	67.9	66	74.0	97.3	370.2	47.3	23.3	19.5
	1018♀	9.0	71.3	64	74.3	95.2	290.7	70.7	27.5	15.5
	1020♀	9.6	83.5	83	66.5	77.8	404.3	74.8	18.2	12.0
	1022♀	10.7	71.8	82	67.9	88.6	360.7	84.8	30.4	23.2
	1024♀	11.0	78.3	71	66.2	85.9	387.3	114.7	7.2	18.0
	Mean ♂	11.1	78.8	68	78.5	98.3	373.2	50.6	23.8	18.2
	Mean ♀	10.1	76.2	75	68.7	86.9	360.8	86.3	20.8	17.2
	Mean	10.6	77.5	72	73.6	92.6	367.0	68.4	22.3	17.7

APPENDIX 7

(Absolute organ weights - continued)

Diet concn. ppm	Dog no.	Prostate /Uterus g	Kidneys R g	Kidneys L g	Thyroids R g	Thyroids L g	Adrenals R g	Adrenals L g	Gonads R g	Gonads L g
Control	1009♂	2.93	32.2	24.9	0.39	0.43	0.65	0.86	7.34	7.65
	1011♂	2.19	26.0	26.1	0.69	0.41	0.75	0.86	9.77	9.79
	1013♂	4.25	23.8	24.9	0.52	0.48	0.69	0.68	12.21	12.36
	1015♂	2.44	27.9	29.3	0.66	0.53	0.86	0.76	1.17	1.43
	1010♀	4.55	23.2	23.6	0.38	0.36	0.75	0.54	0.51	0.65
	1012♀	3.37	30.1	16.5	0.49	0.39	0.64	0.69	0.52	0.59
	1014♀	4.24	25.0	24.9	0.65	0.55	0.83	0.68	0.44	0.49
	1016♀	3.17	27.3	28.6	0.43	0.40	0.82	0.62	0.40	0.45
	Mean ♂	2.95		53.8		1.03		1.53		15.43
	Mean ♀	3.83		49.8		0.91		1.39		1.01
	Mean			51.8		0.97		1.46		
100	1017♂	6.50	24.7	26.1	0.50	0.39	0.88	0.91	10.63	11.22
	1019♂	2.24	27.8	28.3	0.31	0.45	0.79	0.83	10.72	10.21
	1021♂	5.57	27.2	29.1	0.38	0.45	0.79	0.82	10.36	10.46
	1023♂	5.43	30.0	24.8	0.49	0.61	0.71	0.63	8.94	9.86
	1018♀	6.66	22.3	21.9	0.54	0.40	0.67	0.49	0.42	0.43
	1020♀	3.43	24.4	23.8	0.58	0.48	0.71	0.77	0.65	0.69
	1022♀	3.39	25.9	25.9	0.54	0.39	0.63	0.87	0.38	0.44
	1024♀	2.24	26.3	25.8	0.63	0.54	0.76	0.96	0.39	0.32
	Mean ♂	4.94		54.5		0.90		1.59		20.60
	Mean ♀	3.93		49.1		1.03		1.47		0.93
	Mean			51.8		0.96		1.53		

APPENDIX 7

(Absolute organ weights - continued)

Diet concn. ppm	Dog no.	Body wt. kg	Brain g	Pitu-itary mg	Heart g	Lungs g	Liver g	Spleen g	Panc-reas g	Thymus g
300	1025♂	10.7	78.3	66	85.6	82.3	397.1	61.6	25.9	13.6
	1027♂	10.6	72.9	74	81.6	100.8	424.0	38.1	19.1	10.3
	1029♂	11.5	83.7	67	80.5	87.1	367.4	79.5	23.2	19.4
	1031♂	11.7	89.8	79	80.1	101.4	453.7	63.3	31.4	17.1
	1026♀	8.9	76.9	50	77.7	76.0	320.6	76.2	27.8	11.9
	1028♀	10.5	80.3	76	67.6	84.9	403.8	100.0	10.2	22.1
	1030♀	9.9	77.8	72	66.9	88.9	339.3	60.1	22.9	18.0
	1032♀	11.9	76.5	78	80.0	98.7	398.5	74.9	26.9	21.2
	Mean ♂	11.1	81.2	72	82.0	92.9	410.6	60.6	24.9	15.1
	Mean ♀	10.3	77.9	69	73.1	87.1	365.6	77.8	22.0	18.3
	Mean	10.7	79.5	70	77.5	90.0	388.1	69.2	23.4	16.7
1000	1033♂	7.0	67.2	55	55.2	58.9	334.4	69.1	26.2	9.8
	1035♂##	7.5	82.5	67	59.2	89.8	324.6	21.0	17.8	6.3
	1037♂	10.7	74.6	70	63.2	84.3	432.8	49.6	15.5	9.9
	1039♂	12.0	83.7	86	75.8	97.0	523.9	110.8	25.5	21.7
	1034♀	9.6	78.2	82	80.6	70.8	408.4	63.8	24.3	15.9
	1036♀	8.7	72.1	74	58.9	76.3	339.4	54.4	20.4	12.9
	1038♀	10.8	67.8	66	74.1	83.8	391.9	107.8	19.3	18.9
	1040♀	11.0	70.4	67	77.7	86.3	414.6	100.9	20.9	19.8
	Mean ♂	9.9	75.2	70	64.7	80.1	430.4	76.5	22.4	13.8
	Mean ♀	10.0	72.1	72	72.8	79.3	388.6	81.7	21.2	16.9
	Mean	10.0	73.4	71	69.4	79.6	406.5	79.5	21.7	15.6

Animal excluded from calculation of mean and statistical analysis - killed Day 6 of Week 5

APPENDIX 7

(Absolute organ weights - continued)

Diet concn. ppm	Dog no.	Prostate /Uterus g	Kidneys R g	Kidneys L g	Thyroids R g	Thyroids L g	Adrenals R g	Adrenals L g	Gonads R g	Gonads L g
300	1025♂	5.86	27.1	25.7	0.55	0.43	0.79	0.69	11.89	10.75
	1027♂	4.32	28.2	24.5	0.56	0.38	0.78	0.72	9.45	12.41
	1029♂	4.17	28.1	28.2	0.35	0.37	0.78	0.75	10.13	10.85
	1031♂	5.08	30.7	31.3	0.48	0.42	0.88	0.93	11.05	11.00
	1026♀	4.08	22.6	22.9	0.43	0.38	0.63	0.86	0.42	0.53
	1028♀	2.84	29.1	28.7	0.45	0.41	0.68	0.73	0.39	0.46
	1030♀	2.43	23.8	23.9	0.59	0.63	0.82	0.85	0.45	0.63
	1032♀	3.04	30.3	30.1	0.56	0.51	0.72	0.73	0.56	0.72
	Mean ♂	4.86		56.0		0.89		1.58		21.88
	Mean ♀	3.10		52.9		0.99		1.51		1.04
	Mean			54.4		0.94		1.54		
1000	1033♂	3.16	22.5	22.9	0.34	0.30	0.74	0.81	8.19	8.54
	1035♂##	1.07	21.0	20.8	0.35	0.26	0.55	0.52	2.67	3.14
	1037♂	5.17	25.2	27.4	0.63	0.62	0.72	0.83	9.91	12.33
	1039♂	5.35	31.7	31.5	0.59	0.60	0.87	0.89	11.03	10.77
	1034♀	3.39	25.3	25.1	0.48	0.48	0.79	0.85	0.31	0.46
	1036♀	5.89	23.1	23.6	0.39	0.44	0.59	0.60	0.43	0.62
	1038♀	2.70	26.4	26.0	0.65	0.52	0.59	0.68	0.46	0.39
	1040♀	3.59	29.1	28.1	0.47	0.56	0.77	0.68	0.56	0.70
	Mean ♂	4.56		53.7		1.03		1.62		20.26
	Mean ♀	3.89		51.7		1.00		1.39		0.98
	Mean			52.6		1.01		1.49		

Animal excluded from calculation of mean and statistical analysis - killed Day 6 of Week 5

APPENDIX 8

Relative organ weights - individual values
(expressed as a percentage of final bodyweight)

Diet concn. ppm	Dog no.	Body wt kg	Brain	Pituitary	Heart	Lungs	Liver	Spleen
Control	1009♂	10.8	0.65	0.00062	0.74	0.78	3.47	0.52
	1011♂	11.3	0.80	0.00046	0.70	0.77	3.05	0.59
	1013♂	11.2	0.76	0.00067	0.63	0.86	3.15	0.60
	1015♂	11.2	0.72	0.00071	0.72	0.86	3.79	0.57
	1010♀	10.7	0.72	0.00071	0.77	0.88	3.07	0.87
	1012♀	8.9	0.80	0.00083	0.77	1.01	3.45	0.48
	1014♀	10.0	0.78	0.00076	0.74	0.91	3.56	0.62
	1016♀	10.0	0.74	0.00065	0.70	1.07	3.34	0.77
	Mean ♂	11.1	0.73	0.00062	0.70	0.82	3.37	0.57
	Mean ♀	9.9	0.76	0.00074	0.75	0.97	3.36	0.69
	Mean	10.5	0.75	0.00068	0.72	0.89	3.36	0.63
100	1017♂	11.2	0.79	0.00062	0.72	0.88	2.93	0.52
	1019♂	11.3	0.70	0.00063	0.70	0.91	3.58	0.45
	1021♂	10.7	0.75	0.00063	0.75	0.89	3.64	0.42
	1023♂	11.2	0.61	0.00059	0.66	0.87	3.31	0.42
	1018♀	9.0	0.79	0.00071	0.83	1.06	3.23	0.79
	1020♀	9.6	0.87	0.00087	0.69	0.81	4.21	0.78
	1022♀	10.7	0.67	0.00077	0.63	0.83	3.37	0.79
	1024♀	11.0	0.71	0.00065	0.60	0.78	3.52	1.04
	Mean ♂	11.1	0.71	0.00062	0.71	0.89	3.37	0.45
	Mean ♀	10.1	0.76	0.00075	0.69	0.87	3.58	0.85
	Mean	10.6	0.74	0.00068	0.70	0.88	3.47	0.65

APPENDIX 8

(Relative organ weights - continued)

Diet concn. ppm	Dog no.	Panc-reas	Thymus	Prostate /Uterus	Kidneys	Thyroids	Adrenals	Gonads
Control	1009♂	0.15	0.17	0.027	0.53	0.0076	0.0140	0.1388
	1011♂	0.22	0.12	0.019	0.46	0.0097	0.0142	0.1731
	1013♂	0.22	0.12	0.038	0.43	0.0089	0.0122	0.2194
	1015♂	0.15	0.12	0.022	0.51	0.0106	0.0145	0.0232
	1010♀	0.25	0.21	0.043	0.44	0.0069	0.0121	0.0108
	1012♀	0.22	0.10	0.038	0.52	0.0099	0.0149	0.0125
	1014♀	0.20	0.13	0.042	0.50	0.0120	0.0151	0.0093
	1016♀	0.18	0.08	0.032	0.56	0.0083	0.0144	0.0085
	Mean ♂	0.19	0.13	0.027	0.48	0.0092	0.0137	0.1386
	Mean ♀	0.21	0.13	0.039	0.51	0.0093	0.0141	0.0103
	Mean	0.20	0.13		0.49	0.0092	0.0139	
100	1017♂	0.20	0.20	0.058	0.45	0.0079	0.0160	0.1951
	1019♂	0.24	0.17	0.020	0.50	0.0067	0.0143	0.1852
	1021♂	0.20	0.11	0.052	0.53	0.0078	0.0150	0.1946
	1023♂	0.21	0.17	0.048	0.49	0.0098	0.0120	0.1679
	1018♀	0.31	0.17	0.074	0.49	0.0104	0.0129	0.0094
	1020♀	0.19	0.13	0.036	0.50	0.0110	0.0154	0.0140
	1022♀	0.28	0.22	0.032	0.48	0.0087	0.0140	0.0077
	1024♀	0.07	0.16	0.020	0.47	0.0106	0.0156	0.0065
	Mean ♂	0.21	0.16	0.045	0.49	0.0081	0.0143	0.1857
	Mean ♀	0.21	0.17	0.041	0.49	0.0102	0.0145	0.0094
	Mean	0.21	0.17		0.49	0.0091	0.0144	

APPENDIX 8

(Relative organ weights - continued)

Diet concn. ppm	Dog no.	Body wt kg	Brain	Pituitary	Heart	Lungs	Liver	Spleen
300	1025♂	10.7	0.73	0.00062	0.80	0.77	3.71	0.58
	1027♂	10.6	0.69	0.00070	0.77	0.95	4.00	0.36
	1029♂	11.5	0.73	0.00058	0.70	0.76	3.19	0.69
	1031♂	11.7	0.77	0.00068	0.68	0.87	3.88	0.54
	1026♀	8.9	0.86	0.00056	0.87	0.85	3.60	0.86
	1028♀	10.5	0.76	0.00072	0.64	0.81	3.85	0.95
	1030♀	9.9	0.79	0.00073	0.68	0.90	3.43	0.61
	1032♀	11.9	0.64	0.00066	0.67	0.83	3.35	0.63
	Mean ♂	11.1	0.73	0.00065	0.74	0.84	3.70	0.54
	Mean ♀	10.3	0.76	0.00067	0.72	0.85	3.56	0.76
	Mean	10.7	0.75	0.00066	0.73	0.84	3.63	0.65
1000	1033♂	7.0	0.96	0.00079	0.79	0.84	4.78	0.99
	1035♂##	7.5	1.10	0.00089	0.79	1.20	4.33	0.28
	1037♂	10.7	0.70	0.00065	0.59	0.79	4.04	0.46
	1039♂	12.0	0.70	0.00072	0.63	0.81	4.37	0.92
	1034♀	9.6	0.81	0.00085	0.84	0.74	4.25	0.66
	1036♀	8.7	0.83	0.00085	0.68	0.88	3.90	0.63
	1038♀	10.8	0.63	0.00061	0.69	0.78	3.63	1.00
	1040♀	11.0	0.64	0.00061	0.71	0.78	3.77	0.92
	Mean ♂	9.9	0.79	0.00072	0.67	0.81	4.40	0.79
	Mean ♀	10.0	0.73	0.00073	0.73	0.80	3.89	0.80
	Mean	10.0	0.75	0.00073	0.70	0.80	4.11	0.80

Animal excluded from calculation of mean and statistical analysis - killed Day 6 of Week 5

APPENDIX 8

(Relative organ weights - continued)

Diet. concn. ppm	Dog no.	Panc-reas	Thymus	Prostate /Uterus	Kidneys	Thyroids	Adrenals	Gonads
300	1025♂	0.24	0.13	0.055	0.49	0.0092	0.0138	0.2116
	1027♂	0.18	0.10	0.041	0.50	0.0089	0.0142	0.2062
	1029♂	0.20	0.17	0.036	0.49	0.0063	0.0133	0.1324
	1031♂	0.27	0.15	0.043	0.53	0.0077	0.0155	0.1885
	1026♀	0.31	0.13	0.046	0.51	0.0091	0.0167	0.0107
	1028♀	0.10	0.21	0.027	0.55	0.0082	0.0134	0.0081
	1030♀	0.23	0.18	0.025	0.48	0.0123	0.0169	0.0109
	1032♀	0.23	0.18	0.026	0.51	0.0090	0.0122	0.0108
	Mean ♂	0.22	0.14	0.044	0.50	0.0080	0.0142	0.1972
	Mean ♀	0.22	0.18	0.031	0.51	0.0097	0.0148	0.0101
	Mean	0.22	0.16		0.51	0.0088	0.0145	
1000	1033♂	0.37	0.14	0.045	0.65	0.0091	0.0221	0.2390
	1035♂##	0.24	0.08	0.014	0.56	0.0081	0.0143	0.0775
	1037♂	0.14	0.09	0.048	0.49	0.0117	0.0145	0.2079
	1039♂	0.21	0.18	0.045	0.53	0.0099	0.0147	0.1817
	1034♀	0.25	0.17	0.035	0.53	0.0100	0.0171	0.0080
	1036♀	0.23	0.15	0.068	0.54	0.0095	0.0137	0.0121
	1038♀	0.18	0.18	0.025	0.49	0.0108	0.0118	0.0079
	1040♀	0.19	0.18	0.033	0.52	0.0094	0.0132	0.0115
	Mean ♂	0.24	0.14	0.046	0.56	0.0102	0.0171	0.2095
	Mean ♀	0.21	0.17	0.040	0.52	0.0099	0.0140	0.0099
	Mean	0.22	0.16		0.54	0.0101	0.0153	

Animal excluded from calculation of mean and statistical analysis - killed Day 6 of Week 5

APPENDIX 9

Pathology - individual macroscopic and microscopic findings

Group:	1	2	3	4
Compound:	-		Ziram	
Diet concn. ppm:	0	100	300	1000

In this appendix the macroscopic and microscopic findings relating to each animal are listed. These latter findings are presented by an automated data collation system and the following comments should be noted.

Particular care is taken during tissue removal and processing to ensure recovery and sectioning of all protocol-scheduled tissues. Understandably, omissions or irregularities can occasionally occur, the most vulnerable tissues in this regard being parathyroid, thymus, male mammary gland and autolysed portions of the gastro-intestinal tract. For each animal, any tissue so affected is listed as "Not seen".

The following abbreviations are used:

PAS - Periodic acid-Schiff

ORO - Oil Red O

W.N.L. - Within normal limits

Sub.Mandib. - Submandibular

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: Control

Dog No/Sex: 1009♂ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities detected.

MICROSCOPIC FINDINGS

The following observations were noted:
TESTES
Immature
STOMACH
Foci of mineralisation in mucosa

The following tissues were considered normal:
AORTA ; TRACHEA ; LUNGS ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; PROSTATE ; THYROIDS ; PARATHYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: Control

Dog No/Sex: 1011♂ (Terminal)

MACROSCOPIC FINDINGS

HEART
Left ventricle, area of sub-endocardial discolouration at apex (4 mm x 3 mm).

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:
HEART
Area of haemorrhage into endocardium and superficial myocardium
LIVER
Foci of degenerate hepatocytes with inflammatory cell infiltration: (Minimal)

The following tissues were considered normal:
AORTA ; TRACHEA ; LUNGS ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; PROSTATE ; TESTES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: Control

Dog No/Sex: 1013♂ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities detected.

MICROSCOPIC FINDINGS

The following observations were noted:
SPLEEN
Haemosiderosis: (Minimal)
LIVER
Foci of degenerate hepatocytes with inflammatory cell infiltration: (Minimal)

The following tissues were considered normal:
AORTA ; TRACHEA ; LUNGS ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; PROSTATE ; TESTES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: Control

Dog No/Sex: 1015♂ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities detected.

MICROSCOPIC FINDINGS

The following observations were noted:
TESTES
Atrophic tubules
Marked interstitial cell hyperplasia

The following tissues were considered normal:
AORTA ; TRACHEA ; LUNGS ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; PROSTATE ; THYROIDS ; PARATHYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: Control

Dog No/Sex: 1010♀ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities detected.

MICROSCOPIC FINDINGS

The following observations were noted:
STERNUM
Focal haemorrhage: (Possible puncture site)

The following tissues were considered normal:
AORTA ; TRACHEA ; LUNGS ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; UTERUS ; VAGINA ; OVARIES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: Control

Dog No/Sex: 1012♀ (Terminal)

MACROSCOPIC FINDINGS

KIDNEY
Left, small.

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:
STERNUM
Focal haemorrhage: (Possible site of puncture)

The following tissues were considered normal:
AORTA ; TRACHEA ; LUNGS ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS : (W.N.L.) ; URINARY BLADDER ; UTERUS ; VAGINA ; OVARIES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: Control

Dog No/Sex: 1014♀ (Terminal)

MACROSCOPIC FINDINGS

DUODENUM
Diffuse areas of red discolouration.
JEJUNUM
Diffuse areas of red discolouration.

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:
TRACHEA
Inflammatory cell infiltration into lamina propria: (Minimal)
LYMPH NODES - MESENTERIC
Erythrophagocytosis
KIDNEYS
Granulomatous lesion(s): (Probably parasitic)
PITUITARY
Cyst(s) in pars anterior
DUODENUM
Congestion of capillaries of villi
MAMMARY GLAND
Dilated sweat glands
STERNUM
Area of bone remodelling: (Possible site of puncture)

The following tissues were considered normal:
AORTA ; LUNGS ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; SPLEEN ; LIVER ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; URINARY BLADDER ; UTERUS ; VAGINA ; OVARIES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; JEJUNUM : (W.N.L.) ; ILEUM ; CAECUM ; COLON ; RECTUM ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: Control

Dog No/Sex: 1016♀ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities detected.

MICROSCOPIC FINDINGS

The following observations were noted:
TRACHEA
Inflammatory cell infiltration into lamina propria: (Minimal)
LIVER
Foci of degenerate hepatocytes with inflammatory cell infiltration: (Minimal)
KIDNEYS
Focal interstitial inflammatory cell infiltration: (Minimal ; Unilateral)
URINARY BLADDER
Cystitis
PITUITARY
Cyst(s) in pars anterior

The following tissues were considered normal:
AORTA ; LUNGS ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; UTERUS ; VAGINA ; OVARIES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Pathologist: R.M.Read

APPENDIX 9

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: 100 ppm

Dog No/Sex: 1017♂ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities detected.

MICROSCOPIC FINDINGS

The following tissues were considered normal:
AORTA ; TRACHEA ; LUNGS ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; PROSTATE ; TESTES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Pathologist: R.M.Read

APPENDIX 9

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: 100 ppm

Dog No/Sex: 1019♂ (Terminal)

MACROSCOPIC FINDINGS

SPLEEN
Large area of white scarring (25 mm x 5 mm).

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:
SPLEEN
Areas of marked fibrous thickening of capsule

The following tissues were considered normal:
AORTA ; TRACHEA ; LUNGS ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; LIVER ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; PROSTATE ; TESTES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Pathologist: R.M.Read

APPENDIX 9

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: 100 ppm

Dog No/Sex: 1021♂ (Terminal)

MACROSCOPIC FINDINGS

LUNGS
Right cardiac lobe, adhesion to eighth rib.
Right intermediate lobe, area of dark discolouration covering half of lobe.

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:
TRACHEA
Tracheitis: (Minimal)
LUNGS
Foci of homogeneous eosinophilic material in some alveoli: (Minimal)
Bronchopneumonia: (Area)
Pleural adhesions
PITUITARY
Cyst(s) in pars anterior

The following tissues were considered normal:
AORTA ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; PROSTATE ; TESTES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS); TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: 100 ppm

Dog No/Sex: 1023♂ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities detected.

MICROSCOPIC FINDINGS

The following tissues were considered normal:
AORTA ; TRACHEA ; LUNGS ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; PROSTATE ; TESTES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: 100 ppm

Dog No/Sex: 1018♀ (Terminal)

MACROSCOPIC FINDINGS

LUNG

Right apical lobe, area of white discolouration (12 mm x 3 mm).
Left apical lobe, area of white discolouration (5 mm x 2 mm).

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:
LUNGS

Foci of homogeneous eosinophilic material in some alveoli: (Minimal)
Focal pneumonitis: (Minimal)

The following tissues were considered normal:
AORTA ; TRACHEA ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; UTERUS ; VAGINA ; OVARIES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: 100 ppm

Dog No/Sex: 1020♀ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities detected.

MICROSCOPIC FINDINGS

The following tissues were considered normal:
AORTA ; TRACHEA ; LUNGS ; HEART ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; UTERUS ; VAGINA ; OVARIES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Tissues not available for examination were:
THYMUS : (Not seen)

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: 100 ppm

Dog No/Sex: 1022♀ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities detected.

MICROSCOPIC FINDINGS

The following tissues were considered normal:
AORTA ; TRACHEA ; LUNGS ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; UTERUS ; VAGINA ; OVARIES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: 100 ppm

Dog No/Sex: 1024♀ (Terminal)

MACROSCOPIC FINDINGS

DUODENUM
Area of red discolouration covering most of mucosal surface.

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:
LIVER
Foci of degenerate hepatocytes with inflammatory cell infiltration: (Minimal)
DUODENUM
Congestion of capillaries of villi

The following tissues were considered normal:
AORTA ; TRACHEA ; LUNGS ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; UTERUS ; VAGINA ; OVARIES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: 300 ppm

Dog No/Sex: 1025♂ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities detected.

MICROSCOPIC FINDINGS

The following observations were noted:
PITUITARY
Cyst(s) in pars anterior

The following tissues were considered normal:
AORTA ; TRACHEA ; LUNGS ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; PROSTATE ; TESTES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS); TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: 300 ppm

Dog No/Sex: 1027♂ (Terminal)

MACROSCOPIC FINDINGS

LUNGS
Right intermediate lobe, area of dark discolouration with white raised foci.

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:
LUNGS
Foci of homogeneous eosinophilic material in some alveoli: (Minimal)
Bronchopneumonia: (Minimal ; Focal)
LIVER
Focal necrosis: (Minimal)
EPIDIDYMIDES
Vasculitis: (Focal) (on testis slide)

The following tissues were considered normal:
AORTA ; TRACHEA ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; PROSTATE ; TESTES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: 300 ppm

Dog No/Sex: 1029♂ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities detected.

MICROSCOPIC FINDINGS

The following observations were noted:
LUNGS
Focal pneumonitis: (Minimal)
HEART
Mucoid degeneration of atrioventricular valve
LIVER
Pigment in Kupffer cells: (Minimal)
LIVER (PAS STAIN)
Reduced glycogen in periportal hepatocytes

The following tissues were considered normal:
AORTA ; TRACHEA ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER (ORO STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; PROSTATE ; TESTES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: 300 ppm

Dog No/Sex: 1031♂ (Terminal)

MACROSCOPIC FINDINGS

HEART
Large haemocyst (4 mm) on atrioventricular node.

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:
HEART
Haemocyst
LIVER (PAS STAIN)
Reduced glycogen in centrilobular hepatocytes
STERNUM
Area of bone remodelling: (possible site of previous sternal puncture)

The following tissues were considered normal:
AORTA ; TRACHEA ; LUNGS ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER ; LIVER (ORO STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; PROSTATE ; TESTES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: 300 ppm

Dog No/Sex: 1026♀ (Terminal)

MACROSCOPIC FINDINGS

ILEUM

Several areas of marked congestion on mucosal surface (up to 10 mm x 10 mm).

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

PARATHYROIDS

Cyst: (Bilateral)

ILEUM

Congestion of capillaries of the villi

STERNUM

Focal haemorrhage: (possible puncture site)

The following tissues were considered normal:

AORTA ; TRACHEA ; LUNGS ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; UTERUS ; VAGINA ; OVARIES ; THYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: 300 ppm

Dog No/Sex: 1028♀ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities detected.

MICROSCOPIC FINDINGS

The following observations were noted:
LIVER
Pigment in Kupffer cells: (Minimal)
GALL BLADDER
Mucosal inflammatory cell infiltration: (Moderate)
Mucosal hyperplasia: (Moderate)
STERNUM
Focal haemorrhage: (possible site of puncture)

The following tissues were considered normal:
AORTA ; TRACHEA ; LUNGS ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; UTERUS ; VAGINA ; OVARIES ; THYROIDS ; PARATHYROIDS; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: 300 ppm

Dog No/Sex: 1030♀ (Terminal)

MACROSCOPIC FINDINGS

URINARY BLADDER
Multiple raised red foci (1 mm diameter).

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:
URINARY BLADDER
Cystitis

The following tissues were considered normal:
AORTA ; TRACHEA ; LUNGS ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; UTERUS ; VAGINA ; OVARIES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: 300 ppm

Dog No/Sex: 1032♀ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities detected.

MICROSCOPIC FINDINGS

The following observations were noted:
LIVER
Subcapsular focus of inflammatory cells: (probably parasitic)

The following tissues were considered normal:
AORTA ; TRACHEA ; LUNGS ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; UTERUS ; VAGINA ; OVARIES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: 1000 ppm

Dog No/Sex: 1033♂ (Terminal)

MACROSCOPIC FINDINGS

CERVICAL REGION
Subcutis, no abnormalities detected.
SUBMANDIBULAR LYMPH NODE
Enlarged.

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:
LUNGS
Focal acute inflammatory cell infiltration some associated with homogeneous eosinophilic material: (Minimal)
Vacuolated alveolar macrophages: (some associated with eosinophilic foreign material)
LIVER
Pigment in Kupffer cells: (Minimal)
KIDNEYS
Foci of mineralisation in the papilla

The following tissues were considered normal:
AORTA ; TRACHEA ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; LYMPH NODES - SUBMANDIBULAR : (W.N.L.) ; SPLEEN ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; URINARY BLADDER ; PROSTATE ; TESTES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: 1000 ppm

Dog No/Sex: 1035♂ (Intercurrent)

MACROSCOPIC FINDINGS

GENERAL COMMENTS
Animal killed for humane reasons due to convulsion.
LIVER
Pale with accentuated lobular markings, cut surface friable.
HEART
Left ventricle, pallor of endocardium and extending into myocardium, several foci of red discolouration on endocardium.
Left atrioventricular valve, area of red discolouration of endocardial surface (6x3 mm).
LUNGS
Right cardiac lobe and left intermediate lobe congested.

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:
LUNGS
Polymorphonuclear leucocyte infiltration into alveoli: (Areas)
HEART
Focal myocardial necrosis with acute inflammatory cell infiltration and haemorrhage
THYMUS
Involution: (Minimal)
LYMPH NODES - CERVICAL
Sinus histiocytosis
SPLEEN
Contracted
LIVER
Centrilobular hepatocyte degeneration: (Minimal)
LIVER (ORO STAIN)
Fat droplets in periportal hepatocytes: (Moderate)
TESTES
Tubular atrophy: (Bilateral)
EPIDIDYMIDES
No spermatazoa in tubules: (on testes slide)
* PATHOLOGIST'S COMMENTS

The changes seen in the heart were considered to be sufficient to account for some of the clinical signs observed

The following tissues were considered normal:
AORTA ; TRACHEA ; LYMPH NODES - MESENTERIC ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; PROSTATE ; THYROIDS ; PARATHYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: 1000 ppm

Dog No/Sex: 1037♂ (Terminal)

MACROSCOPIC FINDINGS

LUNGS
Left cardiac lobe, pale raised area (6 mm x 2 mm).

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:
LUNGS
Subpleural interstitial fibrosis with epithelialisation
SPLEEN
Haemosiderosis: (Minimal)
LIVER
Pigment in Kupffer cells: (Minimal)
KIDNEYS
Groups of dilated cortical tubules with associated primitive glomeruli

The following tissues were considered normal:
AORTA ; TRACHEA ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; URINARY BLADDER ; PROSTATE ; TESTES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: 1000 ppm

Dog No/Sex: 1039♂ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities detected.

MICROSCOPIC FINDINGS

The following observations were noted:
LUNGS
Alveolar macrophages: (Minimal ; Focal)
PROSTATE
Prominent alveolar activity

The following tissues were considered normal:
AORTA ; TRACHEA ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; TESTES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: 1000 ppm

Dog No/Sex: 1034♀ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities detected.

MICROSCOPIC FINDINGS

The following observations were noted:
LIVER
Foci of degenerate hepatocytes with inflammatory cell infiltration: (Minimal)
THYROIDS
Ectopic thymic tissue
PITUITARY
Cyst(s) in pars anterior

The following tissues were considered normal:
AORTA ; TRACHEA ; LUNGS ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS , KIDNEYS ; URINARY BLADDER ; UTERUS ; VAGINA ; OVARIES ; PARATHYROIDS; ADRENALS ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: 1000 ppm

Dog No/Sex: 1036♀ (Terminal)

MACROSCOPIC FINDINGS

LIVER

All lobes, multiple depressed pale areas on capsule (up to 4 x 4 mm).

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:

LIVER

Focal necrosis: (Minimal ; Foci)
Pigment in Kupffer cells: (Minimal)
Inflammatory cell infiltration adjacent to central veins
Loss of cells with dilated sinusoids

PITUITARY

Cyst(s) in pars anterior

The following tissues were considered normal:
AORTA ; TRACHEA ; LUNGS ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; UTERUS ; VAGINA ; OVARIES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS); TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: 1000 ppm

Dog No/Sex: 1038♀ (Terminal)

MACROSCOPIC FINDINGS

DUODENUM
Diffuse areas of red discolouration.
STOMACH ANTRUM
Multiple fine brown foci (less than 1 mm diameter).

All the other organs and tissues appeared normal.

MICROSCOPIC FINDINGS

The following observations were noted:
LIVER
Foci of degenerate hepatocytes with inflammatory cell infiltration: (Minimal)
EYES
Conjunctivitis: (Minimal ; Focal ; Unilateral)

The following tissues were considered normal:
AORTA ; TRACHEA ; LUNGS ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; UTERUS ; VAGINA ; OVARIES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH : (W.N.L.) ; DUODENUM ; JEJUNUM : (W.N.L.) ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; STERNUM ; FEMUR & JOINT

Pathologist: R.M.Read

(Pathology - continued)

Compound: Ziram Technical

Dosage Level: 1000 ppm

Dog No/Sex: 1040♀ (Terminal)

MACROSCOPIC FINDINGS

No abnormalities detected.

MICROSCOPIC FINDINGS

The following observations were noted:
STERNUM
Area of bone remodelling: (Possible puncture site)

The following tissues were considered normal:
AORTA ; TRACHEA ; LUNGS ; HEART ; THYMUS ; LYMPH NODES - CERVICAL ; LYMPH NODES - MESENTERIC ; SPLEEN ; LIVER ; LIVER (ORO STAIN) ; LIVER (PAS STAIN) ; GALL BLADDER ; PANCREAS ; KIDNEYS ; URINARY BLADDER ; UTERUS ; VAGINA ; OVARIES ; THYROIDS ; PARATHYROIDS ; ADRENALS ; PITUITARY ; SALIVARY GLAND (SUB.MANDIB) ; MUSCLE (BICEPS FEMORIS) ; TONGUE ; OESOPHAGUS ; STOMACH ; DUODENUM ; JEJUNUM ; ILEUM ; CAECUM ; COLON ; RECTUM ; MAMMARY GLAND ; SKIN ; EYES ; SPINAL CORD ; SCIATIC NERVE ; BRAIN ; FEMUR & JOINT

Pathologist: R.M.Read

ADDENDUM 1

THE ANALYSIS OF ZIRAM IN DIETARY FORMULATIONS

Authors:

Alan Anderson,
I. Suzanne Dawe,
Steven F. Johnson,
Sujit Patel.

ADDENDUM 1

(continued)

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ADDENDUM 1

(continued)

9. Calculation

The absorbance for each calibration standard was measured and calibration curves were constructed by linear regression of standard absorbance versus standard concentration. The absorbance of each sample and procedural recovery solution was measured and the concentration of Ziram calculated using the equation below:

$$\text{Concentration, ppm} = \frac{Y - I}{S} \times \frac{V}{W} \times \frac{Mp}{2 \times Mc}$$

Where Y = Absorbance of test solution (corrected for absorbance of control extract)
I = Intercept derived from linear regression of calibration data
S = Slope derived from linear regression of calibration data
V = Dilution volume of sample (ml)
W = Weight of sample (g)
Mp = Molecular weight of Ziram (305.82)
Mc = Molecular weight of carbon disulphide (76.14)

Results were corrected for the appropriate procedural recovery value at analysis.

10. Limit of detection

The limit of detection, defined as the concentration of Ziram in control matrix producing an absorbance of 0.01 AU, was estimated as 1.5 ppm.

ADDENDUM 1

(continued)

RESULTS AND DISCUSSION

The mean concentrations of Ziram in dose formulations analysed during the study and the deviation of mean results from nominal values are summarised in Table 1. Mean results were all within 7% of nominal concentrations. Individual analytical results and associated procedural recovery values are detailed in Table 2.

The results in Table 3 confirm that, at nominal concentrations of 100 ppm and 5000 ppm, Ziram can be homogeneously blended in SDS Diet A.

The stability of Ziram in SDS Diet A was assessed during three trials to optimise storage conditions (Table 4). The data obtained for Trials 1 and 2 indicate that Ziram at a concentration of 5000 ppm is stable in the SDS Diet A matrix for up to 14 days during storage at ambient temperature under animal room conditions. At a concentration of 100 ppm significant losses occurred during storage (approximately 15% after 24 hours and 30% after 14 days) at ambient temperature. However, the data obtained for Trial 3 confirm that no significant change in concentration occurred in the 100 ppm diet during 8 hour storage at ambient temperature under animal room conditions. Therefore, it was recommended that diet formulations should be stored frozen (-20°C), fed on a daily basis and consumed within 8 hours.

The procedural recovery data obtained during method validation (summarised in Table 5) and the homogeneity/stability trials (shown in Tables 3 and 4, summarised in Table 5), confirm that the method of analysis described provides good extraction efficiency for Ziram from the SDS Diet A matrix. A mean procedural recovery value of 92.4% ± 2.88 SD (n=19) was obtained for 100 ppm and 92.7% ± 2.10 SD (n=16) was obtained for 5000 ppm. All results are corrected for the appropriate procedural recovery value at analysis.

ADDENDUM 1

(continued)

TABLE 1

Summary: mean concentrations of Ziram in dose formulations

Week of study	Group	Nominal inclusion (ppm)	Mean analysed concentration (ppm)	RME (%)
1	Control	0	ND	-
	2	100	92.9	-7.1
	3	300	296	-1.3
	4	1000	960	-4.0
13	Control	0	ND	-
	2	100	95.5	-4.5
	3	300	297	-1.0
	4	1000	978	-2.2

ND None detected
RME Relative mean error

ADDENDUM 1

(continued)

TABLE 2

Concentrations of Ziram in dose formulations
(individual values)

Week of study	Group	Nominal inclusion (ppm)	Analysed concentration (ppm)			Procedural recovery (%)
			Analysis 1	Analysis 2	Mean	
1	Control	0	ND	-	ND	-
	2	100	90.8	95.0	92.9	86.2
	3	300	292	300	296	92.7
	4	1000	947	974	960	99.2
13	Control	0	ND	-	ND	-
	2	100	95.5	95.5	95.5	89.9
	3	300	295	298	297	94.5
	4	1000	968	989	978	97.9

ND None detected

Results are corrected for the appropriate procedural recovery value at analysis, given in this Table

ADDENDUM 1

(continued)

TABLE 3

Homogeneity of Ziram blended in dog diet formulations

Nominal inclusion (ppm)	Sample	Analysed concentration (ppm)			Mean of mix (ppm)	CV (%)	Procedural recoveries (%)
		Analysis 1	Analysis 2	Mean			
100	Top	101	101	101	98.9	2.3	91.8
	Top	96.9	94.6	95.7			94.1
	Middle	99.0	98.3	98.6			91.8
	Middle	99.8	100	99.9			91.6
	Bottom	98.6	103.6	101			95.1
	Bottom	96.0	97.9	97.0			95.6
5000	Top	5030	4870	4950	4970	0.7	91.9
	Top	4980	4990	4990			95.5
	Middle	4990	4930	4960			90.8
	Middle	5070	4980	5030			93.6
	Bottom	5000	4890	4940			94.4
	Bottom	4940	4950	4950			96.1

CV Coefficient of variation

Results are corrected for the appropriate procedural recovery value at analysis given in this Table.

ADDENDUM 1

(continued)

TABLE 4

Stability of Ziram in dog diet formulation

4.1. Trial 1

Nominal inclusion (ppm)	Storage time (days)	Analysed concentration (ppm)			RME (%)	Procedural recoveries (%)
		Analysis 1	Analysis 2	Mean		
100	0	-	-	98.9[1]	- 1.1	98.1,93.3, 91.6
	8	70.0,69.5	69.8,70.1	69.8	-30.2	
	14	67.6	69.1	68.3	-31.7	
5000	0	-	-	4970[1]	- 0.6	95.9
	8	4770	4770	4770	- 4.6	92.0
	14	4910	4900	4910	- 1.8	

4.2. Trial 2

Nominal inclusion (ppm)	Storage time (hours)	Analysed concentration (ppm)			RME (%)	Procedural recoveries (%)
		Analysis 1	Analysis 2	Mean		
100	0	97.5	97.6	97.5	- 2.5	93.2
	24	84.2	83.4	83.8	-16.2	94.5
5000	0	4970	4920	4940	- 1.2	94.6
	24	5140	5100	5120	+ 2.4	91.9

4.3. Trial 3

Nominal inclusion (ppm)	Storage time (hours)	Analysed concentration (ppm)			RME (%)	Procedural recoveries (%)
		Analysis 1	Analysis 2	Mean		
100	0	90.8	95.1	92.9	- 7.1	86.2
	8	88.2	86.8	87.5	-12.5	88.0

RME Relative mean error
[1] Represents the mean of homogeneity data given in Table 3

Results are corrected for the appropriate procedural recovery value at analysis given in this Table.

ADDENDUM 1

(continued)

TABLE 5

Procedural recovery data

(results are expressed as percent recovery)

5.1 Obtained during method validation

	Nominal fortification (ppm)	
	100	5000
	89.6 89.7 89.8 94.2 93.3 94.5	89.5 91.2 89.9 90.9 92.2 92.8
Mean (5.1)	91.9	91.1
SD (±)	2.39	1.28
Range	89.6-94.5	89.5-92.8

5.2 Obtained during homogeneity/stability trials

	Nominal fortification (ppm)	
	100	5000
	91.8 94.1 91.8 91.6 95.1 95.6 98.1 93.3 91.6 93.2 94.5 86.2 88.0	91.9 95.5 90.8 93.6 94.4 96.1 95.9 92.0 94.6 91.9
Mean (5.1 + 5.2)	92.4	92.7
SD (±)	2.88	2.10
Range	86.2-98.1	89.5-96.1

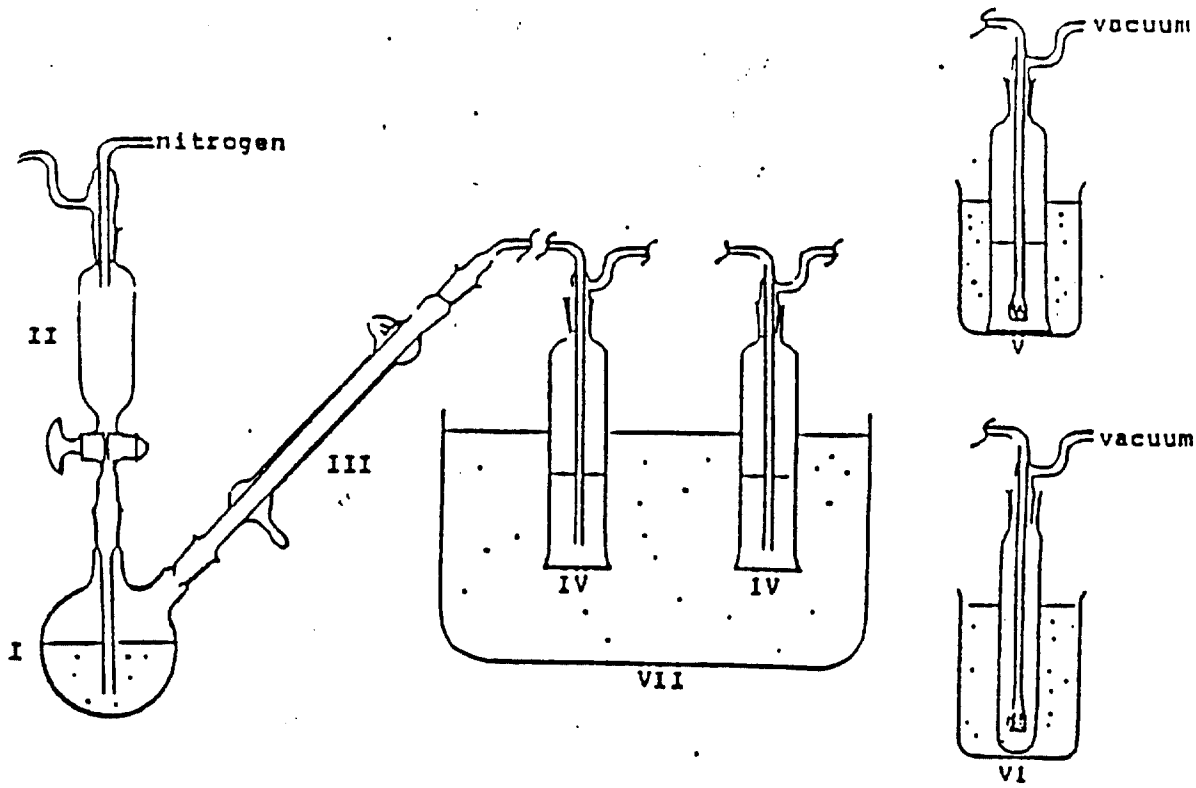
SD Standard deviation

ADDENDUM 1

(continued)

FIGURE 1

Carbon disulphide evolution apparatus



Key

I	Two-necked round-bottomed flask (500 ml)
II	Cylindrical dropping funnel
III	Condenser
IV	Dreschel bottles (250 ml) for H_2S absorption
V	Dreschel bottle (250 ml) for CS_2 absorption
VI	Test tube (60 ml) with Dreschel bottle head for CS_2 absorption
VII	Water bath (90°C)

ADDENDUM 2

Results of stability analysis of Ziram technical grade (Data supplied by Sponsor)

CHEMISCHE SECTOR

MINERALE DIVISIE

VESTIGING TE GENT

Our ref. GM/8862

April 4th, 1990

Ziram technical grade: stability.

The lot number 8331AA, from which a sample has been sent to Huntingdon Research Center for toxicological studies, has been analysed at four different times in this laboratory.
On the other hand a 5 g sample sent back by HRC has also been analysed in this laboratory.
The method used for determinining the active ingredient content is the official CIPAC method [CIPAC 1; 31/1.M.1.2].
The results are tabulated below:

Date of analysis	% ziram
March 88	99.0 %
Nov. 88	98.9 %
Jan. 89	98.5 %
Dec. 89*	96.8 %
March 90	98.5 %

The above results, which are within the accuracy limits of the method, show that the active ingredient content has been stable over the past two years.

G. Marthijs
Head of Laboratory

J. Swinnen
Head of Technical Department

*5 g sample sent back by HRC

ucb n.v.

ADDENDUM 2

(continued)

July 18th, 1990

Ziram technical: stability

A subsample (dated 16.7.90) of lot number 8331AA , used by HRC for toxicological studies was sent back to UCB. It was analysed in this laboratory on July 18th.

The active ingredient was determined in threefold, making use of the official CIPAC method [CIPAC 1; 31/1/M.1.2] .

The values found were:

- 99.0 %
- 98.8 %
- 98.9 %

The average value is 98.9 % , against 98.8 % for a sample sent back by HRC in december 1989.

This leads us to the conclusion that there is no deterioration of the active material.

G. Matthijs
Head of Laboratory

A. Schollaert
Director

ADDENDUM 2

(continued)

January 7th, 1991

Ziram technical: stability

A subsample (dated 30.11.90) of lot number 8331AA , used by HRC for toxicological studies was sent back to UCB. It was analysed in this laboratory on 4.1.91.

The active ingredient was determined in threefold, making use of the official CIPAC method [CIPAC 1; 31/1/M.1.2] .

The values found were:

- 98.6 %
- 98.8 %
- 98.7 %

The average value is 98.7 % , against 98.8 % in december '89 and 98.9 % in June '90.

This leads us to the conclusion that there is no deterioration of the active ingredient.

G. Matthijs
Head of Laboratory

J. Swinnen
Head of Technical Department

ADDENDUM 3

Test Material Safety Data Sheet

UCB chemicals corporation

5365-A Robin Hood Road, Norfolk, Virginia 23513
(804) 857-8610, Fax: (804) 855-7975, Telex: 494-6186 UCB UI

MATERIAL SAFETY DATA SHEET

PRODUCT NAME: Ziram Technical
Minimum 97% Powder Phyto Grade

CHEMICAL NAME: Zinc Dimethyldithiocarbamate
SYNONYMS: Ziram, Bis (Dimethyldithiocarbamato) Zinc
CODE NUMBER: 245150 CAS NUMBER: 137-30-4
FORMULA: $C_6H_{12}S_4N_2Zn$ MOLECULAR WEIGHT: 305.82

SECTION II PHYSICAL DATA

BOILING POINT @ 760 mm Hg):	**
SPECIFIC GRAVITY (H_2O = 1):	1.65
VAPOR PRESSURE (@ 20 C mm Hg):	**
SOLUBILITY (IN H_2O = 1):	Soluble in part
PHYSICAL STATE/APPEARANCE:	Creamy white powder
ODOR:	No specific information
FREEZING POINT:	**
MELTING POINT:	240/464 (°C/°F)
BULK DENSITY (g/cc):	0.25
pH (in 4% aqueous suspension):	7.29

**Not applicable

SECTION III HAZARDOUS COMPONENTS

NAME	TLV	PEL	TOXICOLOGY DATA
Ziram	*	*	See Section VI

*Not Established

SECTION IV FIRE AND EXPLOSION HAZARD DATA

FLASH POINT AND METHOD: Not applicable
FLAMMABLE LIMITS/VOL %: No specific information
AUTOIGNITION TEMPERATURE: No specific information
EXTINGUISHING MEDIA: Small quantities of burning material may be extinguished with CO_2 or dry chemical. For large quantities or where other flammable/combustible materials are present, use flooding amounts of water fog or firefighting foam.

SPECIAL FIRE FIGHTING PROCEDURES:
Wear self-contained breathing apparatus (scba) in pqsitive pressure mode.

UNUSUAL FIRE AND EXPLOSION HAZARDS: No specific information.

AC297588

ADDENDUM 3

(continued)

SECTION V OCCUPATIONAL EXPOSURE LIMITS

ACGIHTLV	Not established
OSHA PEL	Not established

SECTION VI HEALTH HAZARD DATA

INGESTION: Slightly toxic LD_{50} (rat): 1950 mg/kg. (Proprietary study. May not be quoted.) May cause gastritis.

SKIN CONTACT: (1) Primary irritation index with the rabbit was 38 on the Draize scale. (Proprietary study. May not be quoted.) (2) Practically non-toxic. Dermal LD_{50} (rat): >20 g/kg. (Proprietary study. May not be quoted.)

INHALATION: 4h LC_{50}: >0.75 mg/L. Can be very irritating to respiratory tract, lungs and mucous membranes.

EYE CONTACT: Ziram to be classified as severely irritating in the rabbit according to the ETAD code. (Proprietary study. May not be quoted.)

CARCINOGENICITY: Not listed in NTP or IARC monograph or OSHA regulation.

OTHER CHRONIC EFFECTS OF EXPOSURE: NTP Carcinogenesis bioassay resulted in positive finding in rats. IARC - positive animal carcinogen (A-63). Reported to be teratogenic in laboratory animals. May affect metabolism and liver function. Mutagenicity is positive in various in-vitro tests. (Proprietary study. May not be quoted.)

SECTION VII EMERGENCY and FIRST AID PROCEDURES

INGESTION: Seek medical attention immediately.

SKIN CONTACT: Wash affected areas with soap and water for 15 minutes. Seek medical attention immediately.

EYE CONTACT: Flush eyes with plenty of water for at least 15 minutes. Seek medical attention immediately.

INHALATION: If breathing has stopped, administer artificial respiration. Seek medical attention immediately.

ADDENDUM 3

(continued)

SECTION VIII REACTIVITY HAZARD DATA

STABILITY: Stable

HAZARDOUS POLYMERIZATION: Does not occur.

CONDITIONS TO AVOID: Excessive heat

MATERIALS TO AVOID: Acids, alkalis

HAZARDOUS COMBUSTION OR HAZARDOUS DECOMPOSITION PRODUCTS: When heated to decomposition, emits fumes of NO_x and So_x. Inorganic acids break down methyl ziram with evolution of carbon disulphide.

SECTION IX SPECIAL PROTECTION INFORMATION

RESPIRATORY PROTECTION: NIOSH-approved particulate respirator with full face piece, properly fitted, is recommended whenever exposure to dust is likely.

VENTILATION: Local exhaust recommended to control exposure as appropriate. Mechanical not recommended as sole means of controlling employee exposure.

PROTECTIVE CLOTHING: Impervious gloves.

EYE PROTECTION: Chemical splash goggles. DO NOT WEAR CONTACT LENSES.

OTHER PROTECTIVE EQUIPMENT: A safety shower and an eyewash facility should be available.

RN 29766 88

ADDENDUM 3

(continued)

SECTION X SPILL OR LEAK PROCEDURES

ENVIRONMENTAL TOXICITY DATA: 96h LC_{50}: 2.28 mg/L (carp). (Proprietary study. May not be quoted.)

STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED: Sweep spilled materials and recover for disposal. Prevent washings from entering waterways.

WASTE DISPOSAL METHOD: Incineration in accordance with federal, state and local regulations. This material is not defined as a hazardous waste under current RCRA regulations.

SECTION XI SPECIAL PRECAUTIONS

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORING: Keep containers tightly closed and away from excessive heat and moisture.

OTHER PRECAUTIONS: Avoid contamination of skin and eyes. Avoid breathing dust.

SECTION XII OTHER REGULATORY CONTROLS

DOT SHIPPING NAME:	Dithiocarbamate Pesticide, Solid, NOS.
DOT HAZARD CLASSIFICATION:	Not classified
DOT LABELS REQUIRED (49CFR172.101):	None
HAZARDOUS SUBSTANCE (40CFR 116):	Not applicable
REPORTABLE QUANTITY:	Not applicable
DOT PLACARDS REQUIRED:	None
POISON CONSTITUENT (49 CFR173.343):	Not applicable
BILL OF LADING DESCRIPTION:	Ziram Technical (zinc dimethyldithiocarbamate) Minimum 97% Powder Phyto Grade
UN NUMBER:	2771

Approved By: Paul C. Nelson
Paul C. Nelson
Manager, Regulatory Affairs
UCB Chemicals Corporation

Dated: 29 Feb 88

Contains No CBI

CONFIDENTIAL

ZIR 20(c)/901626

THE ACUTE TOXICITY OF ZIRAM TECHNICAL TO BLUEGILL SUNFISH (*Lepomis macrochirus*)

Study completed: 3 September 1991

Regulations: OECD Guideline No. 203 and EPA Pesticide Assessment Guidelines, Subdivision E, Section 72-1

Addressee:

Ziram Task Force,
Consortium No. 62405,
c/o UCB Chemicals Corporation,
5505-A Robin Hood Road,
Norfolk,
VA 23513,
U.S.A.

Report issued: 3 September 1991.

Authors:

Mark T. Douglas,
Robert O. Stonehewer,
Ian A. Macdonald.

Huntingdon Research Centre Ltd.,
P.O. Box 2,
Huntingdon,
Cambridgeshire,
PE18 6ES,
ENGLAND.

SPONSOR

Ziram Task Force,
Consortium No. 62405,
c/o UCB Chemicals Corporation,
5365-A Robin Hood Road,
Norfolk, Virginia 23513 U.S.A.

STUDY TITLE

The Acute Toxicity of Ziram Technical to Bluegill sunfish (*Lepomis macrochirus*)

DATA REQUIREMENT

OECD Guideline No. 203 and EPA Pesticide Guidelines, Subdivision E, Section 72-1

AUTHORS

Mark T. Douglas, Robert O. Stonehewer and Ian A. Macdonald

EXPERIMENTAL PHASE COMPLETED ON

25 May 1990

PERFORMING LAB

Huntingdon Research Centre Ltd.,
P.O. Box 2,
Huntingdon, Cambridgeshire,
PE18 6ES, ENGLAND.

HRC STUDY REPORT NO.

ZIR 20(c)/901626

FINAL REPORT DATE

3 September 1991

STATEMENT OF NO CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10(d) (1) (A), (B), or (C).

COMPANY:	Ziram Task Force c/o UCB Chemicals Corporation
COMPANY AGENT:	Paul R. Larson
DATE:	October 22, 1991
TITLE:	Manager, Agricultural Products
SIGNATURE:	Paul R. Larson

These data are the property of the Ziram Task Force (Consortium No. 62405) and, as such, are considered to be confidential for all purposes other than compliance with FIFRA 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any other statute or in any other country.

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

HRC Report No. ZIR 20(c)/901626

To the best of my knowledge and belief the Study described in this Report was conducted in compliance with the following Good Laboratory Practice Standards:

Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health & Social Security 1986 and subsequent revision, Department of Health, 1989.

United States Food and Drug Administration, Title 21 Code of Federal Regulations Part 58, Federal Register 22 December 1978, and subsequent Amendments.

United States Environmental Protection Agency, (FIFRA), Title 40 Code of Federal Regulations Part 160, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

United States Environmental Protection Agency, (TSCA), Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

Japan Ministry of Health and Welfare, Notification No. YAKUHATSU 313 Pharmaceutical Affairs Bureau, 31 March 1982 and subsequent amendment Notification No. YAKUHATSU 870, Pharmaceutical Affairs Bureau, 5 October 1988.

Japan Ministry of Agriculture, Forestry and Fisheries, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984.

Japan Ministry of International Trade and Industry, Directive 31 March 1984 (Kanpogyo No. 39 Environmental Agency, Kikyoku No. 85 MITI).

Organisation for Economic Co-operation and Development, ISBN 92-64-12367-9, Paris 1982.

3 Sept 91

Mark T. Douglas, B.Sc., Ph.D.,
Study Director,
Huntingdon Research Centre Ltd.

Date

22/8/91

David H. Christopher, Ph.D., D.I.C.,
For Laboratory Management

Date

22/10/91

Submitter/Sponsor

Date

We the undersigned, hereby declare that the work was performed under our supervision according to the procedures herein described, and that this report provides a correct and faithful record of the results obtained.

Mark T. Douglas, B.Sc., Ph.D.,
Head of Unit,
Unit of Fish Toxicology

Robert O. Stonehewer, B.Sc.,
Scientific Officer,
Unit of Fish Toxicology

Ian A. Macdonald, L.R.S.C.,
Unit Head, Pesticide and Environmental Analysis,
Department of Analytical Chemistry

QUALITY ASSURANCE STATEMENT

HRC Report No. ZIR 20(c)/901626

Certain studies such as that described in this report, are conducted at HRC in a setting which involves frequent repetition of similar or identical procedures. At or about the time the study described in this report was in progress, "process-based" inspections were made by the Quality Assurance Department of critical procedures relevant to this study type. For the inspection of any given procedure, at least one study was selected without bias. The findings of these inspections were reported promptly to the Study Director and to HRC Management.

This report has been audited by HRC Quality Assurance Department. It is considered to be an accurate description of the procedures and practices employed during the course of the study and an accurate presentation of the findings.

Date of inspection	04-14.06.90
Date of reporting Inspection Findings to the Study Director and HRC Management	15.06.90
Date of reporting Audit Findings to the Study Director and HRC Management	07.01.91

28.8.91

Peter H.C.V. Richold, B.Sc.,
Senior Systems Compliance Auditor,
Department of Quality Assurance,
Huntingdon Research Centre Ltd.

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SUMMARY

Test Substance	:	Ziram Technical.
Test Species	:	Bluegill sunfish (*Lepomis macrochirus*).
Test Type	:	Acute toxicity. 96 h LC_{50}.
Test Conditions	:	Dynamic conditions according to OECD Guideline No. 203 and EPA Pesticide Assessment Guidelines, Subdivision E, Section 72-1.

Results

Test substance : Ziram Technical.

Time (h)	LC_{50} (mg/l)	95% confidence limits (mg/l)
3	>0.022	-
6	>0.022	-
24	>0.022	-
48	0.016	0.011 - 0.023
72	0.012	0.0099 - 0.014
96	0.0097	0.0086 - 0.011

All results are expressed in terms of measured concentration.

INTRODUCTION

This report contains a description of the methods used and the results obtained during a study to investigate the acute toxicity of Ziram Technical to bluegill sunfish (*Lepomis macrochirus*).

The study was commissioned by Ziram Task Force and undertaken between 21 - 25 May 1990.

Dates of Protocol approval

Study Director	:	20 January 1990.
HRC Ltd. Management	:	20 January 1990.
Sponsor	:	3 April 1990.

MATERIALS AND METHODS

Test Substance

Identification	:	Ziram Technical. Zinc bis(dimethyldithiocarbamate).
Description	:	White powder.
Purity	:	98.9% (Lot No. 8331 AA reanalysed on 18 July 1990).
Major impurities	:	Na_2SO_4 (1%) and water (0.23%).
Date of receipt	:	Received from UCB SA., Brussels, Belgium on 12 April 1989.
Storage	:	In darkness at room temperature.
Expiry date	:	Subsamples analysed periodically to confirm stability of sample (see Appendix 4).
Method of preparation	:	Preliminary solution in dimethylformamide. Five separate solvent stock solutions of 0.112, 0.20, 0.36, 0.64 and 1.12 mg/ml were prepared for automatic dispensing via the multi-channel dosing apparatus (see "Exposure Conditions. Medium renewal").
Stability of test concentrations	:	Ensured by continuous renewal of test media and verified by chemical analysis. Water samples were taken from test and control vessels and sent to the HRC Department of Analytical Chemistry for analysis (see Appendix 2).

Test Species

Name	:	Bluegill sunfish (*Lepomis macrochirus*).
Source	:	SP Inc., Salem, Mass. U.S.A.
Acclimatisation	:	Stock held since 4 April 1990 and acclimatised to test conditions from 4 April to 21 May 1990. Temperature: 21°C. Dissolved oxygen: 8.5 mgO_2/l. Feeding: Daily, with commercial fish flake to repletion.

		Discontinued 48 h prior to test.
		Mortalities: 3% in 7 days prior to study.
		Medication: None.
Mean standard length	:	3.3 cm (SD = 0.6 cm)
Mean weight	:	1.01 g (SD = 0.40 g)

The fish used in this test slightly exceed the range recommended in OECD Guideline No. 203 but this is not considered to have had any significant effect on the results of the test.

Test Water

Laboratory tap water, dechlorinated by the addition of sodium thiosulphate. Total hardness ≈ 350 mg/l as $CaCO_3$. This value is slightly higher than the recommended range but is not considered to have had any significant effects on the results of the test.

Exposure Conditions

Test vessels	:	Glass aquaria holding 20 l of test media.
Experimental design	:	5 test concentrations plus 1 control and 1 solvent control (including 50 µl auxiliary solvent per litre). All in duplicate.
		20 animals per test concentration.
Method of initiation	:	Fish were placed in prepared test media.
Loading	:	0.51 g bodyweight/litre (static volume) 0.01 g bodyweight/litre (volume in 24 hours).
Photoperiod	:	16 h light : 8 h dark.
Temperature	:	21°C.
Aeration	:	None.
Nominal test concentrations	:	0.0056, 0.01, 0.018, 0.032 and 0.056 mg/l.
Measured test concentrations	:	0.0046, 0.0080, 0.015, 0.022 and 0.025 mg/l (mean of samples collected at 0 h, 24 h and 96 h).

Medium renewal	:	Continuous at 118 ml/min delivered by a Watson-Marlow® multichannel variable speed peristaltic pump (diluent) with solvent stock solutions injected by means of a Braun® syringe pump dispensing at a rate of 0.3553 ml/hr (i.e. 50 μl solvent stock per litre of diluent). This stream was divided equally between two replicates to give a renewal rate of 59 ml/minute.
Duration of exposure	:	96 hours.
Criteria of death	:	Absence of (i) respiratory movement and (ii) response to physical stimulation.

Records

All raw data and other documents generated at HRC during the course of this study, together with a copy of this final report, have been lodged in the HRC Archives, Huntingdon, England.

RESULTS

Cumulative mortality data are given in Table 1 and the relationship between percentage mortality and concentration is given in Figure 1. All results are expressed in terms of mean measured concentrations (mean of samples collected at 0h, 24 h and 96 h). However, the mortality data for the 0.025 mg/l solution (0.056 mg/l nominal concentration) has not been used in subsequent calculations owing to erratic chemistry results and the distortive effect on LC_{50} estimates. The narrow range of the 95% confidence limits for the 96 h LC_{50} value confirms that a precise estimate can be obtained without the need to use data from this treatment group.

Analysis of the mortality data by the method of Thompson & Weil (1952) gave the following results:

Time (h)	LC_{50} (mg/l)	95% confidence limits (mg/l)
3	>0.022	-
6	>0.022	-
24	>0.022	-
48	0.016	0.011 - 0.023
72	0.012	0.0099 - 0.014
96	0.0097	0.0086 - 0.011

Highest test concentration resulting in 0% mortality = 0.0046 mg/l
Lowest test concentration resulting in 100% mortality >0.022 mg/l

The relationship between the median lethal concentration and time is presented in Figure 2.

Thompson, W.R. & Weil, C.S. (1952) Biometrics *8*, 51-54.

Observations

Marked reactions to exposure (other than death) were increased pigmentation, swimming at surface, lying on bottom of tank, loss of equilibrium, lethargy and moribundity.

A chronological record of observations is given below (unless stated no adverse reactions were observed):

Concentration mg/l (measured)	Abnormality	Time (hours)					
		3	6	24	48	72	96
0.0046	No abnormalities detected						
0.0080	Lethargic Lying on bottom of tank					1/19	1/19 1/19
0.015	Lying on bottom of tank Lethargic Loss of equilibrium			1/20	1/15 2/15 1/15	1/14 9/14 1/14	8/8
0.022	Lying on bottom of tank Lethargic Swimming at surface Moribund			2/18 17/18 2/18 1/18	7/9 2/9	4/4	1/2 1/2
0.025	Lethargic Moribund Lying on bottom of tank			15/16 1/16	9/9 1/9	1/7 2/7	2/2

TABLE 1

Cumulative mortality data for bluegill sunfish exposed for 96 hours to Ziram Technical

Concentration mg/l (measured)	Cumulative mortality (initial population: 10 per replicate)					
	Hours					
	3	6	24	48	72	96
Control	0	0	0	0	0	0
Solvent control	0	0	0	0	0	0
0.0046	0	0	0	0	0	0
0.0080	0	0	0	1	1	1
0.015	0	0	0	5	6	12
0.022	0	0	2	11	16	18
0.025	0	0	4	11	13	18

FIGURE 1

Concentration-mortality curve for bluegill sunfish exposed for 96 hours to Ziram Technical

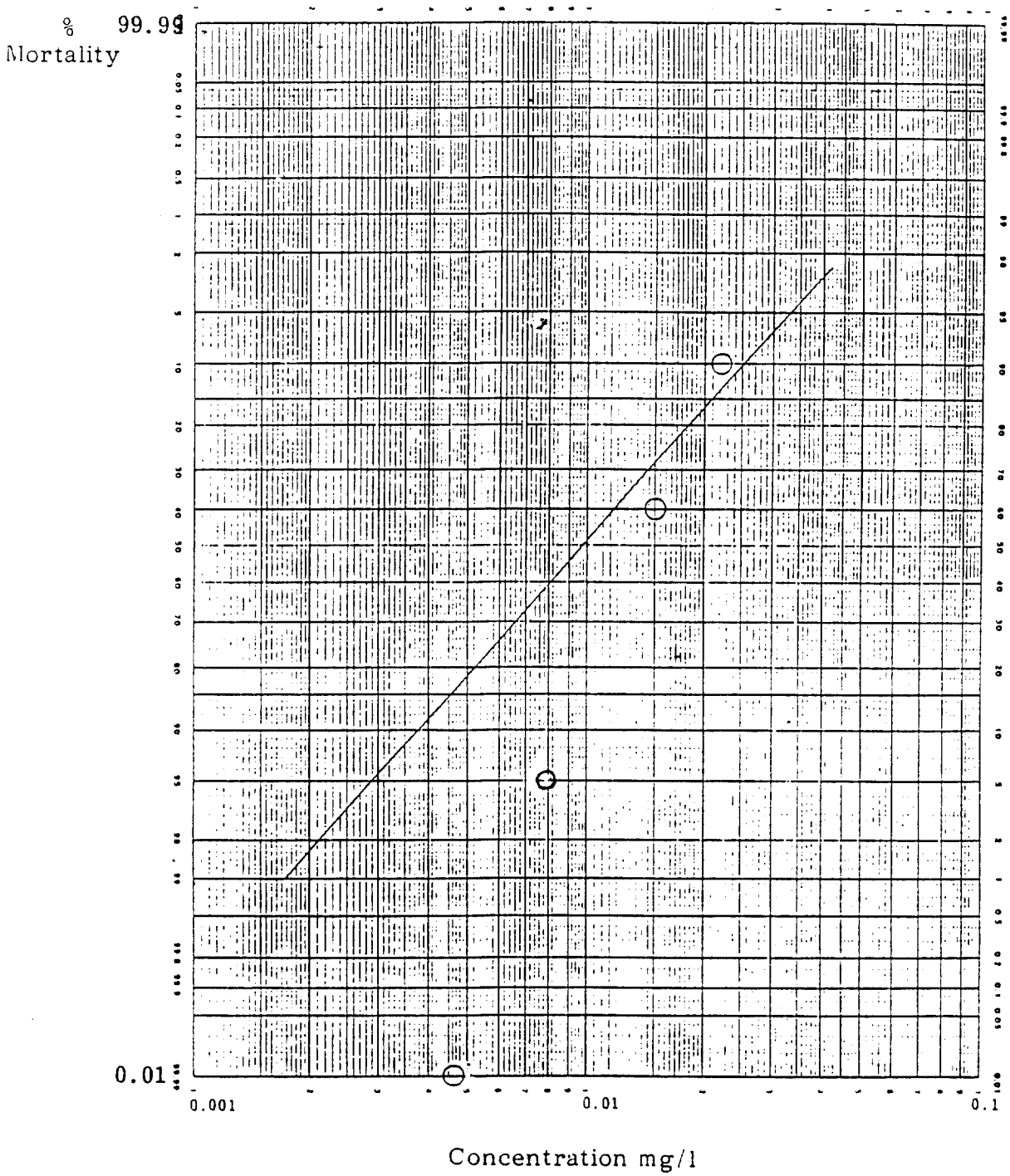
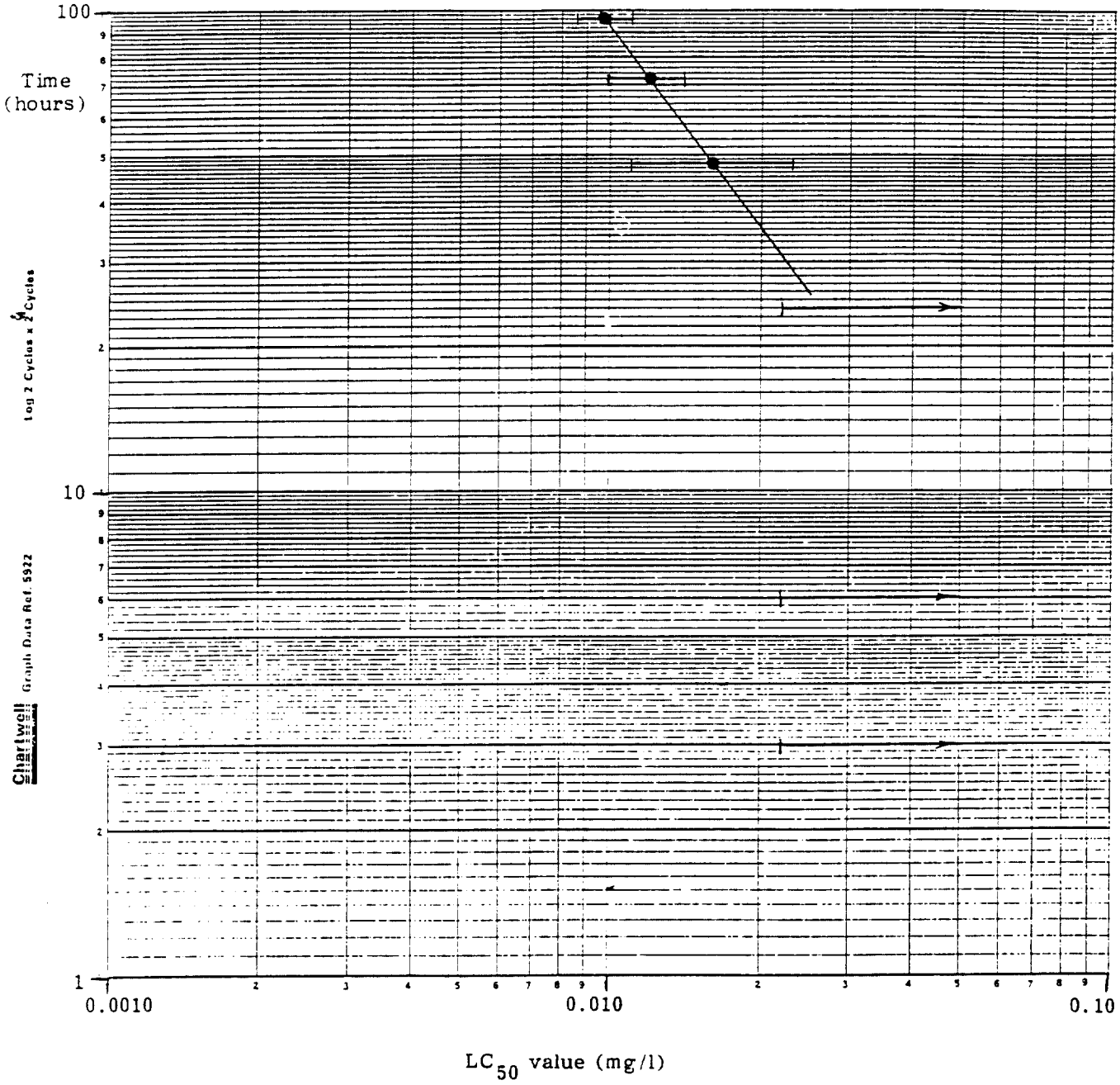


FIGURE 2

Toxicity curve



APPENDICES

APPENDIX 1

Environmental measurements

Concentration mg/l (measured)		0 hours			24 hours			48 hours			72 hours			96 hours		
		pH	mgO_2/l	T°C	pH	mgO_2/l	T°C	pH	mgO_2/l	T°C	pH	mgO_2/l	T°C	pH	mgO_2/l	T°C
Control	R_1	7.5	8.8	21	7.5	8.6	21	7.5	8.6	21	7.5	8.7	21	7.6	8.6	21
	R_2	7.5	8.8	21	7.5	8.6	21	7.5	8.6	21	7.5	8.7	21	7.6	8.6	21
Solvent control	R_1	7.5	8.8	21	7.5	8.6	21	7.5	8.7	21	7.5	8.7	21	7.6	8.6	21
	R_2	7.5	8.8	21	7.5	8.7	21	7.5	8.7	21	7.5	8.7	21	7.6	8.6	21
0.0046	R_1	7.5	8.8	21	7.5	8.7	21	7.5	8.7	21	7.5	8.7	21	7.6	8.6	21
	R_2	7.5	8.8	21	7.5	8.7	21	7.5	8.7	21	7.5	8.7	21	7.6	8.6	21
0.0080	R_1	7.5	8.8	21	7.5	8.7	21	7.5	8.7	21	7.5	8.6	21	7.6	8.6	21
	R_2	7.5	8.8	21	7.5	8.6	21	7.5	8.7	21	7.5	8.6	21	7.7	8.6	21
0.015	R_1	7.5	8.8	21	7.5	8.7	21	7.5	8.7	21	7.5	8.7	21	7.6	8.6	21
	R_2	7.5	8.8	21	7.5	8.7	21	7.5	8.7	21	7.5	8.6	21	7.7	8.6	21
0.022	R_1	7.5	8.8	21	7.5	8.7	21	7.5	8.7	21	7.5	8.7	21	7.6	8.7	21
	R_2	7.5	8.8	21	7.5	8.7	21	7.5	8.7	21	7.6	8.7	21	7.7	8.6	21
0.025	R_1	7.5	8.8	21	7.5	8.7	21	7.5	8.7	21	7.5	8.7	21	A/D	-	-
	R_2	7.5	8.8	21	7.5	8.7	21	7.5	8.7	21	7.6	8.7	21	7.7	8.6	21

R_1 and R_2 Replicates 1 and 2
A/D All fish dead

APPENDIX 2

Verification of test concentrations

Verification of Concentrations of Ziram in Water

Bluegill Study (Schedule No. Zir 20c)

Method of analysis

Samples were analysed by head-space gas analysis following chemical reduction of the Ziram to produce carbon disulphide.

The sample bottles were shaken and aliquots (see table) transferred by bulb pipette to 40 ml vials. The total volume in each vial was made up to 20.0 ml with dechlorinated water (carbon filtered) followed by the addition of 8.0 ml reducing solution (3% stannous chloride dihydrate in 10 M hydrochloric acid). The vials were sealed (screw cap and septum) and heated in a water bath at 80°C for one hour. The vials were then shaken for 30 seconds and allowed to stand at room temperature for one hour to stabilise.

Aliquots (150 mcl) of the head-space gas were withdrawn using a gas-tight syringe and analysed for liberated carbon disulphide (against prepared standards) by gas chromatography using the conditions listed.

Nominal Concentration mg/l	Sample Solution (ml)	Dechlorinated Water (ml)
Untreated	20.0	-
0.0056	20.0	-
0.010	10.0	10.0
0.018	5.0	15.0
0.032	4.0	16.0
0.056	2.0	18.0

APPENDIX 2

(continued)

Calibration solutions	:	Aliquots (5.0 - 50.0 mcl) of a solution of Ziram in acetone (approximately 2.5 mg/l accurately weighed) were injected through the septa of 40 ml vials into dechlorinated water (20.0 ml) and reducing solution (8.0 ml). These solutions were incubated then cooled together with the test solutions.
Blank solution	:	Dechlorinated water (20.0 ml)
Recovery solutions	:	An aliquot (5.0 ml) of a solution of Ziram in acetone (2.5 mg/l) was diluted to 250.0 ml with dechlorinated water (Ziram concentration : 0.05 mg/l). Aliquots (10.0 and 20.0 ml) of this solution were diluted to 100.0 ml to give solutions with concentrations of 0.01 and 0.005 mg/l. These solutions were subsequently treated as test samples with equivalent nominal concentrations of Ziram.

APPENDIX 2

(continued)

Gas Chromatographic Conditions

Instrument	:	Hewlett Packard 5890 gas chromatograph with flame photometric detector operated in the sulphur mode in series with a Spectra-Physics 4270 integrator.
Column	:	2% Carbowax, 20 M + 5% DC 200; 1.8 m x 3 mm i.d.
Injection volume	:	150 mcl (manual injection)
Temperatures:		
Oven	:	70°C
Injector	:	100°C
Detector	:	150°C
Gases:		
Carrier		
Nitrogen/Helium	:	30 ml/min
Detector:		
Air	:	100 ml/min
Hydrogen	:	100 ml/min

Under these conditions carbon disulphide chromatographed as a single peak with a retention time of 0.68 minutes.

The carbon disulphide was quantified by integrated peak area.

Calculations

A linear regression line was calculated using the calibration solution concentrations and the square root of the detector response (peak area).

$$\text{Concentration (Ziram)} = \frac{(\sqrt{\text{peak area of sample}} - A)}{B} \times \frac{20\ \text{ml}}{C}$$

Where A = intercept of regression line on the y axis

B = slope of the regression line

C = sample volume taken (ml)

APPENDIX 2

(continued)

Results Bluegill Study

a) Samples

HRC Reference	Nominal Concentration mg/l	Concentration found mg/l	Concentration expressed as % of nominal
0 Hours			
90/2954	Control	0.0030	-
90/2955	0.0056	0.0035	63
90/2956	0.01	0.0094	94
90/2957	0.018	0.0145	80
90/2958	0.032	0.0237	74
90/2959	0.056	0.0297	53
24 Hours			
90/2997	Control	0.0037	-
90/2998	0.0056	0.0032	58
90/2999	0.01	0.0064	64
90/3000	0.018	0.0152	85
90/3001	0.032	0.0258	81
90/3002	0.056	0.0335	60
96 Hours			
90/3076	Control	0.0012	-
90/3077	0.0056	0.0071	127
90/3078	0.01	0.0081	81
90/3079	0.018	0.0147	82
90/3080	0.032	0.0155	49
90/3081	0.056	0.0108	19

Results not corrected for overall mean recovery (100%).

Limit of detection : 0.0005 mg/l

Reliable limit of determination (samples) : 0.0053 mg/l (2 x mean level found in controls)

APPENDIX 2

(continued)

b) Recoveries

Recovery	Fortification (mg/l)	Recovery
Validation	0.05 0.005	86, 92, 108, 101 92, 95
Procedural	0.01	91, 93, 108, 118, 107, 103
Stability#	0.05	a) 89, 88 b) 89, 94 c) 73, 72

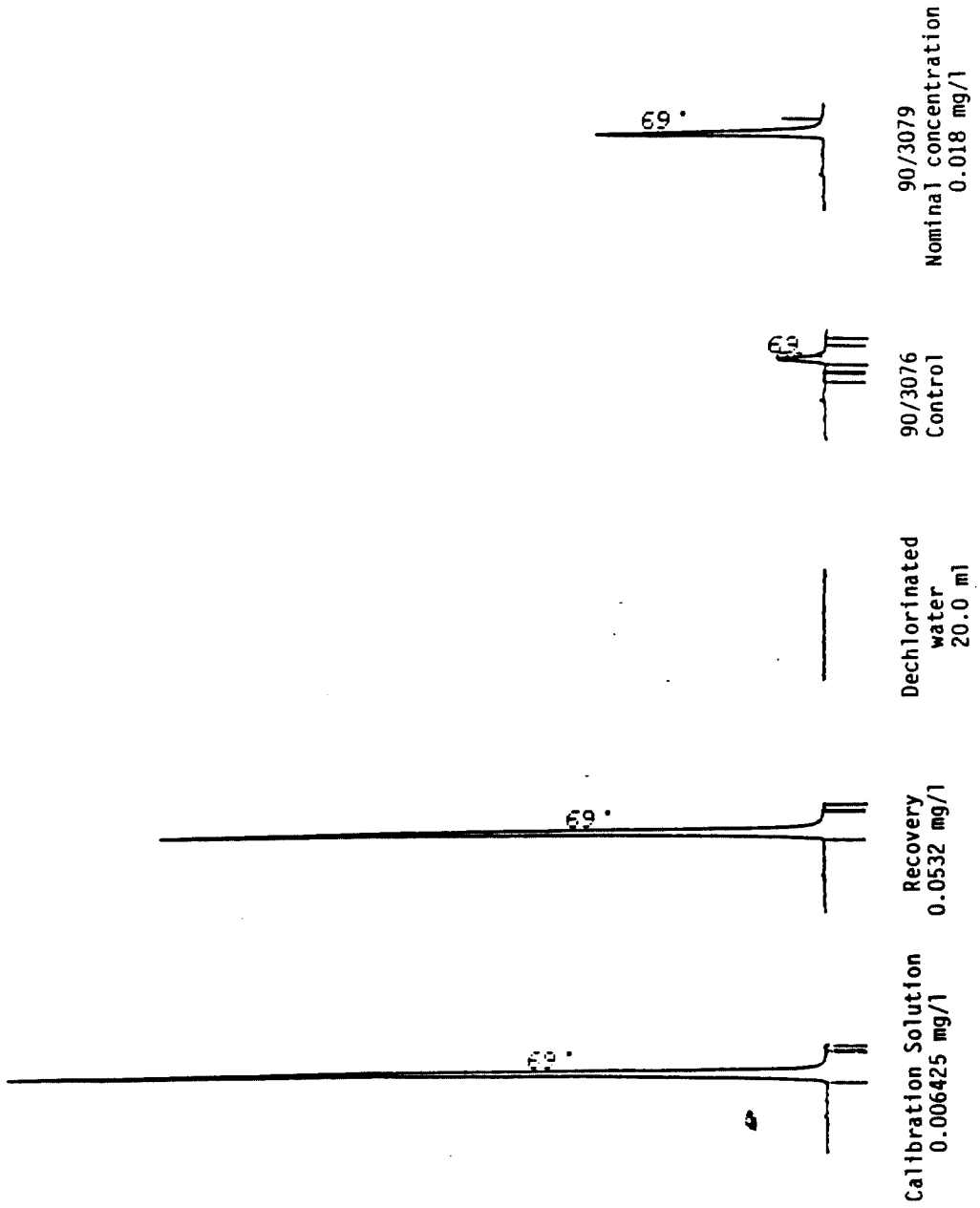
Mean overall recovery (excluding stabilities) 100%.

Solutions stored for 24 hours prior to analysis :
a) In sealed containers in the dark
b) In sealed containers in the light
c) In open containers in the light.

APPENDIX 2

(continued)

Typical Chromatography Bluegill 96 Hours



APPENDIX 3

Typical water quality characteristics

QUALITY OF PUBLIC WATER SUPPLIES.....

PERIOD 01/01/90 TO 31/05/90

HUNTINGDON NORTH SUPPLY ZONE

NAME OF PARAMETER	UNIT	NUMBER OF VALUES	MEAN VALUE	MAXIMUM OR MINIMUM	TYPE OF STATISTIC
ALKALINITY	mg/l	91	200	190	MINIMUM
ALUMINIUM	mg/l	9	<0.0029	0.014	MAXIMUM
AMMONIUM	mg/l	104	< 0.060	0.14	MAXIMUM
ANTIMONY	µg/l	1	*	*	
ARSENIC	µg/l	1	*	*	
BACTERIA-37C	No/ml	13	2.4	6.0	MAXIMUM
BACTERIA-22C 3DAYS	No/ml	13	5.2	11	MAXIMUM
BORON	µg/l	1	360	360	MAXIMUM
CADMIUM	µg/l	1	*	*	
CALCIUM	mg/l	10	130	140	MAXIMUM
CHLORIDE	mg/l	105	62	71	MAXIMUM
CHLORINE RESIDUAL	mg/l	24	0.28	0.20	MINIMUM
CHLOROFORMS TOTAL	µg/l	7	38	46	MAXIMUM
TRIHALOMETHANES	µg/l	7	36	45	MAXIMUM
COBALT	µg/l	1	*	*	
COLIFORMS	No/dl	13	0	0	MAXIMUM
COLOUR	Pt/Co	134	< 4.7	9.0	MAXIMUM
CONDUCTIVITY (20C)	µS/cm	147	900	970	MAXIMUM
COPPER	µg/l	9	45	200	MAXIMUM
CYANIDE	µg/l	1	*	*	
DISSOLVED OXYGEN	%Satn	8	100	94	MINIMUM
E.COLI	No/dl	13	0	0	MAXIMUM
FAECAL STREPTOCOCCI	No/dl	3	0	0	MAXIMUM
FLOORIDE	µg/l	54	250	310	MAXIMUM
FREE CARBON DIOXIDE	mg/l	11	8.7	10	MAXIMUM
HARDNESS TOTAL	mg/l	91	370	350	MINIMUM
HYDROGEN SULPHIDE	µg/l	6	0	0	MAXIMUM
IRON	µg/l	13	32	120	MAXIMUM
LEAD	µg/l	1	3.0	3.0	MAXIMUM
MAGNESIUM	mg/l	9	8.7	8.9	MAXIMUM
MANGANESE	µg/l	12	11	17	MAXIMUM
MERCURY	µg/l	1	*	*	
NICKEL	µg/l	1	6.0	6.0	MAXIMUM
NITRATE	mg/l	107	29	43	MAXIMUM
NITRITE	mg/l	14	< 0.038	0.11	MAXIMUM
ODOUR (25C)	DilNo	6	0	0	MAXIMUM
ORGANIC CARBON TOT.	mg/l	36	4.3	5.0	MAXIMUM
OXIDISABILITY-KMNO4	mg/l	1	2.9	2.9	MAXIMUM
PH	Units	148	7.7	7.9	MAXIMUM
PH	Units	148	7.7	7.4	MINIMUM
POTASSIUM	mg/l	20	8.5	9.6	MAXIMUM
SELENIUM	µg/l	1	*	*	
SILICA	mg/l	117	3.0	7.2	MAXIMUM
SILVER	µg/l	1	*	*	
SODIUM	mg/l	20	43	50	MAXIMUM
SULPHATE	mg/l	119	170	200	MAXIMUM
TASTE (25C)	DilNo	6	0	0	MAXIMUM
TEMPERATURE	Deg-C	114	8.5	14	MAXIMUM
TURBIDITY	FTU	12	0.38	0.60	MAXIMUM
VANADIUM	mg/l	1	*	*	
ZINC	µg/l	9	< 8.9	19	MAXIMUM

* Parameter values below Limit of Detection

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APPENDIX 4

Certificate of analysis

CHEMISCHE SECTOR
MINERALE DIVISIE
VESTIGING TE GENT

Our ref. GM/8862

April 4th, 1990

Ziram technical grade: stability.

The lot number 8331AA, from which a sample has been sent to Huntingdon Research Center for toxicological studies, has been analysed at four different times in this laboratory.
On the other hand a 5 g sample sent back by HRC has also been analysed in this laboratory.
The method used for determinining the active ingredient content is the official CIPAC method [CIPAC 1; 31/1/M.1.2] .
The results are tabulated below:

Date of analysis	% ziram
March 88	99.0 %
Nov. 88	98.9 %
Jan. 89	98.5 %
Dec. 89*	98.8 %
March 90	98.5 %

The above results, which are within the accuracy limits of the method, show that the active ingredient content has been stable over the past two years.

G. Matthijs
Head of Laboratory

J. Swinnen
Head of Technical Department

*5 g sample sent back by HRC

ucb nv
Pantserschipstraat 207
Zeehaven West
Telefoon 091 54 14 11
Telefax 091 54 14 10
B.T.W. 403 053 808
Bank G.B.M. 210-0040887-72

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APPENDIX 4

(continued)

July 18th, 1990

Ziram technical: stability

A subsample (dated 16.7.90) of lot number 8331AA , used by HRC for toxicological studies was sent back to UCB. It was analysed in this laboratory on July 18th.

The active ingredient was determined in threefold, making use of the official CIPAC method [CIPAC 1; 31/1/M.1.2] .

The values found were:

- 99.0 %
- 98.8 %
- 98.9 %

The average value is 98.9 % , against 98.8 % for a sample sent back by HRC in december 1989.

This leads us to the conclusion that there is no deterioration of the active material.

G. Matthijs
Head of Laboratory

A. Schollaert
Director

Contains No CBI

CONFIDENTIAL

ZIR 20(a)/901625

THE ACUTE TOXICITY OF ZIRAM TECHNICAL TO *DAPHNIA MAGNA*

Study completed: 3 September 1991

Regulations: OECD Guideline No. 202 Part 1 and EPA Pesticide Guidelines, Subdivision E, Section 72 - 2

Addressee:

Ziram Task Force,
Consortium No. 62405,
c/o UCB Chemicals Corporation,
5505-A Robin Hood Road,
Norfolk,
VA 23513,
U.S.A.

Report issued: 3 September 1991.

Authors:

Mark T. Douglas,
Robert O. Stonehewer,
Ian A. Macdonald.

Huntingdon Research Centre Ltd.,
P.O. Box 2,
Huntingdon,
Cambridgeshire,
PE18 6ES,
ENGLAND.

SPONSOR

Ziram Task Force,
Consortium No. 62405,
c/o UCB Chemicals Corporation,
5365-A Robin Hood Road,
Norfolk, Virginia 23513 U.S.A.

STUDY TITLE

The Acute Toxicity of Ziram Technical to *Daphnia magna*

DATA REQUIREMENT

OECD Guideline No. 202, Part 1 and EPA Pesticide Guidelines, Subdivision E, Section 72-2

AUTHORS

Mark T. Douglas, Robert O. Stonehewer and Ian A. Macdonald

EXPERIMENTAL PHASE COMPLETED ON

24 August 1990

PERFORMING LAB

Huntingdon Research Centre Ltd.,
P.O. Box 2,
Huntingdon, Cambridgeshire,
PE18 6ES, ENGLAND.

HRC STUDY REPORT NO.

ZIR 20(a)/901625

FINAL REPORT DATE

3 September 1991

STATEMENT OF NO CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10(d) (1) (A), (B), or (C).

COMPANY:	Ziram Task Force c/o UCB Chemicals Corporation
COMPANY AGENT:	Paul R. Larson
DATE:	October 22, 1991
TITLE:	Manager, Agricultural Products
SIGNATURE:	Paul R. Larson

These data are the property of the Ziram Task Force (Consortium No. 62405) and, as such, are considered to be confidential for all purposes other than compliance with FIFRA 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any other statute or in any other country.

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

HRC Report No. ZIR 20(a)/901625

To the best of my knowledge and belief the Study described in this Report was conducted in compliance with the following Good Laboratory Practice Standards:

Good Laboratory Practice, The United Kingdom Compliance Programme, Department of Health & Social Security 1986 and subsequent revision, Department of Health, 1989.

United States Food and Drug Administration, Title 21 Code of Federal Regulations Part 58, Federal Register 22 December 1978, and subsequent Amendments.

United States Environmental Protection Agency, (FIFRA), Title 40 Code of Federal Regulations Part 160, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

United States Environmental Protection Agency, (TSCA), Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August 1989.

Japan Ministry of Health and Welfare, Notification No. YAKUHATSU 313 Pharmaceutical Affairs Bureau, 31 March 1982 and subsequent amendment Notification No. YAKUHATSU 870, Pharmaceutical Affairs Bureau, 5 October 1988.

Japan Ministry of Agriculture, Forestry and Fisheries, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984.

Japan Ministry of International Trade and Industry, Directive 31 March 1984 (Kanpogyo No. 39 Environmental Agency, Kikyoku No. 85 MITI).

Organisation for Economic Co-operation and Development, ISBN 92-64-12367-9, Paris 1982.

Mark T. Douglas, B.Sc., Ph.D.,
Study Director,
Huntingdon Research Centre Ltd.

3 Sept 91
Date

David H. Christopher, Ph.D., D.I.C.,
For Laboratory Management

22/8/91
Date

Submitter/Sponsor

22/10/91
Date

We the undersigned, hereby declare that the work was performed under our supervision according to the procedures herein described, and that this report provides a correct and faithful record of the results obtained.

Mark T. Douglas, B.Sc., Ph.D.,
Head of Unit,
Unit of Fish Toxicology

Robert O. Stonehewer, B.Sc.,
Scientific Officer,
Unit of Fish Toxicology

Ian A. Macdonald, L.R.S.C.,
Unit Head, Pesticide and Environmental Analysis,
Department of Analytical Chemistry

QUALITY ASSURANCE STATEMENT

HRC Report No. ZIR 20(a)/901625

Certain studies such as that described in this report, are conducted at HRC in a setting which involves frequent repetition of similar or identical procedures. At or about the time the study described in this report was in progress, "process-based" inspections were made by the Quality Assurance Department of critical procedures relevant to this study type. For the inspection of any given procedure, at least one study was selected without bias. The findings of these inspections were reported promptly to the Study Director and to HRC Management.

This report has been audited by HRC Quality Assurance Department. It is considered to be an accurate description of the procedures and practices employed during the course of the study and an accurate presentation of the findings.

Date of inspection	04-14.06.90
Date of reporting Inspection Findings to the Study Director and HRC Management	15.06.90
Date of reporting Audit Findings to the Study Director and HRC Management	07.01.91

28.8.91

Peter H.C.V. Richold, B.Sc.,
Senior Systems Compliance Auditor,
Department of Quality Assurance,
Huntingdon Research Centre Ltd.

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SUMMARY

Test Substance	:	Ziram Technical.
Test Species	:	*Daphnia magna* (Straus) tested in the 1st instar.
Test Type	:	Acute toxicity. 48 h EC_{50} (immobilisation).
Test Conditions	:	Static test conditions according to OECD Guideline No. 202 Part 1 and EPA Pesticide Guidelines, Subdivision E, Section 72-2.

Results

Test substance : Ziram Technical.

Time (h)	EC_{50} (mg/l)	95% confidence limits (mg/l)
24	0.15	0.065 - 0.34
48	0.048	0.034 - 0.068

"no effect" level at 24 h = 0.015 mg/l
"no effect" level at 48 h = 0.0026* mg/l

All results are expressed in terms of measured concentration.

* This value is below the reliable limit of determination of Ziram in water and should, therefore, be regarded as an estimate rather than a definitive value.

INTRODUCTION

This report contains a description of the methods used and the results obtained during a study to investigate the acute toxicity of Ziram Technical to *Daphnia magna*.

The study was commissioned by The Ziram Task Force and undertaken between 22 and 24 August 1990.

Dates of Protocol approval

Study Director	:	20 January 1989.
HRC Ltd. Management	:	20 January 1989.
Sponsor	:	3 April 1989.

MATERIALS AND METHODS

Test Substance

Identification	:	Ziram Technical. Zinc bis(dimethyldithiocarbamate).
Description	:	White powder.
Purity	:	98.9% (Lot No. 8331 AA reanalysed on 18 July 1990).
Major impurities	:	Na_2SO_4 (1%) and water (0.23%).
Date of receipt	:	Received from UCB SA., Brussels, Belgium on 12 April 1989.
Storage	:	In darkness at room temperature.
Expiry	:	Subsamples analysed periodically to confirm stability of sample (see Appendix 4).
Method of preparation	:	Direct dispersion in water. A stock solution of 50 mg/l was prepared by dissolving the test sample in test water with the aid of ultrasonic disruption. The test series was prepared by further dilution of this stock with test water. No precipitate was observed during the preparation of stock or test solutions.
Stability of test concentrations	:	Verified by chemical analysis. Water samples were taken from control and test vessels (replicates pooled) at 0 h and 48 h and sent to the HRC Department of Analytical Chemistry for analysis (see Appendix 2).

Test Species

Name	:	*Daphnia magna* (Straus).
Source	:	Laboratory culture originating from a strain supplied by IRCHA, France.

Culture	:	At 20 ± 2°C in glass vessels containing two litres of dechlorinated and aged tap-water. Cultures were fed daily with a mixture of fry fish food (Liquifry®) and a suspension of mixed algae (predominantly *Scenedesmus* or *Selenastrum* spp.). Culture conditions ensure that reproduction is by parthenogenesis.
Selection	:	Gravid adults were isolated 24 hours prior to initiation of the test. Young daphnids produced overnight were used for testing.

Test Water

Dechlorinated (with sodium thiosulphate) and aged laboratory tap water. Total hardness ≈ 350 mg/l as $CaCO_3$. This value is slightly higher than the recommended range but is not considered to have had any significant effects on the results of this test.

Exposure Conditions

Test vessels	:	Glass jars each containing 200 ml test solution.
Experimental design	:	9 test concentrations plus 1 control, each in duplicate. 20 animals per test concentration.
Method of initiation	:	*Daphnia* were placed in the test solutions after addition of the test substance.
Loading	:	20 ml test solution per organism.
Photoperiod	:	16 h light : 8 h dark.
Temperature	:	21°C.
Aeration	:	None.
Nominal test concentrations	:	0.00032, 0.00056, 0.001, 0.0018, 0.0032, 0.0056, 0.01, 0.018, 0.032, 0.056 and 0.1 mg/l.

Measured test concentrations	:	0.0012*, 0.0012*, 0.0026*, 0.0017*, 0.0072, 0.0055, 0.0094, 0.015, 0.032, 0.048 and 0.096 mg/l (mean of samples collected at 0 h and 48 h). * Below the limit of reliable determination (0.0034 mg/l).
Medium renewal	:	None.
Duration of exposure	:	48 hours.
Criterion of effect	:	*Daphnia* were considered to be immobilised if they were unable to swim for approximately 15 seconds after gentle agitation.

Records

All raw data and other documents generated at HRC during the course of this study, together with a copy of this final report, have been lodged in the HRC Archives, Huntingdon, England.

RESULTS

Cumulative immobilisation data are given in Table 1 and the relationships between percentage immobilisation and concentration at 24 h and 48 h are given in Figures 1 and 2. All results are expressed in terms of measured concentration (mean of samples collected at 0h and 48 h). All calculations have been conducted using data obtained from concentrations greater than the reliable limit of determination of Ziram in water (i.e. 0.0034 mg/l).

Analysis* of the immobility data gave the following results:

Time (h)	EC_{50} (mg/l)	95% confidence limits (mg/l)
24	0.15	0.065 - 0.34
48	0.048	0.034 - 0.068

"no-effect" level≠ at 24 h = 0.015 mg/l
"no-effect" level≠ at 48 h = 0.0026* mg/l

* The "no-effect" level at 48 h is below the reliable limit of determination of Ziram in water. The value of 0.0026 mg/l should, therefore, be regarded as an estimate rather than as a definitive value.

≠ Immobilisation less than or equal to 10%
* Thompson, W.R. and Weil, C.S. (1952) Biometrics *8*, 51-54.

TABLE 1

Cumulative immobilisation data for *Daphnia magna* exposed for 48 hours to Ziram Technical

Concentration mg/l (measured)	Cumulative immobilised *Daphnia* (initial population: 10 per replicate)							
	24 hours				48 hours			
	R_1	R_2	Total	%	R_1	R_2	Total	%
Control	0	0	0	0	0	0	0	0
0.0012*	0	0	0	0	0	0	0	0
0.0012*	0	0	0	0	0	0	0	0
0.0026*	0	0	0	0	0	0	0	0
0.0017*	0	0	0	0	0	0	0	0
0.0072	1	0	1	5	2	1	3	15
0.0055	1	1	2	10	1	2	3	15
0.0094	0	1	1	5	2	2	4	20
0.015	1	0	1	5	2	3	5	25
0.032	2	1	3	15	4	3	7	35
0.048	1	2	3	15	6	6	12	60
0.096	6	5	11	55	10	10	20	100

R_1 Replicate 1
R_2 Replicate 2
* Below the limit of reliable determination

FIGURE 1

Concentration-response curve for *Daphnia magna* exposed for 24 hours to Ziram Technical

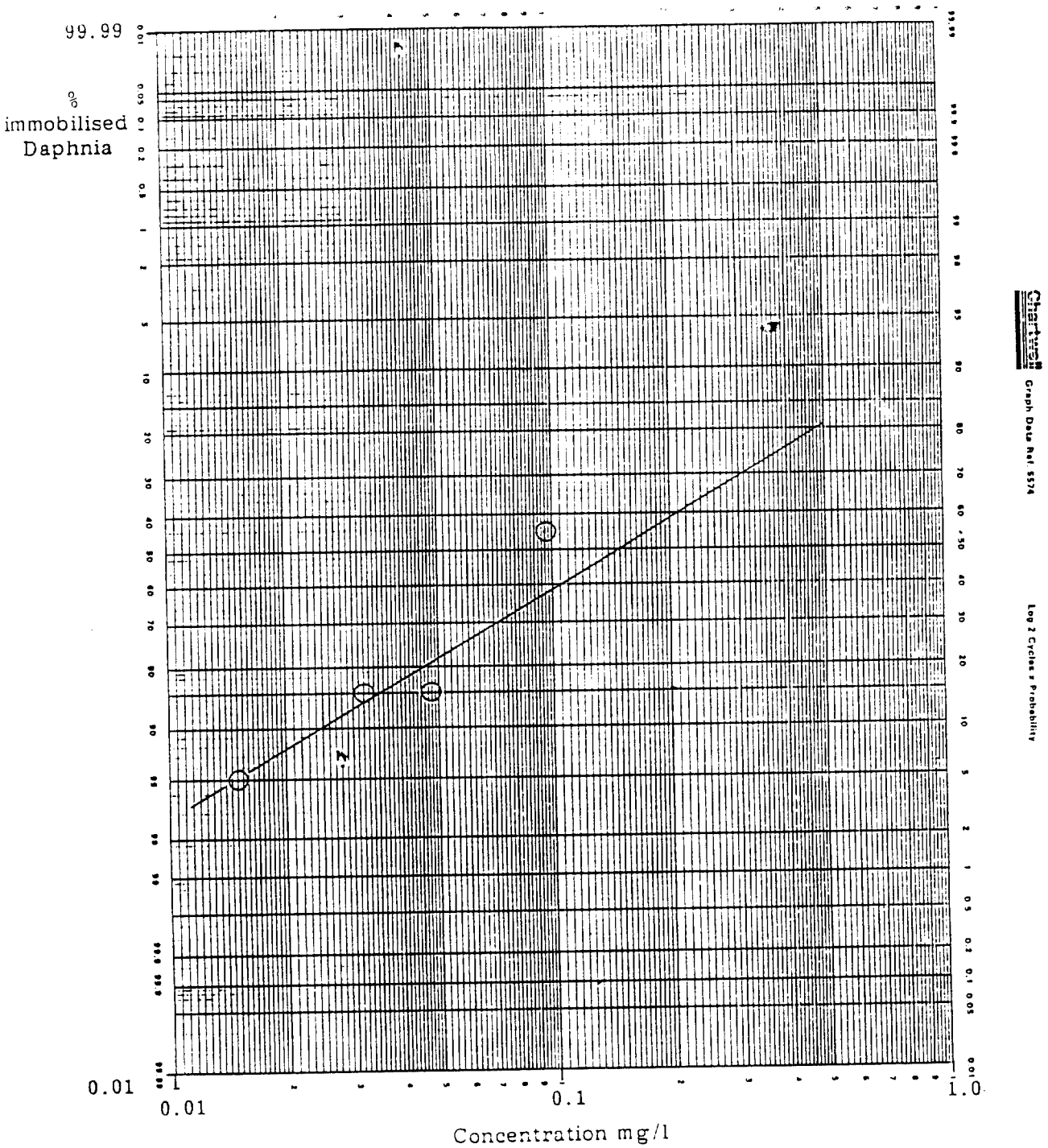
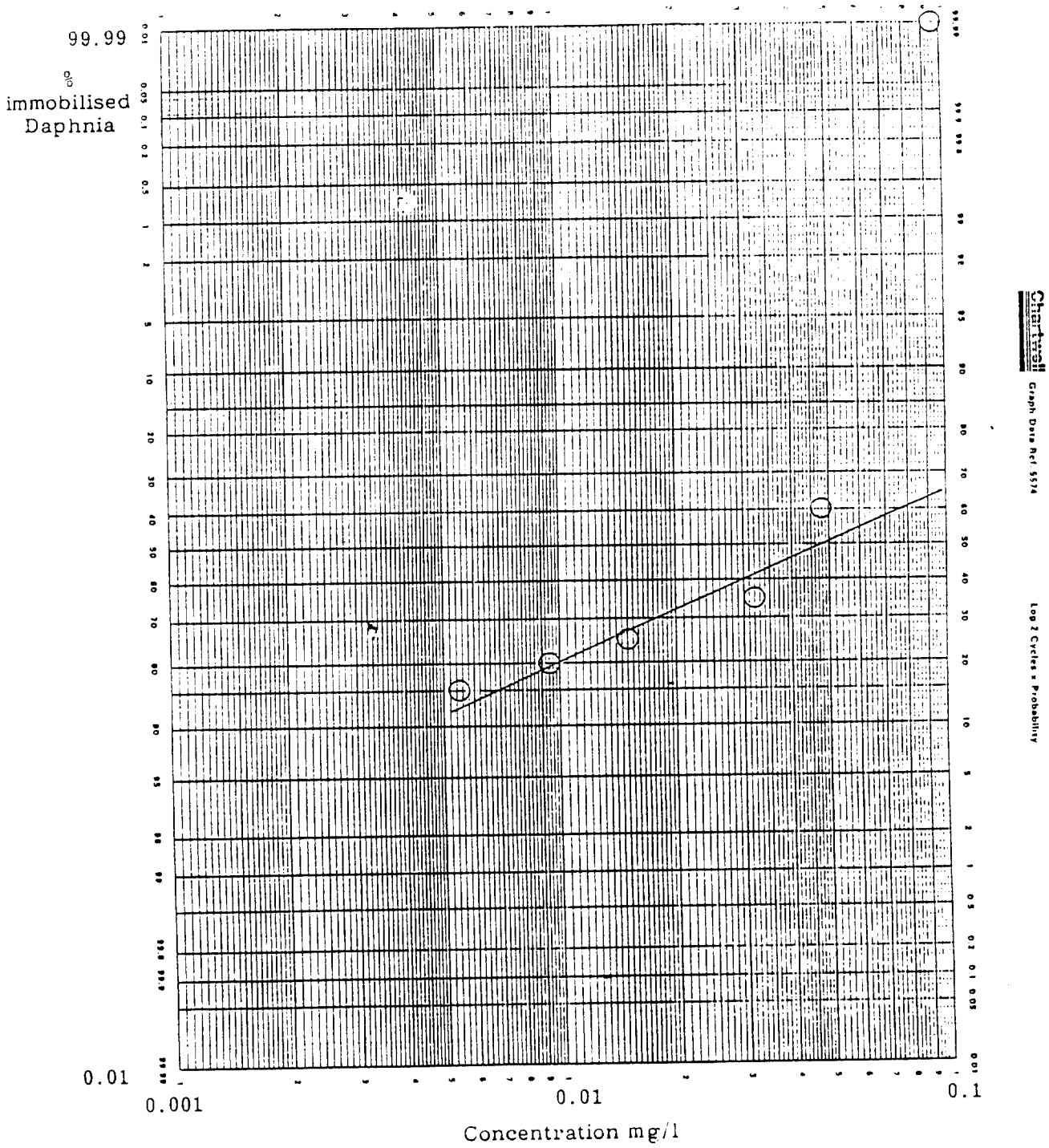


FIGURE 2

Concentration-response curve for *Daphnia magna* exposed for 48 hours to Ziram Technical



APPENDICES

APPENDIX 1

Environmental measurements

Concentration mg/l (measured)		0 hours			24 hours	48 hours		
		pH	mgO_2/l	T°C	T°C	pH	mgO_2/l	T°C
Control	R_1	8.0	8.9	21	21	8.0	8.7	21
	R_2	8.0	8.9	21	21	8.0	8.7	21
0.0012*	R_1	8.0	8.9	21	21	8.0	8.6	21
	R_2	8.0	8.9	21	21	8.0	8.6	21
0.0012*	R_1	8.0	8.9	21	21	8.0	8.7	21
	R_2	8.0	8.9	21	21	8.0	8.6	21
0.0026*	R_1	8.0	8.9	21	21	8.0	8.6	21
	R_2	8.0	8.9	21	21	8.0	8.6	21
0.0017*	R_1	8.0	8.9	21	21	8.0	8.6	21
	R_2	8.0	8.9	21	21	8.0	8.6	21
0.0072	R_1	8.0	8.9	21	21	8.0	8.6	21
	R_2	8.0	8.9	21	21	8.0	8.6	21
0.0055	R_1	8.0	8.9	21	21	8.0	8.6	21
	R_2	8.0	8.9	21	21	8.0	8.6	21
0.0094	R_1	8.0	8.9	21	21	8.0	8.6	21
	R_2	8.0	8.9	21	21	8.0	8.6	21
0.015	R_1	8.0	8.9	21	21	8.0	8.6	21
	R_2	8.0	8.9	21	21	8.0	8.6	21
0.032	R_1	8.0	8.9	21	21	8.0	8.6	21
	R_2	8.0	8.9	21	21	8.0	8.6	21
0.048	R_1	8.0	8.9	21	21	8.0	8.6	21
	R_2	8.0	8.9	21	21	8.0	8.6	21
0.096	R_1	8.0	8.9	21	21	8.0	8.6	21
	R_2	8.0	8.9	21	21	8.0	8.6	21

R_1 Replicate 1
R_2 Replicate 2
* Below the limit of reliable detection

APPENDIX 2

Verification of test concentrations

Verification of Concentrations of Ziram in Water

Daphnia Study (Schedule No. ZIR 20a)

Method of analysis

Samples were analysed by head-space gas analysis following chemical reduction of the Ziram to produce carbon disulphide.

The sample bottles were shaken and aliquots (see table) transferred by bulb pipette to 40 ml vials. The total volume in each vial was made up to 20.0 ml with dechlorinated water (carbon filtered) followed by the addition of 8.0 ml reducing solution (3% stannous chloride dihydrate in 10 M hydrochloric acid). The vials were sealed (screw cap and septum) and heated in a water bath at 80°C for one hour. The vials were then shaken for 30 seconds and allowed to stand at room temperature for one hour to stabilise.

Aliquots (150 mcl) of the head-space gas were withdrawn using a gas-tight syringe and analysed for liberated carbon disulphide (against prepared standards) by gas chromatography using the conditions listed.

Nominal Concentration (mg/l)	Sample Solution (ml)	Dechlorinated Water (ml)
Untreated	20.0	-
0.00032	20.0	-
0.00056	20.0	-
0.001	20.0	-
0.0018	20.0	-
0.0032	15.0	5.0
0.0056	10.0	10.0
0.01	5.0	15.0
0.018	3.0	17.0
0.032	1.5	18.5
0.056	1.0	19.0
0.1	0.5	19.5

APPENDIX 2

(continued)

Calibration solutions	:	0.00030-0.003125 mg/l Aliquots (5.0-50.0 mcl) of a solution of Ziram in acetone (1.25 mg/l) were injected through the septa of 40 ml vials into dechlorinated water (20.0 ml) and reducing solution (8.0 ml). These solutions were incubated then cooled together with the test solutions.
Blank solution	:	Dechlorinated water (20.0 ml).
Recovery solutions	:	A solution of Ziram in acetone (1.25 mg/l) was serially diluted with dechlorinated water to give solutions with concentrations of 0.1, 0.03, 0.003 and 0.0003 mg/l. These solutions were subsequently treated as test samples with equivalent nominal concentrations of Ziram.

APPENDIX 2

(continued)

Gas Chromatographic Conditions

Instrument	:	Hewlett Packard 5890 gas chromatograph with flame photometric detector operated in the sulphur mode in series with a Spectra-Physics 4270 integrator.
Column	:	2% Carbowax, 20 M + 5% DC 200; 1.8 m x 3 mm i.d.
Injection volume	:	200 mcl (manual injection)
Temperatures:		
Oven	:	70°C
Injector	:	100°C
Detector	:	150°C
Gases:		
Carrier Nitrogen/Helium:		40 ml/min
Detector:		
Air	:	100 ml/min
Hydrogen	:	75 ml/min

Under these conditions carbon disulphide chromatographed as a single peak with a retention time of 0.58 minutes.

The carbon disulphide was quantified by integrated peak area.

Calculations

A linear regression line was calculated using the calibration solution concentrations and the square root of the detector response (peak area).

$$\text{Concentration (Ziram)} = \frac{(\sqrt{\text{peak area of sample}} - A)}{B} \times \frac{20\ \text{ml}}{C}$$

Where A = intercept of regression line on the y axis

B = slope of the regression line

C = sample volume taken (ml)

APPENDIX 2

(continued)

Results - Daphnia Study

a) Samples

HRC Reference	Nominal Concentration (mg/l)	Concentration found (mg/l)	Concentration expressed as % of nominal
0 Hours			
90/4751	Control	0.0013	-
90/4752	0.00032	0.0009	281
90/4753	0.00056	0.0015	268
90/4754	0.001	0.0026	260
90/4755	0.0018	0.0022	122
90/4756	0.0032	0.0050	156
90/4757	0.0056	0.0066	118
90/4758	0.01	0.0100	100
90/4759	0.018	0.0167	93
90/4760	0.032	0.0302	94
90/4761	0.056	0.0483	86
90/4762	0.1	0.1096	110
48 Hours			
90/4870	Control	0.0021	-
90/4871	0.00032	0.0015	469
90/4872	0.00056	0.0009	161
90/4873	0.001	0.0027	270
90/4874	0.0018	0.0013	72
90/4875	0.0032	0.0093	291
90/4876	0.0056	0.0044	79
90/4877	0.01	0.0087	87
90/4878	0.018	0.0134	74
90/4879	0.032	0.0335	105
90/4880	0.056	0.0473	84
90/4881	0.1	0.0822	82

Results not corrected for overall mean recovery (107%).
Limit of detection : 0.0001 mg/l
Reliable limit of determination (samples) : 0.0034 mg/l
(2 x mean level found in controls)

APPENDIX 2

(continued)

b) Recoveries

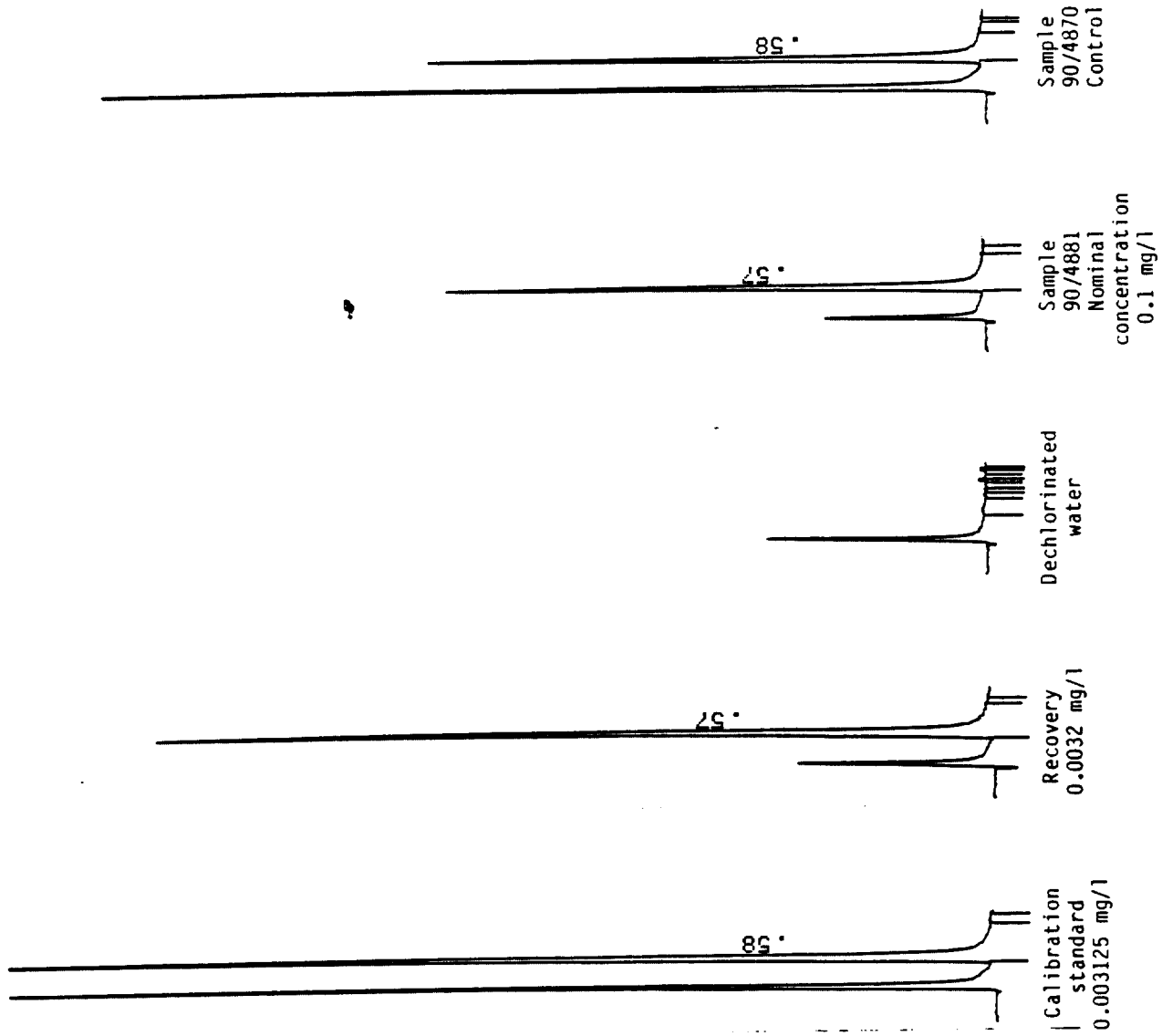
Recovery	Fortification (mg/l)	Recovery (%)
Validation	0.1 0.0003*	105, 107 74, 45, 69
Procedural	0.03 0.003	105 109
Stability#	0.05	a) 89, 88 b) 89, 94 c) 73, 72

Mean overall recovery (excluding stabilities and 0.0003 mg/l level) 107%.
* Below reliable limit of determination.
# Solutions stored for 24 hours prior to analysis:
a) In sealed containers in the dark
b) In sealed containers in the light
c) In open containers in the light

APPENDIX 2

(continued)

Typical Chromatography : Daphnia 48 Hours



APPENDIX 3

Typical water quality characteristics

QUALITY OF PUBLIC WATER SUPPLIES.....

PERIOD 01/01/90 TO 31/05/90

HUNTINGDON NORTH SUPPLY ZONE

NAME OF PARAMETER	UNIT	NUMBER OF VALUES	MEAN VALUE	MAXIMUM OR MINIMUM	TYPE OF STATISTIC
ALKALINITY	mg/l	91	200	190	MINIMUM
ALUMINIUM	mg/l	9	<0.0029	0.014	MAXIMUM
AMMONIUM	mg/l	104	< 0.060	0.14	MAXIMUM
ANTIMONY	µg/l	1	*	*	
ARSENIC	µg/l	1	*	*	
BACTERIA-37C	No/ml	13	2.4	6.0	MAXIMUM
BACTERIA-22C 3DAYS	No/ml	13	5.2	11	MAXIMUM
BORON	µg/l	1	360	360	MAXIMUM
CADMIUM	µg/l	1	*	*	
CALCIUM	mg/l	10	130	140	MAXIMUM
CHLORIDE	mg/l	105	62	71	MAXIMUM
CHLORINE RESIDUAL	mg/l	24	0.28	0.20	MINIMUM
CHLOROFORMS TOTAL	µg/l	7	38	46	MAXIMUM
TRIHALOMETHANES	µg/l	7	36	45	MAXIMUM
COBALT	µg/l	1	*	*	
COLIFORMS	No/dl	13	0	0	MAXIMUM
COLOUR	Pt/Co	134	< 4.7	9.0	MAXIMUM
CONDUCTIVITY (20C)	µS/cm	147	900	970	MAXIMUM
COPPER	µg/l	9	45	200	MAXIMUM
CYANIDE	µg/l	1	*	*	
DISSOLVED OXYGEN	%Satn	8	100	94	MINIMUM
E.COLI	No/dl	13	0	0	MAXIMUM
FAECAL STREPTOCOCCI	No/dl	3	0	0	MAXIMUM
FLUORIDE	µg/l	54	250	310	MAXIMUM
FREE CARBON DIOXIDE	mg/l	11	8.7	10	MAXIMUM
HARDNESS TOTAL	mg/l	91	370	350	MINIMUM
HYDROGEN SULPHIDE	µg/l	6	0	0	MAXIMUM
IRON	µg/l	13	32	120	MAXIMUM
LEAD	µg/l	1	3.0	3.0	MAXIMUM
MAGNESIUM	mg/l	9	8.7	8.9	MAXIMUM
MANGANESE	µg/l	12	11	17	MAXIMUM
MERCURY	µg/l	1	*	*	
NICKEL	µg/l	1	6.0	6.0	MAXIMUM
NITRATE	mg/l	107	29	43	MAXIMUM
NITRITE	mg/l	14	< 0.038	0.11	MAXIMUM
ODOUR (25C)	DilNo	6	0	0	MAXIMUM
ORGANIC CARBON TOT.	mg/l	36	4.3	5.0	MAXIMUM
OXIDISABILITY-KMNO4	mg/l	1	2.9	2.9	MAXIMUM
PH	Units	148	7.7	7.9	MAXIMUM
PH	Units	148	7.7	7.4	MINIMUM
POTASSIUM	mg/l	20	8.5	9.6	MAXIMUM
SELENIUM	µg/l	1	*	*	
SILICA	mg/l	117	3.0	7.2	MAXIMUM
SILVER	µg/l	1	*	*	
SODIUM	mg/l	20	43	50	MAXIMUM
SULPHATE	mg/l	119	170	200	MAXIMUM
TASTE (25C)	DilNo	6	0	0	MAXIMUM
TEMPERATURE	Deg-C	114	8.5	14	MAXIMUM
TURBIDITY	FTU	12	0.38	0.60	MAXIMUM
VANADIUM	mg/l	1	*	*	
ZINC	µg/l	9	< 8.9	19	MAXIMUM

* Parameter values below Limit of Detection

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APPENDIX 4

Certificate of analysis

CHEMISCHE SECTOR

MINERALE DIVISIE

VESTIGING TE GENT

Our ref. GM/8862

April 4th, 1990

Ziram technical grade: stability.

The lot number 8331AA, from which a sample has been sent to Huntingdon Research Center for toxicological studies, has been analysed at four different times in this laboratory.
On the other hand a 5 g sample sent back by HRC has also been analysed in this laboratory.
The method used for determinining the active ingredient content is the official CIPAC method [CIPAC 1; 31/1/M.1.2] .
The results are tabulated below:

Date of analysis	% ziram
March 88	99.0 %
Nov. 88	98.9 %
Jan. 89	98.5 %
Dec. 89*	98.8 %
March 90	98.5 %

The above results, which are within the accuracy limits of the method, show that the active ingredient content has been stable over the past two years.

G. Matthijs
Head of Laboratory

J. Swinnen
Head of Technical Department

*5 g sample sent back by HRC

ucb nv

Pantserschipstraat 207
Deenaven West

Telefoon 091 54 14 11
Telefax 091 54 14 10

B.T.W. 403 [illegible]
Bank G.B.M. [illegible]

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APPENDIX 4

(continued)

July 18th, 1990

Ziram technical: stability

A subsample (dated 16.7.90) of lot number 8331AA , used by HRC for toxicological studies was sent back to UCB. It was analysed in this laboratory on July 18th.

The active ingredient was determined in threefold, making use of the official CIPAC method (CIPAC 1; 31/1/M.1.2) .

The values found were:

- 99.0 %
- 98.8 %
- 98.9 %

The average value is 98.9 % , against 98.8 % for a sample sent back by HRC in december 1989.

This leads us to the conclusion that there is no deterioration of the active material.

G. Matthijs
Head of Laboratory

A. Schollaert
Director

Contains No CBI

ZIRAM

PRELIMINARY DIETARY TOXICITY STUDY IN BEAGLE DOGS

(Final report -

repeated daily dosage for 4 weeks)

Addressee:

Ziram Task Force,
c/o UCB Chemicals Corporation,
5365-A Robin Hood Road,
Norfolk,
VA 23513,
U.S.A.

Report issued: 30 April 1992.

Authors:

Timothy A. McLean,
Steven A. Horner,
David P. Buist.

Huntingdon Research Centre Ltd.,
P.O. Box 2,
Huntingdon,
Cambridgeshire,
PE18 6ES,
ENGLAND.

SPONSOR

Ziram Task Force,
c/o UCB Chemicals Corporation,
5365-A Robin Hood Road,
Norfolk,
VA 23513,
U.S.A.

STUDY TITLE

ZIRAM PRELIMINARY DIETARY TOXICITY STUDY IN BEAGLE DOGS

AUTHORS

Timothy A. McLean,
Steven A. Horner,
David P. Buist.

STUDY COMPLETED ON

30 April 1992.

PERFORMING LAB

Huntingdon Research Centre Ltd.,
P.O. Box 2,
Huntingdon,
Cambridgeshire,
PE18 6ES,
ENGLAND.

HRC STUDY REPORT NO.

ZIR 1-G/89637

FINAL REPORT DATE

30 April 1992.

CONFIDENTIALITY STATEMENT

This report contains the unpublished results of research sponsored by ZIRAM TASK FORCE. These results may not be published, either wholly or in part, or reviewed or quoted in any other publication without the prior authorisation of the Sponsor.

We the undersigned, hereby declare that the work was performed under our supervision according to the procedures herein described, and that this report provides a correct and faithful record of the results obtained.

Timothy A. McLean, B.Sc. (Hons.), M.Sc., C.Biol., M,I,Biol.,
Study Director, (from 29 May 1991)
Department of Non-Rodent Toxicology.

Steven A. Horner, B.Sc.,(Hons.), M.Sc., Ph.D., C.Biol., M.I.Biol.,
Study Director, (until 29 May 1991)
Department of Dog Toxicology

David P. Buist, B.Vet.Med., M.R.C.V.S.,
Veterinary Director,
Veterinary Services

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Test material:	Ziram.
Test species:	Pure-bred beagle dogs (three pairs, each of 1♂ and 1♀).
Route of administration:	Oral, by admixture with the diet.
Duration of dosing:	Up to 4 weeks.
Treatment levels:	Pair I - 2000 ppm Pair II - 5000 ppm Pair III - 1000 ppm
Dosing commenced:	19 January 1989 - Pair I 2 February 1989 - Pair II 23 February 1989 - Pair III

Results

Mortalities:	**Dog 594♀ (2000 ppm) was killed for humane reasons on Day 22 of treatment following convulsive episodes.** **Dogs 595♂ and 596♀ (5000 ppm) were killed for humane reasons on Day 14 of treatment following inappetence, loss of bodyweight and loss in condition.**
Clinical signs:	**Liquid faeces were passed by Dog 593♂ (2000 ppm) on one occasion on Days 1 and 16 of treatment, for Dog 594♀ (2000 ppm) on one occasion on Day 14, for Dog 595♂ (5000 ppm) on two occasions on Day 1 and for Dog 596♀ (5000 ppm) on one occasion on Days 1 and 2 of dosing.**

Vomiting was observed on Day 1 of dosing for Dogs 593♂ (2000 ppm), 595♂ and 596♀ (5000 ppm). Vomiting was also seen on two occasions for Dog 596♀ (5000 ppm) on Day 8 and on one occasion for Dog 598♀ (1000 ppm) on Day 28 of treatment.

Liquid faeces were usually produced from 1½ - 5½ hours after feeding, whilst vomiting was usually seen within 1½ - 2½ hours of feeding.

On Day 22, two convulsive episodes of up to 5 minutes duration were seen for Dog 594♀ (2000 ppm) at approximately 2½ and 4 hours after feeding. Following each convulsion the dog was subdued and unsteady. Recovery had not occurred at the end of the working day and following veterinary advice it was considered necessary to kill this dog for humane reasons.

Bodyweight:

Bodyweight gain was generally considered to be satisfactory for all animals prior to the commencement of treatment and for Dogs 593♂ (2000 ppm) and 597♂ (1000 ppm) throughout the treatment period, and for Dog 594♀ (2000 ppm) prior to its death on Day 22. For Dog 598♀ (1000 ppm), a slight loss in bodyweight of 0.2 kg was seen over the treatment period.

For both dogs receiving 5000 ppm, however, bodyweight loss of up to 1.5 kg was seen over the first 14 days of treatment and was associated with inappetence and/or loss in clinical condition.

Food consumption:

Food consumption was generally considered to be satisfactory for all animals during the pre-dosing period, although Dog 598♀ consistently ate less food than other dogs.

During the treatment period, food consumption was satisfactory for Dog 593♂ (2000 ppm) and Dog 597♂ (1000 ppm). Food consumption for Dog 594♀ (2000 ppm), Days 1 - 22, and Dog 598♀ (1000 ppm) was slightly reduced in comparison to pre-dosing values.

Slightly reduced food intake, in comparison to pre-dose values, was also seen for Dog 595♂ (5000 ppm) during the first 2 weeks of treatment. Inappetence was seen for Dog 596♀ (5000 ppm) over the first 2 weeks of treatment and was associated with bodyweight loss and loss of condition for this animal.

Terminal studies:

Dog 594♀ (2000 ppm) was killed on Day 22 following convulsive episodes. Dogs 595♂ and 596♀ (5000 ppm) were killed on Day 14 following inappetence, loss in bodyweight and/or loss of condition. Dogs 593♂ (2000 ppm), 597♂ and 598♀ (1000 ppm) were killed on completion of 4 weeks treatment.

Macroscopic post mortem findings:

Macroscopic post mortem findings included:

At 2000 ppm

Dog 593♂ - several foci of red mucosal congestion in jejunum.
Dog 594♀ - red streak on aortic valve of heart;
- cervical lymph node congested.

At 5000 ppm

Dog 595♂ - thyroids and muscles generally pale in colour.
Dog 596♀ - fluid-filled cyst on right ovary;
- area of red discolouration on left diaphragmatic lobe of lungs.

There were no macroscopic post mortem findings for dogs receiving 1000 ppm.

Organ weights:

Liver weight for Dog 593♂ (2000 ppm), Dog 595♂ (5000 ppm) and Dog 597♂ (1000 ppm) exceeded our normal upper limit of 4.0% of bodyweight.

All other organ weights were considered to be within normal limits for dogs of this age.

Histopathology:

Histopathological examination of tissues was not performed.

Conclusion

Following daily oral administration of Ziram to beagle dogs at dietary concentrations of 1000, 2000 or 5000 ppm for up to 4 weeks, notable changes were as follows:

At 1000 ppm
- vomiting on 1 occasion for the female;
- very slight bodyweight loss and slightly reduced food consumption for the female;
- high liver weight, exceeding our normal upper limit of 4.0% of bodyweight, for the male.

At 2000 ppm
- convulsive episodes for the female, requiring the animal to be killed for humane reasons;
- occasional liquid faeces for both animals and vomiting for the male, on 1 occasion;
- slightly reduced food consumption for the female;
- high liver weight, exceeding our normal upper limit of 4.0% of bodyweight, for the male.

At 5000 ppm - occasional liquid faeces and vomiting for both animals;
- inappetence, bodyweight loss and associated loss in clinical condition, requiring both animals to be killed for humane reasons;
- high liver weight, exceeding our normal upper limit of 4.0% of bodyweight, for the male.

In conclusion, based on these findings, it was considered that inappetence/bodyweight loss and loss in clinical condition at 5000 ppm and convulsive episodes at 2000 ppm would be limiting factors in the selection of a high dosage for use in a 13-week study. It is therefore concluded that dosages of 0, 100, 300 and 1000 ppm could be suitable for use in a 13-week dietary toxicity in the beagle dog.

The purpose of this study, performed at the Huntingdon Research Centre Ltd., Huntingdon, England, was to obtain preliminary information on the toxicity of Ziram when administered orally to beagle dogs for 4 weeks and to select dosages for use in a subsequent 13-week toxicity study in the beagle dog.

The test compound, Ziram, was understood to be a fungicide.

Three pairs of dogs (each containing 1♂ and 1♀) were allocated to this study and received the test material at dietary concentrations of 1000, 2000 and 5000 ppm. Treatment continued for up to 4 weeks.

The procedural details and all relevant information obtained during the study are presented in this report.

RELEVANT CALENDAR DATES

Protocol Approval

by Study Director:	5 January 1989.
by Sponsor:	9 January 1989.

Start of pre-dosing period:	Pair I - 5 January 1989 Pair II - 19 January 1989 Pair III - 9 February 1989
Start of dosing period:	Pair I - 19 January 1989 Pair II - 2 February 1989 Pair III - 23 February 1989
Post mortem examinations:	Pair I - 9, 16 February 1989 Pair II - 15 February 1989 Pair III - 23 March 1989

Animal management

Six pure-bred beagle dogs (3 males and 3 females) were supplied by Interfauna UK Ltd., Abbots Ripton Road, Wyton, Huntingdon, PE17 2DT.

Documentation provided by the Supplier included details of the litter of origin, ear tattoo, date of birth, confirmation of inoculation and details relating to any other treatment.

Shortly after arrival, each animal was examined by a veterinary surgeon. Dogs were acclimatised to conditions in the kennel unit for at least 2 weeks before treatment was started, during which time they were subject to our routine examination and acceptance procedures. During the acclimatisation period the dogs were re-inoculated against canine distemper virus, canine adeno virus (hepatitis), para influenza virus, canine parvovirus, Leptospira canicola, Leptospira icterohaemorrhagiae and Bordetella bronchiseptica. The animals also received a course of treatment with the anthelmintic piperazine.

During the study, all ailments were brought to the attention of a veterinary officer. The treatment of any such ailments was documented in the raw data.

The study was performed in the Department of Canine Toxicology, Units 4, 7 and 10. The dogs were housed individually in kennels. Each kennel measured approximately 6 ft x 2 ft with 5 ft high dividing partitions. The kennels were constructed of galvanised metal and were provided with food and water containers.

All kennels were heated by an underfloor hot water system and daily records of maximum and minimum air temperature were maintained. A range of 4 to 23°C was recorded during this study. Natural light was supplemented by fluorescent lighting during normal working hours when necessary.

The unit in which the animals were maintained had an outside exercise area. The dogs were exercised either outside or in the kennel unit for at least 10 minutes during each full working day unless the clinical signs made this impracticable.

Graded whitewood sawdust, Lignocel, supplied by RS Supplies was used as litter and changed daily.

Details relating to the age and bodyweight of individual animals at the time dosing commenced, have been presented in Table 1.

Diet

Each animal in the treated groups received 400 grams of ground treated dry diet at treatment levels of 2000, 5000 or 1000 ppm in the morning. Tap water was available in the kennels at all times.

Analyses were made on all batches of diet used to establish levels of basic nutrients and of specified substances and micro-organisms likely to be present in the feed components and which, if in excess of specified amounts, might have an undesirable effect on the test system. All batches of diet conformed with the acceptable standards agreed by the Study Director and Head, HRC Quality Assurance.

Results of the routine physical and chemical examination of drinking water at source (Grafham Final Water) as conducted usually weekly by the supplier, Anglian Water Authority, are made available to HRC as quarterly summaries. Additionally, levels of specified substances known to be present from time to time in local water and which if in excess of the maxima recommended (for humans) might have an undesirable effect on the test system, are determined in the tap water at approximately 6-monthly intervals.

All analytical data have been lodged in HRC Archives.

There was no information available to indicate that any non-nutrient substance likely to influence the effect of the test compound could reasonably be expected to be present in the diet or drinking water.

Study design

Prior to the commencement of dosing. The animals were given new identity numbers (odd numbers for males, even numbers for females). The identity number was displayed on a disc attached to a neck chain and was tattooed on the inner aspect of the left hind leg.

Each kennel was identified by a label coloured according to the occupant's group. Each label displayed the animal's identity number, dosage level and study schedule number.

The group identification was as follows:

Pair	Dosage (ppm)	Colour code	Animal numbers
I	2000	Green	593♂, 594♀
II	5000	Blue	595♂, 596♀
III	1000	Yellow	597♂, 598♀

Test compound

The test compound, Ziram technical, a white powder, was received at the Huntingdon Research Centre as follows:

25 kg Batch no. 8331 AA, purity 98.5%, on 12 January 1989.

The test material was stored at room temperature, protected from light, and was stated to be stable by the Sponsor.

Administration of the test compound

The test material was administered by admixture with the diet. Each dog was offered 400 grams diet each day and the food remaining approximately 24 hours later was removed, weighed and recorded.

Fresh treated diet was prepared weekly, at the beginning of the dose week. The required amount of test compound was weighed and ground diet added until the desired concentration of pre-mix was achieved. This was blended by shaking in an air-filled polythene bag for a minimum of 3 minutes. Diets for each group were prepared by direct dilution of the pre-mix. Homogeneity of all diets was achieved by mixing for at least 7 minutes in a double cone blender.

Formulated diets were stored at room temperature, protected from light, for Pair I and Pair II dogs throughout the study and for Pair III animals during Weeks 1 and 2 of treatment.

HRC Department of Analytical Chemistry determined the homogeneity and stability of Ziram in dog diet. Preliminary results indicated some instability when diets were stored at room temperature. On receipt of this information formulated diets for Pair III animals on this study were stored at -20°C, protected from light, during Weeks 3 and 4. The results of these analyses are presented in a separate report (HRC Report No. ZIR 13/14/16/23/9188 : 'Analytical Validation Study for the Determination of Ziram in Dose Formulations'). From these results it is apparent that, although the diets given to Pairs I and II (2000 and 5000 ppm, respectively) were stored at room temperature for 1 week following preparation, the 5000 ppm diet is stable for 14 days under these conditions and it is also likely that significant degradation would not have occurred at a concentration of 2000 ppm. Consequently it is considered that the storage of these diets at room temperature would not have affected the integrity of this study.

Observations

The following observations were made during the course of the study:

Clinical signs

All signs of ill health or reaction to treatment were recorded.

Except on Saturdays and Sundays all animals were checked regularly throughout the working day from 0900 to 1700 hours. Dated and signed records of all activities relating to the day by day running and maintenance of the study within the animal unit, as well as to the group observations and examinations outlined below, were recorded in the Study Day Book. In addition, observations relating to individual animals made throughout the day were recorded on the kennel sheets.

On Saturdays and Sundays a similar procedure was carried out except that the final check was performed at approximately mid-day.

Mortalities

The circumstances of any death were recorded in detail and a full post mortem examination was performed as described later.

Bodyweight

All animals were weighed, prior to feeding, twice a week throughout the pre-dosing and dosing periods.

Food consumption

The quantity of food left by individual animals was recorded daily throughout the experimental period.

Terminal studies

Post mortem examination

On completion of 4 weeks dosing each animal was killed by exsanguination under pentobarbitone anaesthesia (Expiral, Ceva Ltd., 200 mg/ml pentobarbitone sodium). All animals were fasted overnight prior to post mortem examination.

At autopsy the macroscopic appearance of the tissues was noted and the weights of the following organs recorded (paired organs being weighed separately):

adrenals
brain
heart
kidneys
liver
lungs
pancreas
pituitary
spleen
testes or ovaries
thymus
thyroids (and parathyroids)
uterus or prostate

Tissues for histopathological study

Samples (or the whole) of the following tissues from each animal were preserved together with any tissue showing macroscopic abnormality:

adrenals
alimentary tract (oesophagus, stomach body and antrum, duodenum, jejunum, ileum, caecum, colon, rectum)
aorta (arch and abdominal)
brain (cerebral cortex, thalamic nuclei, mid-brain, medulla and cerebellum)
eyes
gall bladder
heart
kidneys
liver
lungs (with bronchi)
lymph nodes (cervical and mesenteric)
mammary gland
ovaries
pancreas
pituitary
prostate
salivary gland (submandibular)
sciatic nerve
skeletal muscle (biceps femoris)
skin
spleen
sternum
tattoo
testes (and epididymis)
thymus
thyroids (and parathyroids)
tongue
trachea
urinary bladder
uterus
vagina

In addition, spinal cord (cervical, thoracic and lumbar regions) was preserved for Pair I and Pair II animals.

The fixative routinely used was 10% buffered formalin. The eyes were preserved in Davidson's fixative whilst additional pieces of liver and kidney were placed in formol calcium.

All tissues listed above were stored for possible future histopathological examination.

Records and Archives

Records were maintained of all tests, measurements, investigations and analyses together with other records of observations and procedures associated with the study, as required by the official HRC Procedures Manual. This material was stored in the department during the conduct of the study.

All specimens, raw data and other documents generated at HRC during the course of this study, together with a copy of this final report, have been lodged in the Huntingdon Research Centre Archives, Huntingdon, England, to be retained for 10 years.

The observations and full details for each pair of animals have been described below.

Bodyweight data for individual animals are presented in Appendix 1 and plotted graphically in Figures 1 to 3. Food consumption data for individual animals are presented in Appendix 2. Individual organ weights, expressed as absolute values and as a percentage of bodyweight, are presented in Appendices 3 and 4, respectively. Macroscopic post mortem findings have been detailed in Appendix 5.

PAIR I - 2000 ppm

Dogs 593♂ and 594♀ commenced dosing on 19 January 1989. Dog 594♀ was killed for humane reasons on Day 22 of treatment following convulsive episodes. Dog 593♂ survived the 4-week treatment period.

On Day 22 convulsive episodes of up to 5 minutes duration were seen for Dog 594♀ at approximately 2½ and 4 hours after feeding. Following each convulsion the dog was subdued and unsteady. Recovery had not occurred at the end of the working day (approximately 4½ hours after feeding) and it was considered necessary to kill this dog for humane reasons.

The following clinical signs were also observed for dogs at this dosage:

Liquid faeces were passed by Dog 593♂ on one occasion on Days 1 and 16 of treatment and by Dog 594♀ on one occasion on Day 14 only. The liquid faeces passed by Dog 593♂ on Day 1 were dark green in colour.

Vomiting was seen for Dog 593♂ on one occasion on Day 1 only.

Bodyweight gain was considered satisfactory for both dogs prior to dosing, for Dog 593♂ throughout the treatment period and for Dog 594♀ prior to its death on Day 22, although slight fluctuations in bodyweight were seen for both of these animals during the treatment period.

Food consumption was maximal for both dogs prior to dosing and for Dog 593♂ throughout the treatment period. Maximal or near maximal food consumption was also seen for Dog 594♀ during Days 1 to 13 of treatment, however, from Day 14 food intake for this animal was slightly reduced in comparison to pre-dosing values.

Dog 594♀ was killed on Day 22 (9 February 1989). Dog 593♂ was killed on 16 February 1989 on completion of 4 weeks treatment. Macroscopic post mortem findings included a red streak on the aortic valve of the heart and congestion of the cervical lymph node of Dog 594♀, and several foci of red mucosal congestion in the jejunum of Dog 593♂.

All organ weights were considered to be within normal limits for dogs of this age with the exception of liver weight for Dog 593♂, which at 4.38% exceeded our normal upper limit of 4.0% of bodyweight.

Histological examination of the tissues was not performed.

PAIR II - 5000 ppm

Dogs 595♂ and 596♀ commenced dosing on 2 February 1989. Both dogs were killed for humane reasons on Day 14 of treatment following inappetence, loss in bodyweight and/or deterioration in clinical condition.

The following clinical signs were observed for dogs at this dosage:

Liquid faeces were passed on two occasions on Day 1 by Dog 595♂ and on one occasion on Days 1 and 2 of treatment by Dog 596♀. In addition, dark green coloured faeces were passed by Dog 596♀ on Day 8 of treatment.

Vomiting was observed on one occasion on Day 1 of treatment for Dogs 595♂ and 596♀, and on two occasions on Day 8 of treatment for Dog 596♀.

Subdued behaviour and/or unsteady gait were also seen for Dog 595♂ on Days 12 - 14 of treatment.

Bodyweight gain was considered satisfactory for both dogs prior to the commencement of dosing. During Days 1 to 14 a bodyweight loss of 1.2 kg and 1.5 kg was observed for Dogs 595♂ and 596♀, respectively.

Food consumption was satisfactory for both dogs prior to the commencement of treatment. Slightly reduced food intake, in comparison to pre-dose values, was seen for Dog 595♂ during the first 2 weeks of treatment. Inappetence was seen for Dog 596♀ throughout the treatment period.

Both dogs were killed on Day 14 (15 February 1989). Macroscopic post mortem findings including pale coloured thyroids and general pallor of muscles for Dog 595♂, and a fluid-filled cyst on right ovary and red discolouration on left diaphragmatic lobe of the lungs of Dog 596♀.

All organ weights were considered to be within normal limits for dogs of this age, with the exception of the liver weight of Dog 595♂, which at 4.02% exceeded our normal upper limit of 4.0% of bodyweight.

Histological examination of the tissues was not performed.

PAIR III - 1000 ppm

Dogs 597♂ and 598♀ commenced dosing on 23 February 1989 and both dogs survived the 4-week treatment period.

Vomiting was observed on one occasion on Day 28 for Dog 598♀ only. There were no other clinical signs.

Bodyweight gain was considered to be satisfactory for both dogs prior to dosing and for Dog 597♂ throughout the treatment period. Bodyweight for Dog 598♀ fluctuated throughout the treatment period and after 4 weeks of treatment a slight loss in bodyweight of 0.2 kg was seen for this dog.

Food consumption was satisfactory for both dogs during the pre-dosing period, although Dog 598♀ consistently ate less food. During the treatment period food consumption for Dog 597♂ was maximal, whilst food intake for Dog 598♀ was slightly reduced in comparison to pre-dosing values.

Both dogs were killed on 23 March 1989 on completion of 4 weeks treatment. There were no macroscopic post mortem findings. All organ weights were considered to be within normal limits for dogs of this age, with the exception of the liver weight of Dog 597♂, which at 4.39% exceeded our normal upper limit of 4.0% of bodyweight.

Histological examination of the tissues was not performed.

FIGURE 1

Individual bodyweights - Pair I - 2000 ppm

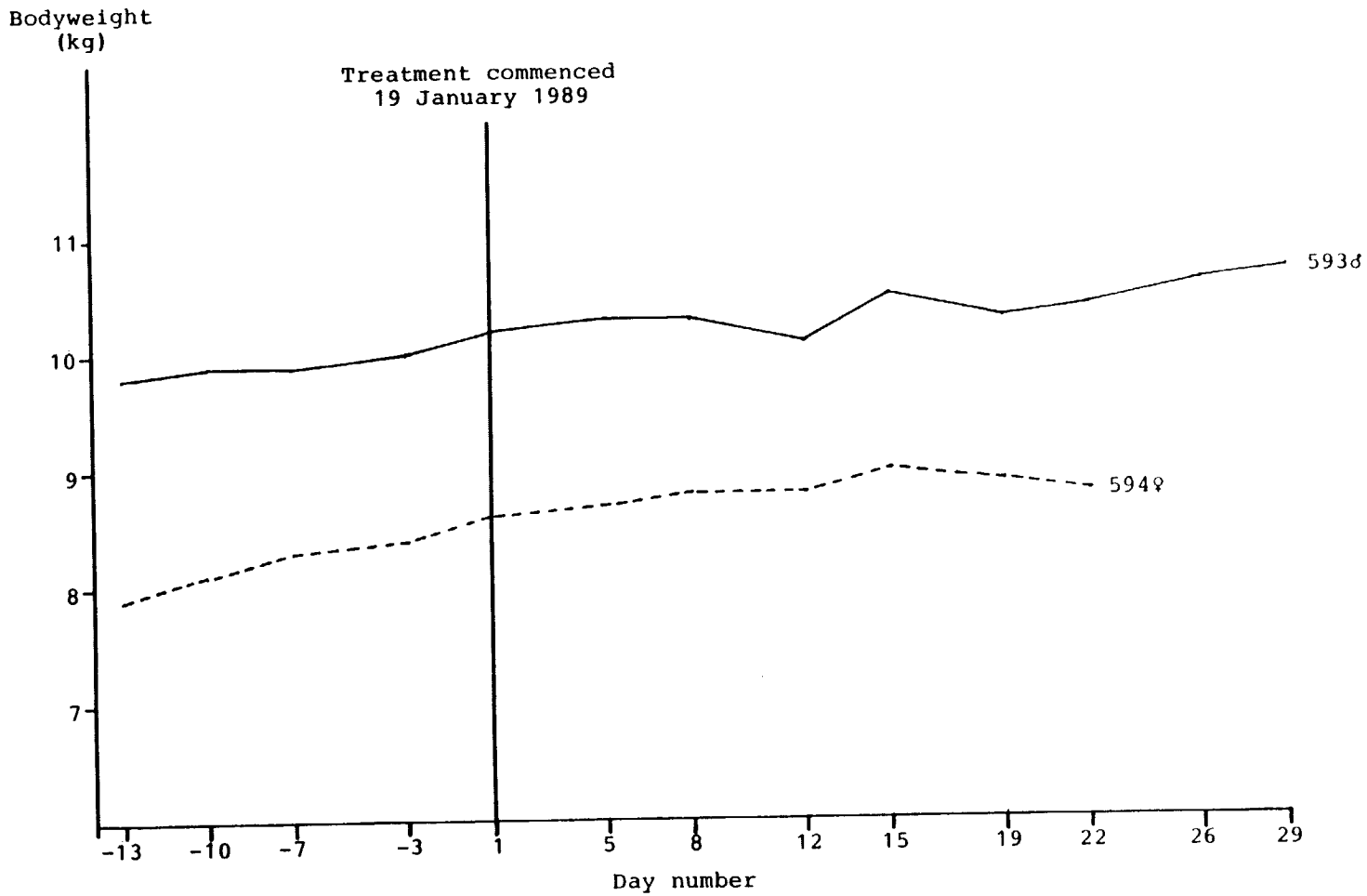

FIGURE 2

Individual bodyweights - Pair II - 5000 ppm

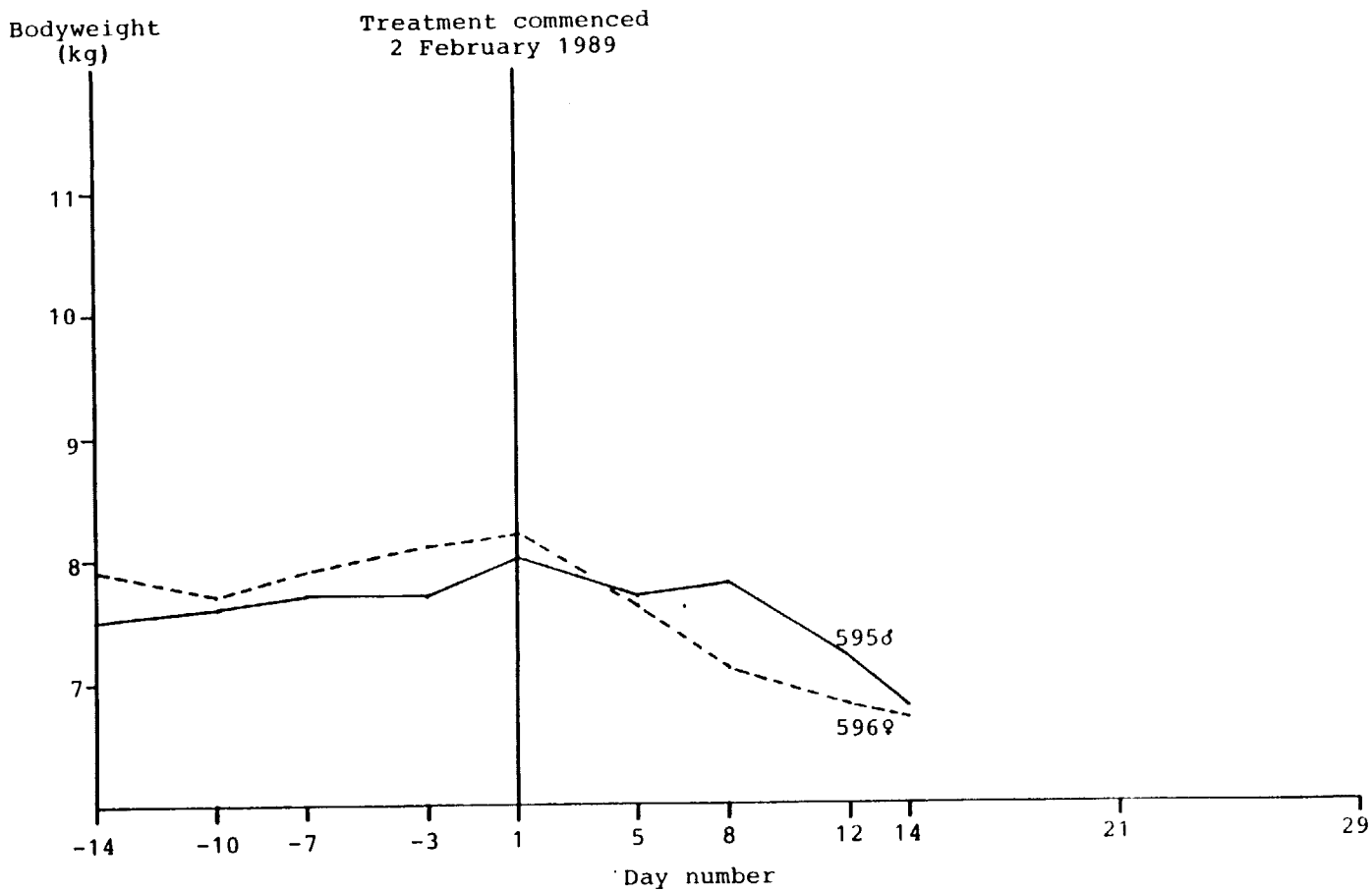

FIGURE 3

Individual bodyweights - Pair III - 1000 ppm

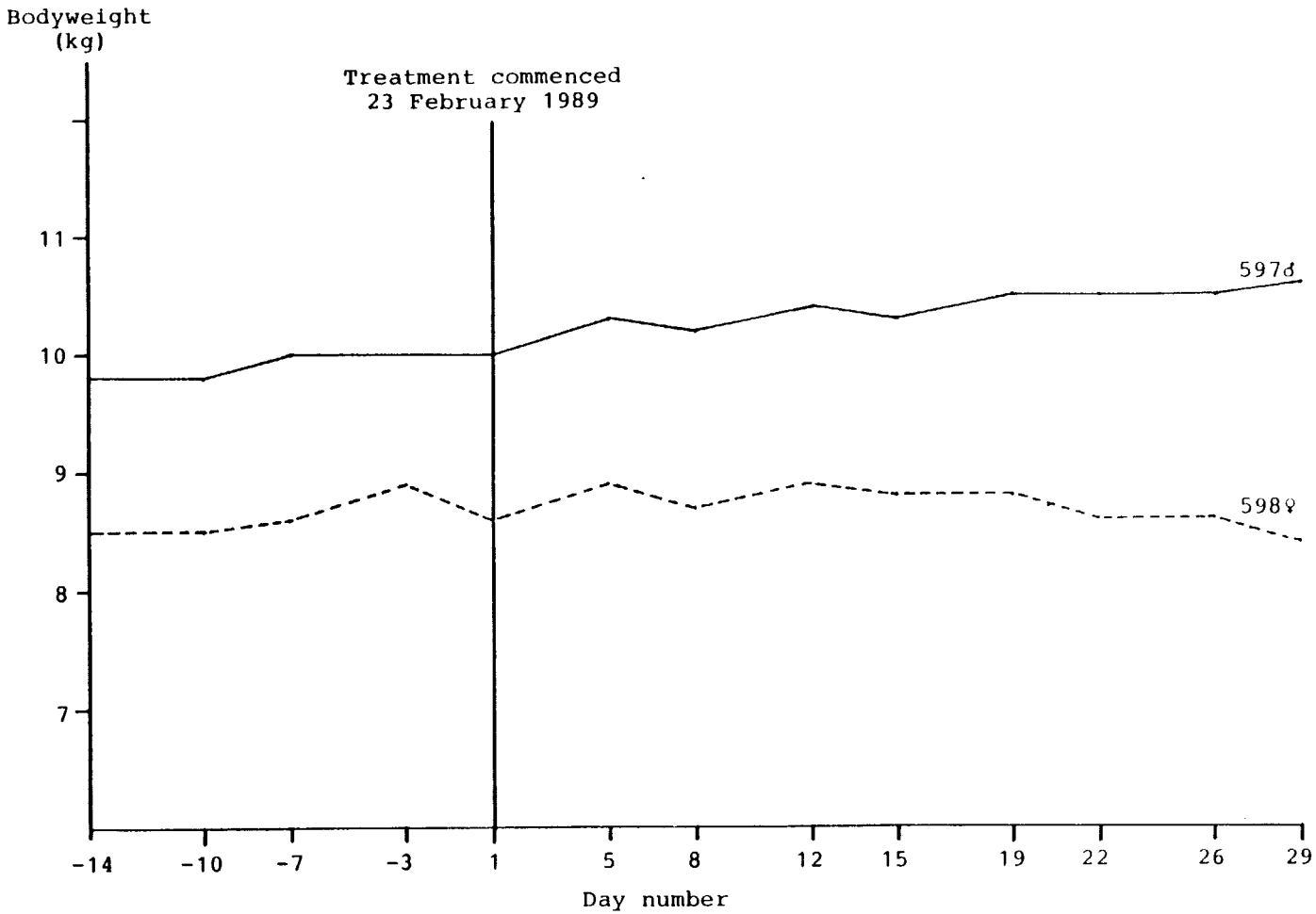

TABLE 1

Data relating to dogs at commencement of dosing

Diet concentration ppm	Males				Females			
	Dog no.	Ear no.	Age (weeks)	Body-weight (kg)	Dog no.	Ear no.	Age (weeks)	Body-weight (kg)
19 January 1989								
Pair I 2000	593	4315	24	10.2	594	4356	23	8.6
2 February 1989								
Pair II 5000	595	4332	25	8.0	596	4306	26	8.2
23 February 1989								
Pair III 1000	597	4350	28	10.0	598	4278	30	8.6

APPENDICES

APPENDIX 1

Bodyweight (kg)

Day	Pair I 2000 ppm		Pair II 5000 ppm		Pair III 1000 ppm	
	593♂	594♀	595♂	596♀	597♂	598♀
Pre-dose						
-14	NR	NR	7.5	7.9	9.8	8.5
-13	9.8	7.9	NR	NR	NR	NR
-10	9.9	8.1	7.6	7.7	9.8	8.5
- 7	9.9	8.3	7.7	7.9	10.0	8.6
- 3	10.0	8.4	7.7	8.1	10.0	8.9
Dosing						
1#	10.2	8.6	8.0	8.2	10.0	8.6
5	10.3	8.7	7.7	7.6	10.3	8.9
8	10.3	8.8	7.8	7.1	10.2	8.7
12	10.1	8.8	7.2	6.8	10.4	8.9
14	NR	NR	6.8*	6.7*	NR	NR
15	10.5	9.0	DEAD	DEAD	10.3	8.8
19	10.3	8.9			10.5	8.8
22	10.4	8.8			10.5	8.6
26	10.6	DEAD			10.5	8.6
29	10.7				10.6	8.4
Change 1 - 29	+0.5	-	-	-	+0.6	-0.2

NR Not recorded
* Weights taken at post mortem
\# Dosing commenced: Pair I - 19 January 1989
Pair II - 2 February 1989
Pair III - 23 February 1989

Food consumption (g/day) when 400 g/day offered

Day	Pair I 2000 ppm		Pair II 5000 ppm		Pair III 1000 ppm	
	593♂	594♀	595♂	596♀	597♂	598♀
Pre-dose						
-14	400	400	400	260	400	370
-13	400	400	400	390	400	250
-12	400	400	400	400	400	230
-11	400	400	400	400	400	140
-10	400	400	400	400	400	350
- 9	400	400	400	400	400	310
- 8	400	400	400	400	400	360
- 7	400	400	400	400	400	160
- 6	400	400	400	400	400	400
- 5	400	400	400	400	400	200
- 4	400	400	400	400	400	400
- 3	400	400	400	400	400	400
- 2	400	400	400	400	400	250
- 1	400	400	400	400	400	300
Pre-dose mean	400	400	400	389	400	294
Dosing						
1	400	400	400	150	400	130
2	400	400	400	60	400	310
3	400	400	360	110	400	240
4	400	400	360	70	400	370
5	400	400	320	90	400	270
6	400	400	360	40	400	250
7	400	400	270	20	400	280
8	400	400	290	100	400	270
9	400	400	400	10	400	290
10	400	360	400	0	400	200
11	400	400	370	60	400	260
12	400	380	400	80	400	350
13	400	400	50	70	400	160
14	400	310	DEAD	DEAD	400	210
15	400	280			400	200
16	400	110			400	200
17	400	390			400	350
18	400	370			400	250
19	400	370			400	110
20	400	330			400	190
21	400	370			400	240
22	400	170			400	310
23	400	DEAD			400	270
24	400				400	130
25	400				400	330
26	400				400	90
27	400				400	200
28	400				400	140
Mean days 1 - 28	400				400	236

APPENDIX 3

Organ weights - individual values

Diet concentration ppm	Dog no.	Body wt. kg	Brain g	Pituitary mg	Heart g	Lungs g	Liver g	Spleen g	Pancreas g	Thymus g	Prostate /Uterus g
Pair I - 2000	593♂	10.5	79.1	74	73.1	105.1	460.0	71.9	30.6	13.3	3.36
	594♀	9.0	77.8	74	70.5	76.1	354.1	85.3	25.3	9.9	2.52
Pair II - 5000	595♂	6.8	78.6	45	47.1	67.8	273.3	26.7	16.1	4.5	1.54
	596♀	6.7	70.1	56	52.2	67.6	210.2	61.3	17.6	5.4	1.35
Pair III - 1000	597♂	10.3	80.7	67	78.0	96.0	451.8	80.6	27.1	10.8	3.32
	598♀	8.3	71.7	42	66.6	74.7	252.0	85.3	13.5	9.8	3.25

Diet concentration ppm	Dog no.	Kidneys		Thyroids		Adrenals		Testes/Ovaries	
		R g	L g	R g	L g	R g	L g	R g	L g
Pair I - 2000	593♂	26.5	28.1	0.75	0.59	0.73	0.90	11.81	11.16
	594♀	24.1	24.9	0.53	0.38	0.64	0.63	0.29	0.32
Pair II - 5000	595♂	18.0	18.4	0.47	0.40	0.52	0.49	2.13	2.21
	596♀	16.5	16.5	0.27	0.32	0.59	0.56	0.31	0.28
Pair III - 1000	597♂	29.8	32.0	0.69	0.39	0.77	0.65	12.60	11.89
	598♀	18.4	18.2	0.34	0.34	0.50	0.57	0.45	0.54

APPENDIX 4

Relative organ weights - individual values - expressed as a percentage of bodyweight

Diet concentration ppm	Dog no.	Body wt. kg	Brain	Pituitary	Heart	Lungs	Liver	Spleen	Pancreas	Thymus
Pair I - 2000	593♂	10.5	0.75	0.00070	0.70	1.00	4.38	0.68	0.29	0.13
	594♀	9.0	0.86	0.00082	0.78	0.85	3.93	0.95	0.28	0.11
Pair II - 5000	595♂	6.8	1.16	0.00066	0.69	1.00	4.02	0.39	0.24	0.07
	596♀	6.7	1.05	0.00084	0.78	1.01	3.14	0.91	0.26	0.08
Pair III - 1000	597♂	10.3	0.78	0.00065	0.76	0.93	4.39	0.78	0.26	0.10
	598♀	8.3	0.86	0.00051	0.80	0.90	3.04	1.03	0.16	0.12

Diet concentration ppm	Dog no.	Prostate/ Uterus	Kidneys	Thyroids	Adrenals	Testes/ Ovaries
Pair I - 2000	593♂	0.032	0.52	0.0128	0.0155	0.2188
	594♀	0.028	0.54	0.0101	0.0141	0.0068
Pair II - 5000	595♂	0.023	0.54	0.0128	0.0149	0.0638
	596♀	0.020	0.49	0.0088	0.0172	0.0088
Pair III - 1000	597♂	0.032	0.60	0.0105	0.0138	0.2378
	598♀	0.039	0.44	0.0082	0.0129	0.0119

APPENDIX 5

Pathology - individual macroscopic findings

Pair:	I	II	III
Compound:		Ziram	
Level (ppm):	2000	5000	1000

In this appendix, the macroscopic findings relating to each animal are listed. Histopathological examination of the tissues was not performed.

(Pathology - continued)

Dog 593♂ - Killed 16 February 1989

2000 ppm

Macroscopic findings:

Jejunum:	Several foci of red mucosal congestion (up to 2 mm diameter).

(Pathology - continued)

Dog 594♀ - Killed 9 February 1989

2000 ppm

Macroscopic findings:

Heart:	Red streak (7 x 1 mm) on central cusp of aortic valve.
Cervical lymph node:	Congested.

(Pathology - continued)

Dog 595♂ - Killed 15 February 1989

5000 ppm

Macroscopic findings:

Thyroids:	Pale.
Muscles:	Generally pale.

(Pathology - continued)

Dog 596♀ - Killed 15 February 1989

5000 ppm

Macroscopic findings:

Ovary:	Fluid-filled cyst (1 mm diameter) on right ovary.
Lungs:	Area of red discolouration (20 x 15 mm) on left diaphragmatic lobe.

APPENDIX 5

(Pathology - continued)

Dog 597♂ - Killed 23 March 1989

1000 ppm

Macroscopic findings: No abnormalities detected.

(Pathology - continued)

Dog 598♀ - Killed 23 March 1989

1000 ppm

Macroscopic findings: No abnormalities detected.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

C. H. Farr, PhD, DABT
Manager, Product Safety and Toxicology
Atochem North America, Inc.
900 First Avenue
P.O. Box 1536
King of Prussia, Pennsylvania 19406-0018

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PREVENTION, PESTICIDES AND
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APR 0 6 1995

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Terry R. O'Bryan

Terry R. O'Bryan
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Submission # 8EHQ- 1092 -12586 SEQ. A

TYPE: INT SUPP FLWP

SUBMITTER NAME: Elf Atochem North America, Inc.

INFORMATION REQUESTED: FLWP DATE:
0501 NO INFO REQUESTED
0502 INFO REQUESTED (TECH)
0503 INFO REQUESTED (VOL ACTIONS)
0504 INFO REQUESTED (REPORTING RATIONALE)

DISPOSITION:
0639 REFER TO CHEMICAL SCREENING
0678 CAP NOTICE

VOLUNTARY ACTIONS:
0401 NO ACTION REPORTED
0402 STUDIES PLANNED/UNDERWAY
0403 NOTIFICATION OF WORKER/OTHERS
0404 LABEL/MSDS CHANGES
0405 PROCESS/HANDLING CHANGES
0406 APP./USE DISCONTINUED
0407 PRODUCTION DISCONTINUED
0408 CONFIDENTIAL

SUB. DATE: 10/20/92 OTS DATE: 10/26/92 CSRAD DATE: 02/09/95

CHEMICAL NAME: Ziram

CAS# 137-30-4

INFORMATION TYPE:		P F C
0201	ONCO (HUMAN)	01 02 04
0202	ONCO (ANIMAL)	01 02 04
0203	CELL TRANS (IN VITRO)	01 02 04
0204	MUTA (IN VITRO)	01 02 04
0205	MUTA (IN VIVO)	01 02 04
0206	REPRO/TERATO (HUMAN)	01 02 04
0207	REPRO/TERATO (ANIMAL)	01 02 04
0208	NEURO (HUMAN)	01 02 04
0209	NEURO (ANIMAL)	01 02 04
0210	ACUTE TOX. (HUMAN)	01 02 04
0211	CHR. TOX. (HUMAN)	01 02 04
0212	ACUTE TOX. (ANIMAL)	01 02 04
0213	SUB ACUTE TOX (ANIMAL)	01 02 04
0214	SUB CHRONIC TOX (ANIMAL)	01 02 04
0215	CHRONIC TOX (ANIMAL)	01 02 04

INFORMATION TYPE:		P F C
0216	EPI/CLIN	01 02 04
0217	HUMAN EXPOS (PROD CONTAM)	01 02 04
0218	HUMAN EXPOS (ACCIDENTAL)	01 02 04
0219	HUMAN EXPOS (MONITORING)	01 02 04
0220	ECO/AQUA TOX	01 02 04
0221	ENV. OCCC/REL/FATE	01 02 04
0222	EMER INCI OF ENV CONTAM	01 02 04
0223	RESPONSE REQEST DELAY	01 02 04
0224	PROD/COMP/CHEM ID	01 02 04
0225	REPORTING RATIONALE	01 02 04
0226	CONFIDENTIAL	01 02 04
0227	ALLERG (HUMAN)	01 02 04
0228	ALLERG (ANIMAL)	01 02 04
0239	METAB/PHARMACO (ANIMAL)	01 02 04
0240	METAB/PHARMACO (HUMAN)	01 02 04

INFORMATION TYPE:		P F C
0241	IMMUNO (ANIMAL)	01 02 04
0242	IMMUNO (HUMAN)	01 02 04
0243	CHEM/PHYS PROP	01 02 04
0244	CLASTO (IN VITRO)	01 02 04
0245	CLASTO (ANIMAL)	01 02 04
0246	CLASTO (HUMAN)	01 02 04
0247	DNA DAM/REPAIR	01 02 04
0248	PROD/USE/PROC	01 02 04
0251	MSDS	01 02 04
0299	OTHER	01 02 04

TRIAGE DATA: NON-CBI INVENTORY
YES
CAS SR NO
DETERMINE

ONGOING REVIEW
YES (DROP/REFER)
NO (CONTINUE)
REFER

SPECIES
Fish
Daphnia
RAT
DOG

TOXICOLOGICAL CONCERN:
LOW
MED
HIGH
Not ranked - acc. oral tox

USE: Fungicide

PRODUCTION:

COMMENTS 8(e)'s on 7/9/92 & 6/26/92

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0501 NO INFO REQUESTED
0502 INFO REQUESTED (TECH)
0503 INFO REQUESTED (VOL ACTIONS)
0504 INFO REQUESTED (REPORTING RATIONALE)

DISPOSITION:
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0678 CAP NOTICE

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0401 NO ACTION REPORTED
0402 STUDIES PLANNED/UNDERWAY
0403 NOTIFICATION OF WORKER/OTHERS
0404 LABEL/MSDS CHANGES
0405 PROCESS/HANDLING CHANGES
0406 APP./USE DISCONTINUED
0407 PRODUCTION DISCONTINUED
0408 CONFIDENTIAL

SUB. DATE: 10/20/92 OTS DATE: 10/26/92 CSRAD DATE: 02/09/95

CHEMICAL NAME: Ziram

CAS# 137-30-4

INFORMATION TYPE:		P F C
0201	ONCO (HUMAN)	01 02 04
0202	ONCO (ANIMAL)	01 02 04
0203	CELL TRANS (IN VITRO)	01 02 04
0204	MUTA (IN VITRO)	01 02 04
0205	MUTA (IN VIVO)	01 02 04
0206	REPRO/TERATO (HUMAN)	01 02 04
0207	REPRO/TERATO (ANIMAL)	01 02 04
0208	NEURO (HUMAN)	01 02 04
0209	NEURO (ANIMAL)	01 02 04
0210	ACUTE TOX. (HUMAN)	01 02 04
0211	CHR. TOX. (HUMAN)	01 02 04
0212	ACUTE TOX. (ANIMAL)	01 02 04
0213	SUB ACUTE TOX (ANIMAL)	01 02 04
0214	SUB CHRONIC TOX (ANIMAL)	01 02 04
0215	CHRONIC TOX (ANIMAL)	01 02 04

INFORMATION TYPE:		P F C
0216	EPI/CLIN	01 02 04
0217	HUMAN EXPOS (PROD CONTAM)	01 02 04
0218	HUMAN EXPOS (ACCIDENTAL)	01 02 04
0219	HUMAN EXPOS (MONITORING)	01 02 04
0220	ECO/AQUA TOX	01 02 04
0221	ENV. OCCC/REL/FATE	01 02 04
0222	EMER INCI OF ENV CONTAM	01 02 04
0223	RESPONSE REQEST DELAY	01 02 04
0224	PROD/COMP/CHEM ID	01 02 04
0225	REPORTING RATIONALE	01 02 04
0226	CONFIDENTIAL	01 02 04
0227	ALLERG (HUMAN)	01 02 04
0228	ALLERG (ANIMAL)	01 02 04
0239	METAB/PHARMACO (ANIMAL)	01 02 04
0240	METAB/PHARMACO (HUMAN)	01 02 04

INFORMATION TYPE:		P F C
0241	IMMUNO (ANIMAL)	01 02 04
0242	IMMUNO (HUMAN)	01 02 04
0243	CHEM/PHYS PROP	01 02 04
0244	CLASTO (IN VITRO)	01 02 04
0245	CLASTO (ANIMAL)	01 02 04
0246	CLASTO (HUMAN)	01 02 04
0247	DNA DAM/REPAIR	01 02 04
0248	PROD/USE/PROC	01 02 04
0251	MSDS	01 02 04
0299	OTHER	01 02 04

TRIAGE DATA:	NON-CBI INVENTORY	ONGOING REVIEW	SPECIES	TOXICOLOGICAL CONCERN:	USE:	PRODUCTION:
	YES	YES (DROP/REFER)	Fish	LOW	Fungicide	
CAS SR	NO	NO (CONTINUE)	Daphnia	MED		
	DETERMINE	REFER	RAT	HIGH		
			DOG			

COMMENTS 8(e)'s on 7/9/92 & 6/26/92
Develop
NOAEL maternal - 1 mg/kg
fetus - 16 mg/kg

64 mg/kg - inc water consumption, dec food consumption + reduced BW gain
reduced litter & mean fetal wt
16 mg/kg - same effects on parents, no effect on fetuses
4 mg/kg - increased water consumption
1 mg/kg - no effect

12586A

Not ranked

The acute oral toxicity of ziram to rats was not ranked as these data were collected as part of a reproductive toxicity study. Pregnant female rats (25/dose) receive gavage doses of 0, 1, 4, 16, or 64 mg/kg/day on day 6 through day 15 of pregnancy. There were no treatment-related deaths. Signs of toxicity at 16 and 64 mg/kg included salivation post-dosing (2/25 and 8/25, respectively), increased water consumption and decreased food consumption, and dose-related weight loss. At 64 mg/kg, hair loss was seen in 6/25 animals.