# RESPONDENT'S EXHIBIT 10

## Annex 3 Protocol and amendments

Huntingdon
Life Sciences
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# PROTOCOL

# DCPA (CHLORTHAL DIMETHYL):

# Acute Oral Toxicity ($LD_{50}$) to the Zebra finch

| | |
|---|---|
| **HLS study number:** | BDG0198 |
| **HLS enquiry number:** | 53284/13 |
| **Version ID:** | Final Protocol |
| **Issue date:** | 10 April 2014 |

# PROTOCOL

## DCPA (Chlorthal Dimethyl): Acute Oral Toxicity ($LD_{50}$) to the Zebra finch

## Test facility

Huntingdon Life Sciences
Huntingdon Research Centre
Woolley Road
Alconbury
Huntingdon
Cambridgeshire
PE28 4HS
UK

# Table of Contents

# 1. Introduction

## 1.1 Management of study

| | |
|---|---|
| Study Director | Adrian Bull |
| Consulting Toxicologist | David Cameron |

In the temporary absence of the Study Director, routine duties and technical questions will be attended to by Vanessa Ross. Formal GLP responsibilities will be assumed by Management.

## 1.2 Objective

The purpose of this study is to assist in the assessment of the safety to wildlife species. The study is designed to determine the oral $LD_{50}$ of DCPA to the Zebra finch.

## 1.3 Regulatory compliance

The protocol followed is based on that given in the U.S. Environmental Protection Agency Ecological Effects Test Guidelines; OCSPP 850.2100: Avian Acute Oral Toxicity Test, dated January 2012.

The Environmental Protection Agency included the requirement for acute oral toxicity testing in a passerine species in the Federal Register Volume 72, Number 207, October 26, 2007.

## 1.4 Good laboratory practice

The study will be conducted in compliance with principles of Good Laboratory Practice Standards as set forth in:

> The UK Good Laboratory Practice Regulations (Statutory Instrument 1999 No. 3106, as amended by Statutory Instrument 2004 No. 994).
>
> OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17.
>
> EC Commission Directive 2004/10/EC of 11 February 2004 (Official Journal No L 50/44).

These principles of Good Laboratory Practice are accepted by the regulatory authorities of the United States of America and Japan on the basis of intergovernmental agreements.

## 1.5 Animals (Scientific Procedures) Act 1986 compliance

The in-life experimental procedures to be undertaken during the course of this study are subject to the provisions of the United Kingdom Animals (Scientific Procedures) Act 1986 (the Act). The Act, administered by the UK Home Office, regulates all scientific procedures in living animals which may cause pain, suffering, distress or lasting harm and provides for the designation of establishments where procedures may be undertaken, the licensing of trained individuals who perform the practical techniques and the issue of project licences for specified programmes of work.

This study will comply with all applicable sections of the Act and the associated Codes of Practice for the Housing and Care of Animals used in Scientific Procedures and the Humane Killing of Animals under Schedule 1 to the Act, issued under section 21 of the Act.

The number of animals used will be the minimum that is consistent with scientific integrity and regulatory acceptability, consideration having been given to the welfare of individual animals in terms of the number and extent of procedures to be carried out on each animal.

**Implementation of humane endpoints:** In order to comply with the specific project licence under which this study is conducted, any bird showing a substantial adverse effect thought unlikely to regress will be humanely killed. Specifically, birds that are in a moribund state and/or showing signs of excessive suffering as a result of acute toxicity following dose administration will be killed without delay (and classified as dose-related mortality).

## 1.6 Reason for selection of animal species

The selected test species is the Zebra finch, as a passerine species considered to be representative of species at potential risk following commercial use of the test substance.

## 1.7 Reason for selection of route and doses

It is not possible to include detailed information as to the number of groups, number of birds and dose levels as these will be determined by the toxicity of the test substance. Full details will be included in the final report and will be the subject of a protocol amendment.

The test substance will be administered by oral gavage as suggested in the guidelines and as ingestion is the likely route of exposure.

# 2. Study Schedule

## 2.1 Duration of study

14-day pre-treatment period

14-day post-treatment observation period (which may be extended as appropriate to allow complete resolution of any persistent signs of toxicity or reaction to treatment (see section 5.3), or if it is considered that further relevant information could be gained)

## 2.2 Scheduled time plan

| | |
|---|---|
| Experimental start date | 16 April 2014 |
| Start of acclimation period | Week beginning 05 May 2014 |
| Day of dosing | Week beginning 20 May 2014 |
| End of observation period | Week beginning 03 June 2014 |
| Draft report to be issued | 01 August 2014 |

# 3. Test Substance and Formulation

## 3.1 Test substance

| | |
|---|---|
| Company code/name: | DCPA (Chlorthal Dimethyl) |
| Alternative names: | Dacthal |
| | Dacthal technical |
| Storage conditions: | At ambient temperature and keep dry |
| Supplier: | Sponsor |
| Batch/Lot: | 120904-1 |
| Expiry: | 1 July 2018 |
| Purity: | 99.3% |
| Correction factor: | Not required |
| Reserve sample: | A reserve sample of the batch of test substance used for the study will not be taken unless requested by the Sponsor. Unused test substance will be disposed of according to the instructions supplied by the Sponsor (see test substance data sheet). |

| | |
|---|---|
| Additional data: | Any additional information regarding the test substance is contained in the test substance data sheet. |
| Pharmacy management: | Pristima |

## 3.2 Vehicle

To be confirmed.

## 3.3 Preparation of dose

A single preparation of dose will be made on each occasion of dosing, following one of the methods below.

| Treatment | Nominal concentration |
|---|---|
| Group 1 | Vehicle; 0 mg/mL |
| Group 2 | DCPA; X mg/mL. |
| Group 3 | DCPA; X mg/mL. |
| Group 4 | DCPA; X mg/mL. |
| Group 5 | DCPA; X mg/mL. |
| Group 6 | DCPA; X mg/mL. |

X to be confirmed in a protocol amendment

### 3.3.1 Solutions

A single preparation in the appropriate vehicle will be prepared with up to five dose concentrations so that all birds receive the same dose volume per unit of bodyweight. The test substance will be dissolved in the vehicle by stirring and/or shaking. A high shear homogeniser may be used to aid dissolution. Warming and/or ultrasonication may also be required. Details will be recorded in the study data and included in the final report.

### 3.3.2 Suspensions

A single preparation in the appropriate vehicle will be prepared with up to five dose concentrations so that all birds receive the same dose volume per unit of bodyweight. The test substance will be ground in a mortar with the vehicle until a smooth paste is formed. The formulation will then be gradually made up to volume and mixed using a high shear homogeniser.

## 3.4 Analysis

| | |
|---|---|
| Liquid formulation | Before commencement of the main study, the suitability of the proposed mixing procedures will be determined and specimen formulations will be analysed to assess the |

stability and homogeneity of the test substance in the liquid matrix.

At specified intervals during treatment, the test formulations will be analysed for achieved concentration of the test substance.

Analysis

The method of analysis will be documented in the study data and a summary included in the Final report.

The analytical method will involve the dilution of the test formulations with a suitable solvent followed by chromatographic assay.

The method will be validated with respect to the determination of the specificity of analysis, limit of detection and quantification, linearity of detector response, repeatability, method accuracy and precision.

Calibration standard solutions and reagents may be shared with other related and concurrent studies. Where this does occur, appropriate details will be documented in the raw data and the Final report.

**Stability and homogeneity**

Sampling and determination:

Specimen formulations (typically 200 mL) will be prepared at 400 mg/mL (limit test assumed based on available information; additional concentrations may be included in the event that lower doses need to be evaluated) and equally split between two amber glass screw-capped bottles. The formulations will be analysed following fresh preparation at 0 hour and after 1 hour continuous stirring (Bottle 1), and following storage at both ambient temperature (nominally 21°C) and refrigeration (nominally 4°C) for 48 hours (Bottles 1 and 2).

Prior to initial sampling on each day, the formulation will be mixed by 20-fold inversion and magnetic stirring for a minimum of 5 minutes. At each time-point, single samples (nominally 1 mL) will be taken for assay from the top, middle and bottom of the continuously stirred formulation. Homogeneity will be determined by analysis of top, middle and bottom samples. Stability will be determined from the mean concentration of test substance in the vehicle at each sampling point.

Two containers of control vehicle (2 × 50 mL) will be supplied with the trial formulations, one to be stored at ambient temperature and one in the refrigerator.

**Acceptance limits:**

- Mean analysed concentrations of the freshly-prepared formulations are expected to be within ±20%[1] of the nominal values.
- For a formulation to be considered homogenous, sample results at each time-point should have a coefficient of variation of ≤5% (n=3). This limit may be increased to ≤10% for formulations where the test materials are at low concentrations. In this case, the justification to increase the limit will be made during the stability trial, documented in the data and will be applied to all analyses. Where this limit has increased, each of the individual results should still be within acceptable limits from nominal (±20%).
- The stability will be determined from the mean concentration of test substance in the vehicle at each time point. Results are expected to be within ±20%[1] of the initial time zero concentration value.

**Achieved concentration**

| | |
|---|---|
| Assay sampling | Test formulations will be analysed for the occasions given below.<br><br>Other sampling regimens may be specified by the Sponsor. |
| Sample storage | Stored samples will be retained as contingency for analysis at the temperature determined by the stability information if any result requires confirmation. |
| Sample disposal | Samples (contingency/residual) will be disposed of once satisfactory results are obtained. |
| Acceptance limits | Achieved concentration: ±20%[1] of the nominal value<br>Precision (measured by deviation from mean): ≤5% |

Table of required achieved concentration samples

| | | Pharmacy | | FIA | |
|---|---|---|---|---|---|
| Occasion | Groups | Sample volume (mL) | Samples taken | Samples assayed | Samples stored |
| $LD_{50}$ | 1-2 | 1.0 | 4 (middle) | 2 | 2 |

[1] Acceptance limits for stability and achieved concentration are based on Ecological Effects Test Guidelines; OCSPP 850.2000: Background and Special Considerations – Tests with Terrestrial Wildlife, dated January 2012.

# 4. Animals and Animal Husbandry

## 4.1 Animals

| | |
|---|---|
| Species | Zebra finch *(Taeniopygia guttata)* |
| Number and sex | Due to the study design the number of birds used will be determined during the course of the study and will be documented in the study records. |
| Bodyweight range | Approximately 20g ± 5 g at the start of the pretreatment period |
| Age of birds | Young adult (all birds will be of approximately the same age; age at study start will be documented in the study data file) |
| Source | Huntingdon Life Sciences stock. Batches of birds are periodically brought in to stock from the breeder/supplier. They are maintained in conditions equivalent to those described in this protocol for birds being used in studies, and clinical health is monitored (at least once daily observations) prior to allocation to study. |

## 4.2 Acclimatisation

Birds will be allowed a minimum acclimatisation period of fourteen days prior to performing any experiment. Only healthy birds will be placed on a study. No prophylactic or therapeutic treatment will be administered during the acclimatisation period.

## 4.3 Animal management

The birds will be housed in groups of five (males and females separately) according to treatment in commercially available powder-coated wire mesh cages suitable for small finches, within a building designed to provide suitable environmental conditions for the species. Each cage of five birds (dimensions 75 cm × 45.5 cm × 46 cm) will contain one or more feeders and drinkers and appropriately sited perches; the same cages will be used for both the acclimation and testing phases. The maximum and minimum ambient temperatures and the relative humidity will be recorded once daily and will be maintained as far as possible within the target ranges of 18-25°C and 45-70% respectively (small fluctuations outside of the range are permissible). Ventilation fans will be adjusted as necessary to maintain relative humidity within acceptable limits as far as possible. Lighting will be provided by means of overhead fluorescent tubes (emission spectrum approximating to natural daylight), and a controlled artificial lighting pattern of 10 hours light and 14 hours darkness will be adopted.

## 4.4 Diet and water supply

A proprietary pelleted mixture formulated for finches will be offered to the birds *ad libitum* (except for a starvation period of 3 - 4 hours before dosing when all feed will be withdrawn). The diet contains ground corn, soybean meal, wheat flour, oat groats, cane molasses, dried whole egg and canola oil. The mixture nominally contains no added antibiotic or other non-nutritional feed additive, and is not anticipated to contain contaminants capable of interfering with the integrity of the study; therefore it is not intended to conduct any routine contaminant analyses. A 100g sample of each batch of feed used will be retained frozen as a contingency sample and may subsequently be analysed at the Study Director's discretion in case of equivocal results.

Fresh drinking water will be available to all birds *ad libitum* throughout the study. Water supply to the birds is potable water from the public supply and is analysed periodically by the supplier.

Feed and water will be offered to the birds in appropriate containers designed to minimise spillage.

## 4.5 Randomisation and identification

Healthy birds will be randomly allocated to groups by sex (five males and five females) at the commencement of the acclimatisation period. Treatments (dose levels) will subsequently be randomly allocated to the groups of birds with the aid of random number tables at the beginning of pre-treatment baseline measurements (Day -14). The birds will be identified by means of leg rings numbered sequentially. The study number followed by the bird number will constitute a unique number. On the day of dosing, birds will be additionally marked temporarily by a coloured spot (using natural food dyes) on the breast to allow accurate recognition following dosing; this will enable birds showing clinical signs or regurgitation to be identified readily without handling the birds.

# 5. Experimental Procedures

## 5.1 Treatment groups

There will be a negative control group of ten birds and a minimum of five† treatment groups each of ten birds. Each group will consist of five males and five females. In addition, six spare birds (three males and three females) will be maintained for use as replacements for any birds which die or show signs of ill health during the pre-treatment observation period.

†except for test substances of low toxicity where a limit test is determined from range finding tests to be appropriate; in this case, there will be a negative control group of ten birds and a single test group of ten birds treated at the limit dose of 2000 mg/kg or at a dose equivalent to the calculated field exposure level, whichever is the higher.

Range finding will be carried out on groups of two birds (one male and one female) at dose levels up to a maximum of 2000 mg/kg (unless environmental residues are expected to result in a higher level of exposure). Depending on the outcome for each pair of birds, further pairs may be dosed at higher or lower dose levels until sufficient data are obtained to enable dose level selection for the definitive test. If regurgitation is observed during range-finding, the Study Director, in consultation with the Sponsor, will determine an appropriate course of action and suitable amendments to the study plan, including consideration of whether capsule dosing may be used as an alternative method of dosing. The US EPA recommendations on decision-making in the event of regurgitation, published as a flowchart by EFED (Environmental Fate and Effects Division), will also be taken into consideration. All details will be documented by protocol amendment.

## 5.2 Procedure

Prior to dosing, birds will be starved for 3-4 hours following a brief early morning feeding period when room lights are turned on (approximately 30 minutes). Birds will be caught singly by hand and weighed quickly in a small plastic container to prevent unnecessary stress. A single dose will be administered to each bird during the morning. The doses will be given by oral intubation into the crop, as specified in the EPA Guidelines. All birds will be dosed at a constant volume rate of 5 mL/kg bodyweight (dose precision to the nearest 0.01 mL). Birds in the control group will be dosed with an equivalent amount of vehicle only.

At termination of the study all birds will be sacrificed by cervical dislocation.

## 5.3 Observations

There will be a fourteen day post-treatment observation period during which bird health, signs of toxicity and mortalities will be observed daily. On the day of dosing each bird will be observed continuously in the period immediately after dosing for a minimum of one hour and on at least three additional occasions, and on all other days at least twice daily for the duration of the study. If signs of toxicity persist during the14-day observation period the study will be extended until at least 21 days or until at least 72 hours after signs of toxicity have fully resolved. Veterinary examinations will be performed when deemed necessary and full details will be recorded.

Individual bodyweights will be recorded on Days -14, -7, 0 (immediately prior to dosing), 7 and 14 of the study (and at the end of any extended observation period).

Food consumption will be recorded weekly over the following periods:

Days -14 to -8
-7 to -1
1 to 7
8 to 14
and during any extended observation period.

## 5.4 Macroscopic *post mortem* examination

The following birds will be examined:

all birds which die during the study;

all control birds

ten dosed birds

The dosed birds will be taken from the highest dose groups in which there are survivors at termination. If changes are seen in these birds, the next highest dose group will be examined. Further birds will be examined until no abnormalities are detected in a complete group.

Tissues examined will include:- digestive tract, liver, kidneys, heart, spleen, muscle and subcutaneous fat. Gross pathological changes will be reported.

# 6. Data treatment

## 6.1 Data analysis

If required $LD_{50}$ analysis will be performed using the SAFEStat application[2]. Alternative statistical analysis may be performed, details of which will be held in the raw data and included in the report.

## 6.2 Computer systems

The computer systems used may include (but not be limited to) those listed below:

| | |
|---|---|
| Pristima: | pharmacy test substance management |
| Waters Empower: | formulation analysis |
| Sample Registry System: | sample analysis tracking |

Computer systems used by Huntingdon Life Sciences will be documented in the Huntingdon Life Sciences report.

# 7. Reporting

## 7.1 Report content

A complete report will be prepared and will contain, but need not be limited to, the following information:

1. Dates on which experimental work was started and completed.
2. Name and address of research laboratory including location where study was performed.
3. Name and address of the Sponsor.
4. Name and signature of the Study Director responsible for the study.
5. A statement of the objectives of the study.
6. A statement of the relevant regulatory guideline(s) followed.
7. Information on the test substance (including identification/chemical name, batch purity, storage and handling).
8. Dose levels and description of dose preparation and administration.
9. Information on the test birds including species (common and scientific names), sex, age, weight, acclimatisation period, husbandry, food consumption and general health.

[2] Brammer R.J. (2003) LD50 SAS Application. Internal document, Department of statistics, Huntingdon Life Sciences.

10. Information on husbandry, including facility type, cage accommodation, environmental conditions, diet, drinking water.
11. Full description of experimental design and procedures, including allocation, treatment and observations.
12. Full description of results obtained, including mortality, clinical signs, body weight, food consumption, necropsy findings, dose formulation analytical results.
13. Description of statistical methods used to analyse mortality data and/or other quantitative data, with appropriate reference to any validated computer systems used.
14. Description of all circumstances that may have affected the quality or integrity of the data; description of any deviations from the protocol together with a statement of assessment of any impact of the deviation(s) on study quality or integrity.
15. Summary and analysis of data, and statement of conclusions.
16. Quality Assurance Statement.
17. Statement of GLP compliance signed by the research laboratory Study Director.
18. Location of archives.

## 7.2 Report issue

| | |
|---|---|
| Study progress | Periodic verbal and/or written updates on study progress will be provided by the Study Director. |
| Draft final report | For review by the Sponsor. |
| Authorised final report | After approval from the Sponsor. |

Routinely, the following number of reports will be supplied on A4 paper (unless otherwise requested by the Sponsor):

| | |
|---|---|
| Draft report: | 1 electronic (PDF or MS Word) |
| Final report: | 1 unbound, printed single-sided with original signatures<br>1 electronic (PDF) copy |

Any additions or corrections to an authorised final report will be documented as a formal addendum/amendment to the final report.

In the absence of ongoing communications, Huntingdon Life Sciences reserves the right to finalise, sign and issue the final report from this study six months after issue of the draft. In such an event, all materials will be transferred to the archive. Any subsequent requests for modifications, corrections or additions to the final report will be the subject of a formal report amendment (or new study, as appropriate) and will be subject to additional cost.

# 8. Quality Assurance and Archiving Procedures

## 8.1 Quality assurance

The following will be inspected or audited in relation to this study.

| | |
|---|---|
| Protocol Audit | Authorised protocol and any amendments. |
| Process based inspections | Procedures will be inspected on representative studies, not necessarily on this study. |
| Report Audit | The draft report and study data will be audited before issue of the draft report to the Sponsor. |

QA findings will be reported to the Study Director and Company Management promptly on completion of each action, except for process based inspections, which will be reported to appropriate Company Management only.

## 8.2 Archiving

Records and documentation relating to this study (including electronic records) will be maintained in the archives of Huntingdon Life Sciences for a period of one year from the date on which the Study Director signs the final report. This will include but may not be limited to the Study Protocol, raw data, a copy of the final report, test material and other samples and specimens generated during the course of this study. If requested, Huntingdon Life Sciences will continue to retain the materials at additional cost.

Samples and specimens that no longer afford evaluation will be discarded in accordance with Standard Operating Procedures.

Huntingdon Life Sciences will retain in its archive the Quality Assurance records relevant to this study for a period of 20 years and a copy of the final report indefinitely.

## 8.3 Amendment to protocol

Any planned deviation from this protocol will be documented in the form of a protocol amendment.

# 9. Health and Safety

## 9.1 Statutory requirements

In order for Huntingdon Life Sciences to comply with the Health and Safety at Work etc. Act 1974, and the current Control of Substances Hazardous to Health Regulations, it is a condition of undertaking the study that the Sponsor shall provide Huntingdon Life Sciences with all information available to it regarding known or potential hazards associated with the handling and use of any substance supplied by the Sponsor to Huntingdon Life Sciences. The Sponsor shall also comply with all current legislation and regulations concerning shipment of substances by road, rail, sea or air.

Such information in the form of a completed Huntingdon Life Sciences test substance data sheet must be received by Safety Management Services at Huntingdon Life Sciences before the test substance can be handled in the laboratory. At the discretion of Safety Management Services at Huntingdon Life Sciences, other documentation containing the equivalent information may be acceptable.

## 9.2 Department safety procedures

Safety precautions to be adopted will be documented in the study records.

## Contact Details

| | |
|---|---|
| **Sponsor** | AMVAC CHEMICAL CORPORATION<br>4695 MacArthur Court, Suite 1200<br>Newport Beach<br>California 92660-1706<br>USA |
| **Test facility** | Huntingdon Life Sciences<br>Huntingdon Research Centre<br>Woolley Road<br>Alconbury<br>Huntingdon<br>Cambridgeshire<br>PE28 4HS<br>UK |
| **Study Director** | Adrian Bull<br>Huntingdon Life Sciences<br>Huntingdon Research Centre<br>Woolley Road<br>Alconbury<br>Huntingdon<br>Cambridgeshire<br>PE28 4HS<br>UK<br><br>Tel: +44 (0) 1480 892506<br>Email: bullad@ukorg.huntingdon.com |
| **Huntingdon Life Sciences Toxicologist** | David Cameron<br>Tel: +44 1480 892097<br>Email: CameronD@UKOrg.Huntingdon.com |
| **Alternative Study contact** | Vanessa Ross<br>Tel: +44 (0) 1480 892506<br>Email: rossv@ukorg.huntingdon.com |
| **Study Monitor** | Ann Jonynas, M.Sc., DipRCPath (Tox)<br>Director of Toxicology<br>AMVAC Chemical Corporation<br>2110 Davie Avenue<br>City of Commerce<br>CA90040<br>USA<br><br>Tel: +1 323 890 1263<br>Fax: +1 323 727 1185<br>Email: annj@amvac-chemical.com |

# Protocol Approval

## DCPA (Chlorthal Dimethyl): Acute Oral Toxicity ($LD_{50}$) to the Zebra finch

A. Bull — 10 April 2014.

Adrian Bull
Study Director
Huntingdon Life Sciences

Date

The signature of the Study Director confirms this protocol as the working document for the study. Any changes made subsequent to the date of the Study Director's signature will be documented in formal amendments.

D G Coleman — 10 April 2014

David Coleman
Management
Huntingdon Life Sciences

Date

Approval received: A Jonynas — 10 April 2014

(Study Monitor)

Date

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# PROTOCOL AMENDMENT 1

# DCPA (CHLORTHAL DIMETHYL): Acute Oral Toxicity ($LD_{50}$) to the Zebra finch

| | |
|---|---|
| **HLS study number:** | BDG0198 |
| **Version ID:** | Protocol Amendment 1 |
| **Issue date:** | 24 April 2014 |

# Amendment approval

## DCPA (CHLORTHAL DIMETHYL): Acute Oral Toxicity (LD50) to the Zebra finch

Study Director Adrian Bull

The signature of the Study Director authorises the implementation of this amendment to protocol. In this amendment, deleted statements are struck through and new statements are underlined. Any changes to the study design after the date of this authorising signature will be documented in a further formal amendment.

**For Huntingdon Life Sciences Ltd**

A.J. Bull — 24 April 2014.

Study Director — Date

**For Sponsor**

(Approved by email (See attached email)) — 24 April 2014

Sponsor (email held with raw data) — Date

**DCPA (CHLORTHAL DIMETHYL): Acute Oral Toxicity (LD50) to the Zebra finch**

Reasons for amendment

**Section 3.2:** Addition of vehicle

**Section 3.3:** Addition of main study dosing concentrations.

**Section 8.2:** Removal of archive storage for the final report for one year (as this is stored indefinitely).

**Section 5.1:** Confirmation of individual bird numbers.

# 3. Test Substance and Formulation

## 3.2 Vehicle

1% w/v methylcellulose.

## 3.3 Preparation of dose

A single preparation of dose will be made on each occasion of dosing, following one of the methods below.

| Treatment | Nominal concentration |
|---|---|
| Group 1 | Vehicle only; 0 mg/mL |
| Group 2 | DCPA; ~~X~~ 400 mg/mL |
| ~~Group 3~~ | ~~DCPA; X mg/mL~~ |
| ~~Group 4~~ | ~~DCPA; X mg/mL~~ |
| ~~Group 5~~ | ~~DCPA; X mg/mL~~ |
| ~~Group 6~~ | ~~DCPA; X mg/mL~~ |

~~X to be confirmed in a protocol amendment~~

### 3.3.1 Solutions

A single preparation in the appropriate vehicle will be prepared with up to five dose concentrations so that all birds receive the same dose volume per unit of bodyweight. The test substance will be dissolved in the vehicle by stirring and/or shaking. A high shear homogeniser may be used to aid dissolution. Warming and/or ultrasonication may also be required. Details will be recorded in the study data and included in the final report.

### 3.3.2 Suspensions

A single preparation in the appropriate vehicle will be prepared with up to five dose concentrations so that all birds receive the same dose volume per unit of bodyweight. The test substance will be ground in a mortar with the vehicle until a smooth paste is formed. The formulation will then be gradually made up to volume and mixed using a high shear homogeniser.

# 5. Experimental Procedures

## 5.1 Treatment groups

~~There will be a negative control group of ten birds and a minimum of five† treatment groups each of ten birds. Each group will consist of five males and five females. In addition, six spare birds (three males and three females) will be maintained for use as replacements for any birds which die or show signs of ill health during the pre-treatment observation period.~~

~~†except for test substances of low toxicity where a limit test is determined from range finding tests to be appropriate; in this case, there will be a negative control group of ten birds and a single test group of ten birds treated at the limit dose of 2000 mg/kg or at a dose equivalent to the calculated field exposure level, whichever is the higher.~~

Range finding will be carried out on groups of two birds (one male and one female) at dose levels up to a maximum of 2000 mg/kg (unless environmental residues are expected to result in a higher level of exposure). Depending on the outcome for each pair of birds, further pairs may be dosed at higher or lower dose levels until sufficient data are obtained to enable dose level selection for the definitive test. If regurgitation is observed during range-finding, the Study Director, in consultation with the Sponsor, will determine an appropriate course of action and suitable amendments to the study plan, including consideration of whether capsule dosing may be used as an alternative method of dosing. The US EPA recommendations on decision-making in the event of regurgitation, published as a flowchart by EFED (Environmental Fate and Effects Division), will also be taken into consideration. ~~All details will be documented by protocol amendment.~~ A range finding test was undertaken using one male and one female at the dose level of 2000 mg/kg.

The range finding test indicated that the LD50 was greater than 2000 mg/kg therefore in this study the limit test is determined as the most appropriate and in this case, there will be a negative control group of ten birds and a single test group of ten birds treated at the limit dose of 2000 mg/kg

| Group | Treatment | Dose concentration (mg/mL) | Dose level (mg/kg) | Dose volume mL | Bird numbers | |
|---|---|---|---|---|---|---|
| | | | | | Males | Females |
| 1 | Control | 0 | 0 | 5 | 81, 83, 85, 87, 89 | 82, 84, 86, 88, 90 |
| 2 | DCPA (Chlorthal Dimethyl) | 400 | 2000 | 5 | 91, 93, 95, 97, 99 | 92, 94, 96, 98, 100 |
| Spares | | | | | 67 | 68 |

# 8. Quality Assurance and Archiving Procedures

## 8.2 Archiving

Records and documentation relating to this study (including electronic records) will be maintained in the archives of Huntingdon Life Sciences for a period of one year from the date on which the Study Director signs the final report. This will include but may not be limited to the Study Protocol, raw data, ~~a copy of the final report,~~ test material and other samples and specimens generated during the course of this study. If requested, Huntingdon Life Sciences will continue to retain the materials at additional cost.

Samples and specimens that no longer afford evaluation will be discarded in accordance with Standard Operating Procedures.

Huntingdon Life Sciences will retain in its archive the Quality Assurance records relevant to this study for a period of 20 years and a copy of the final report indefinitely.

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# PROTOCOL AMENDMENT 2

# DCPA (CHLORTHAL DIMETHYL): Acute Oral Toxicity ($LD_{50}$) to the Zebra finch

| | |
|---|---|
| **HLS study number:** | BDG0198 |
| **Version ID:** | Protocol Amendment 2 |
| **Issue date:** | 29 May 2014 |

# Amendment approval

## DCPA (CHLORTHAL DIMETHYL): Acute Oral Toxicity (LD50) to the Zebra finch

Study Director Adrian Bull

The signature of the Study Director authorises the implementation of this amendment to protocol. In this amendment, deleted statements are struck through and new statements are underlined. Any changes to the study design after the date of this authorising signature will be documented in a further formal amendment.

**For Huntingdon Life Sciences Ltd**

A.I. Bull — Study Director

29 May 2014 — Date

**For Sponsor**

Approved by email (See attached email) — Sponsor (email held with raw data)

03 June 2014 — Date

**DCPA (CHLORTHAL DIMETHYL): Acute Oral Toxicity (LD50) to the Zebra finch**

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|---|---|
| Reasons for amendment | Main study dates were rescheduled due to a delay in receiving pelleted diet from the supplier. |

# 2. Study Schedule

## 2.2 Scheduled time plan

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|---|---|
| Experimental start date (Range finder) | 16 April 2014 |
| Start of acclimation period (Main phase) | Week beginning ~~05 May~~ 02 June 2014 |
| Day of dosing (Main phase) | Week beginning ~~20 May~~ 16 June 2014 |
| End of observation period | Week beginning ~~03~~ 30 June 2014 |
| Draft report to be issued | ~~01~~ 29 August 2014 |