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Momentive Specialty Chemicals Inc.
180 East Broad Street
Columbus, OH 43215
momentive.com

MR# 352340

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OFFICE

2013 FEB 25 10:54

February 21, 2013

TSCA Confidential Business Information Center (7407M)
EPA East – Room 6428
Attn: Section 8(e)
U.S. Environmental Protection Agency
Ariel Rios Building
1200 Pennsylvania Ave., NW
Washington, DC 20460-0001



RE: TSCA Section 8(e) Notification of Substantial Risk: 2,3-epoxypropyl
neodecanoate, CAS No. 26761-45-5

Dear TSCA Section 8(e) Coordinator:

Momentive Specialty Chemicals Inc. is submitting the attached final study report under the Toxic Substances Control ACT (TSCA) Section 8(e), and as a follow up to our original submission dated September 19, 2012. At this time, Momentive Specialty Chemicals continues to believe the data does not represent a significant risk.

Regards,

Sydney Lindquist
Global Product Safety Leader
Momentive Specialty Chemicals Inc. • 180 E. Broad St. • Columbus, OH 43212 • (614)225-4778
E-mail: sydney.lindquist@momentive.com



CONTAINS NO CBI

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Final Report

Study Title Induction of *lacZ*- mutations in the liver, kidney, bone marrow and spermatazoa of treated Muta™Mice

Test Article CARDURA™E10P

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Covance Client Identifier 1009830

Covance Study Number 8259749

Report Issued February 2013

Page Number 1 of 62

**STUDY DIRECTOR AUTHENTICATION
AND GLP COMPLIANCE STATEMENT**

**CARDURA™E10P: Induction of *lacZ*- mutations in the liver, kidney, bone
marrow and spermatazoa of treated Muta™Mice**

I, the undersigned, hereby declare that the work was performed under my supervision and that the findings provide a true and accurate record of the results obtained.

The study was performed in accordance with the agreed protocol and with Covance Laboratories Limited Standard Operating Procedures, unless otherwise stated, and the study objectives were achieved.

The study was conducted in compliance with the United Kingdom Good Laboratory Practice Regulations 1999, Statutory Instrument No. 3106 as amended by the Good Laboratory Practice (Codification Amendments Etc.) Regulations 2004 and the OECD Principles on Good Laboratory Practice (revised 1997, issued January 1998) ENV/MC/CHEM (98) 17.



C Beevers PhD
Study Director

16 February 2013
Date

QUALITY ASSURANCE STATEMENT

CARDURA™E10P: Induction of *lacZ*- mutations in the liver, kidney, bone marrow and spermatazoa of treated Muta™Mice

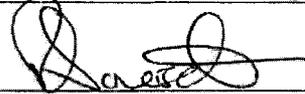
This study has been reviewed by the GLP Quality Assurance Unit of Covance and the report accurately reflects the raw data. The following inspections were conducted and findings reported to the Study Director (SD) and associated management.

Critical procedures, which are performed routinely in an operational area, may be audited as part of a "process" inspection programme. This can be in addition to phases scheduled on an individual study basis. Selected process inspections conducted and considered applicable to this study are included below.

In addition to the inspection programmes detailed below, a facility inspection programme is also operated. Details of this programme, which covers all areas of the facility annually (at a minimum), are set out in standard operating procedures.

Inspection Dates		Phase	Date Reported to SD and SD Management
From	To		
21 Feb 2012	21 Feb 2012	Protocol Review	21 Feb 2012
18 Apr 2012	18 Apr 2012	Protocol Amendment Review	18 Apr 2012
28 May 2012	28 May 2012	Protocol Amendment Review	28 May 2012
28 May 2012	28 May 2012	Protocol Amendment Review	28 May 2012
06 Jun 2012	06 Jun 2012	Dose Preparation	06 Jun 2012
20 Aug 2012	20 Aug 2012	Sample Preparation	20 Aug 2012
29 Nov 2012	06 Dec 2012	Draft Report and Data Review	06 Dec 2012
11 Feb 2013	11 Feb 2013	Final Report Review	11 Feb 2013

Inspection Dates		Phase	Date Reported to SD and SD Management
From	To		
29 Mar 2012	29 Mar 2012	Data Lodging	29 Mar 2012
30 Mar 2012	30 Mar 2012	Sample Loading	30 Mar 2012
10 Apr 2012	10 Apr 2012	Sample Preparation	10 Apr 2012
11 Apr 2012	11 Apr 2012	Treatment	11 Apr 2012
19 Apr 2012	19 Apr 2012	Dose Preparation	19 Apr 2012
03 May 2012	03 May 2012	Standard Preparation	03 May 2012
08 May 2012	08 May 2012	Dose Preparation	08 May 2012
09 May 2012	09 May 2012	Cell line checks	09 May 2012
10 May 2012	10 May 2012	eArchiving Procedure	10 May 2012
17 May 2012	17 May 2012	eArchiving Procedure	17 May 2012

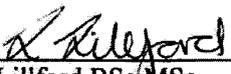

M Somerset
Quality Assurance Unit

14 February 2013
Date

REVIEWING SCIENTIST'S STATEMENT

CARDURA™E10P: Induction of *lacZ*- mutations in the liver, kidney, bone marrow and spermatazoa of treated Muta™Mice

I, the undersigned, hereby declare that I have reviewed this report in conjunction with the Study Director and that the interpretation and presentation of the data in the report are consistent with the results obtained.



L Lillford BSc MSc
Scientist

4 February 2013
Date

RESPONSIBLE PERSONNEL

CARDURA™E10P: Induction of *lacZ*- mutations in the liver, kidney, bone marrow and spermatazoa of treated Muta™Mice

The following personnel were responsible for key elements of the study:

Study Director	C Beevers
Study Supervisor	G Pearce
Animal House Supervisor	A Wronska
Formulations Analyst	P Cox
Statistics	J Saul
Sponsor Representative ¹	T Barfknecht

¹ Located at Momentive Speciality Chemicals, USA.

ARCHIVE STATEMENT

CARDURA™E10P: Induction of *lacZ*- mutations in the liver, kidney, bone marrow and spermatazoa of treated Muta™Mice

All primary data, or authenticated copies thereof, will be retained for 1 year in the Covance Laboratories Limited archives after issue of the Final Report. At this time, the Sponsor Representative will be contacted to determine whether the data should be returned, retained or destroyed on their behalf. The Sponsor Representative will be notified of the financial implications of each of these options at that time. One copy of the protocol and final report will be held in the Covance Laboratories Limited archives as per Covance company policy.

Specimens (formulation samples) if taken during the experimental phase will be retained for as long as the material permits further evaluation, up to issue of the final report. At this time, all samples will be discarded. Specimens or samples will not be destroyed without prior approval of the Study Director.

Any remaining DNA and/or frozen tissue samples will be retained for 1 year in the Department of Genetic and Molecular Toxicology at Covance Laboratories Limited after issue of the Final Report. At this time, the Sponsor Representative will be contacted to determine whether the samples should be transferred to an alternative storage area, retained or destroyed on their behalf.

ABBREVIATIONS

°C	Degrees Celsius
ANOVA	Analysis of variance
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
ECHA	European Chemicals Agency
ENU	EthylNitrosurea
EU	European Union
g	Gram
g	Gravity
GLP	Good laboratory practice
IWGT	International Working group on Genotoxicity Testing
kg	Kilogram
LB	Luria broth
M	Molar
µg	Microgram
µL	Microlitre
mg	Milligram
mL	Millilitre
MF	Mutant frequency
MTD	Maximum tolerated dose
NaCl	Sodium chloride
OECD	Organization for Economic Cooperation and Development
P-gal	Phenyl-galactose
PBS	Phosphate buffered saline
pfu	Plaque forming unit
QA	Quality Assurance
SD	Study Director
SDS	Sodium dodecyl sulfate
SOP	Standard operating procedure
TRAID	Transgenic Rodent Assays Information Database

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SUMMARY

CARDURA™E10P (2,3-epoxypropyl neodecanoate) was tested for its ability to induce gene mutation in the *lacZ* transgene in the liver, kidney, bone marrow and developing sperm cells (taken from the seminiferous tubules) of treated male transgenic mice.

Strain / Species:	Muta™Mice (CD ₂ -lacZ80/HazfBR strain).
Vehicle:	Corn oil.
Administration route:	Oral gavage for all test article and vehicle administrations. Intraperitoneal injection for administration of the positive control.
Dosing regimen:	Range-Finder Experiment: Test article administered daily for 15 consecutive days. Main Experiment: Test article and vehicle control administered daily for 42 consecutive days. Positive control administered daily on Days 1-5 and Days 23-24.
Gender:	Males.
Dose levels:	250, 500 and 1000 mg/kg/day.
Maximum dose:	Maximum recommended dose level.
Positive control:	EthylNitrosurea (ENU), 100 mg/kg.
Animals per group:	Seven.
Dose volume:	10 mL/kg.
Formulation analysis:	Achieved concentration and homogeneity within specification.
Clinical signs of toxicity:	No signs of toxicity observed throughout the dosing period.
Tissues sampled:	The liver, kidney, bone marrow and developing sperm cells (taken from the seminiferous tubules) were taken from all animals on Day 45 (i.e. 3 days after the final test article administration).
Assay validity:	The concurrent vehicle control data were comparable with either the laboratory's historical control data (liver, kidney and bone marrow) or published literature (developing sperm cells (taken from the seminiferous tubules)). The concurrent positive control, ENU, induced a significant increase in mutant frequency compared to the vehicle control animals in all tissues examined. The assay was therefore accepted as valid.

Statistically significant, dose-related increases in mutant frequency (MF) were observed in the liver, kidney and bone marrow (analysis performed using ANOVA at the 5% level) of treated mice. In the liver at the high dose, the MF was 3.1-fold the mean concurrent vehicle control MF. No significant increases in MF were observed in the developing sperm cells (taken from the seminiferous tubules) of these animals.

It was concluded that CARDURATME10P induced mutation in the *lacZ* transgene in the liver, kidney and bone marrow of male MutaTMMice that had been dosed daily for 42 days at up to 1000 mg/kg/day (the maximum recommended dose for this study). No evidence of mutation was observed in the developing sperm cells.

OBJECTIVE

The objective of this study was to evaluate the potential of CARDURA™E10P to induce gene mutations in the *lacZ* transgene in the liver, kidney, bone marrow and developing sperm cells (taken from the seminiferous tubules) of treated male Muta™Mice.

INTRODUCTION

The Muta™Mouse (*lacZ/galE*) assay was developed in 1989 (Gossen *et al.*, 1989) and further refined by the development of a positive selection system developed by Ingeny BV in The Netherlands (Gossen & Vijg, 1993) and described by Dean & Myhr (1994).

The Muta™Mouse (*lacZ/galE*) assay is one of the few *in vivo* genotoxicity assays that is capable of detecting the induction of point mutations and small deletions (as opposed to gross chromosomal damage/loss) and has been widely demonstrated to detect mutation in a range of tissues using known mutagens/carcinogens (Lambert *et al.*, 2005). The ability to detect such changes in a variety of tissues, means this assay is useful for evaluating the genotoxic potential of chemicals that might interact at the site of contact (Dean *et al.*, 1999) and therefore has distinct advantages over conventional *in vivo* mutagenicity tests in which exposure of an appropriate target tissue or site of contact to the test article cannot always be guaranteed.

The genome of the Muta™Mouse strain contains 40 copies of a transgenic lambda gt10 vector, each of which contains a bacterial *lacZ* gene, and is therefore present in every DNA-bearing cell of the animal. Treatments are performed *in vivo*, with sufficient treatment and expression time to permit any mutations to be expressed. Tissues are taken at necropsy, cellular DNA extracted and subsequently packaged into lambda bacteriophage. The packaged bacteriophage are then used to transfect a culture of *E. coli* C *lac*⁻ *galE* Kan^r (*galE* Amp^r). Only bacteriophage units containing a *lacZ* gene from the transgenic vector in the mouse DNA are viable and capable of transfecting a bacterial cell. Successful transfection enables replication of the bacteriophage and transfection of neighbouring bacterial cells. This results in the formation of a visible plaque, that is an area of bacterial cell lysis in a lawn of uninfected viable bacterial cells. Furthermore, due to the *galE*⁻ status of the host bacterial strain cells, only cells infected with a non-functional (i.e. mutated) *lacZ* gene can form plaques on the positive selection plates.

Mutant frequency (MF) is calculated using the total number of viable bacteriophage (i.e. phage that have been successfully packaged with the *lacZ* transgene and visible as plaques on titre plates), compared with the number of bacteriophage which contain mutated *lacZ* transgenes (i.e. plaques on positive selection plates).

This study was performed according to the protocol and three amendments with the exception of the minor deviations detailed in Appendix 4, none of which prejudiced the validity of the study.

The study was initiated on 17 February 2012. Experimental work started on 19 March 2012 and was completed on 26 October 2012. The study completion date is considered to be the date the Study Director signs the final report.

EXPERIMENTAL DESIGN

Regulatory test guidelines

The test methodology used in this study was in accordance with the OECD Guideline 488 (OECD, 2011), the Report of the Transgenic Working group (TWG) at the 2002 International Workshop on Genotoxicity Testing (IWGT: Thybaud *et al.*, 2003) and current literature (as reviewed by Lambert *et al.*, 2005).

Justification for selection of the test system

Transgenic rodent mutation assays, such as MutaTMMouse are recommended by various regulator authorities, including the E.C.H.A, as an appropriate test to determine the genotoxic potential of a compound *in vivo* (COM, 2000).

CARDURATME10P (2,3-epoxypropyl neodecanoate) was positive in multiple Ames/*Salmonella* gene-mutation assays. However, the test substance was negative in other *in vitro* assays including chromosome aberrations. Furthermore, CARDURATME10P did not induce DNA single-strand breaks or DNA repair (UDS) *in vivo* in rat liver following oral gavage. This study was conducted at the specific request of the E.C.H.A. and several E.U. Competent Authorities.

MutaTMMouse was selected as the test system as it is the most commonly reported transgenic system in use and forms the majority of entries in the Transgenic Rodent Assays Information Database (TRAID; Lambert *et al.*, 2005). There is also a large volume of background data in this transgenic strain in this laboratory.

The tissues selected for evaluation were possible target organs and had been selected at the specific request of the E.C.H.A.

Test article and vehicle control administration

All test article and vehicle treatments were given via oral gavage in order to maximise exposure of the target organ to the test article and at the specific request of the E.C.H.A. Animals were not fasted prior to dose administration.

In the Range-Finder Experiment, animals were dosed on each of 15 consecutive days.

In the Mutation Experiment, animals were dosed on each of 42 consecutive days (Days 1–42) and sacrificed on Day 45, i.e. 3 days after the final administration (OECD, 2011; Lambert *et al.*, 2005). DNA was extracted from liver, kidney, bone marrow and developing sperm cells (taken from the seminiferous tubules) of all animals dosed and evaluated for mutant frequency.

The test article and vehicle control were administered at a dose volume of 10 mL/kg. Individual dose volumes were based on individual body weight.

Positive control administration

Positive control animals were dosed with ethylnitrosurea (ENU) once daily on Days 1–5 and 23–24. Animals were sacrificed on Day 45.

The positive control was administered via intraperitoneal injection at a dose volume of 10 mL/kg. Individual dose volumes were based on individual body weight.

Justification for dose selection

The Sponsor confirmed that the published LD₅₀ for CARDURA™E10P was >2000 mg/kg .

Based on this information a dose of 1000 mg/kg/day (the recommended maximum dose in accordance with current regulatory guidelines) was administered in a preliminary Range-Finder Experiment. As this dose was seen to be tolerated over the observation period of the assay, no subsequent lower or higher doses were tested (OECD, 2011; ICH-S2R1, 2011; Fielder *et al.*, 1993).

From the data generated in the Range-Finder Experiment, a dose level of 1000 mg/kg/day CARDURA™E10P was used as the maximum dose for the Main Experiment. Two lower dose levels of 250 and 500 mg/kg/day CARDURA™E10P (equivalent to 25% and 50% of the high dose respectively) were also tested.

As no gender differences in toxicity were identified in previous studies and as information on induction of germ cell mutations was required, testing was performed solely in male animals (OECD, 2011).

Dose levels

The following dose levels were tested in this study:

Table 1: Dose Levels - Range-Finder Experiment

Group No.	Group Description	Dose level (mg/kg/day)	No of animals tested
1RF	CARDURA™E10P	1000	3M

M Male

Table 2: Dose Levels - Main Experiment

Group No.	Group Description	Dose level (mg/kg/day)	Animal ID	Day of administration
			Main Experiment animals	
			Male	
1	Vehicle ^a	0	1-7	1 – 42
2	CARDURA™E10P	250	8-14	1 – 42
3	CARDURA™E10P	500	15-21	1 – 42
4	CARDURA™E10P	1000	22-28	1 – 42
5	Positive control ^b	100	29-35	1 – 5 and 23 – 24

M Male

a Corn oil

b Ethylnitrosurea (ENU)

Tissues for mutation analysis

The following tissues were retained for immediate analysis of mutant frequency:

Liver

Kidney

Bone marrow

Developing sperm cells from seminiferous tubules.

In addition, samples of spermatozoa from the vas deferens were taken but not analysed. This tissue was retained for possible future analysis.

Proof of exposure

No concurrent proof of exposure was performed at the Sponsor's request.

TEST AND CONTROL ARTICLES

Test article

CARDURA™E10P (also known as 2,3-epoxypropyl neodecanoate and Glycidyl neodecanate), batch number PH1H0558 was a colourless liquid. It was received on 14 March 2012 and stored at 15-25°C protected from light. Purity was stated as approximately 89% and the expiry date was given as 30 August 2012. The test article was used as supplied with no correction for purity.

The certificate of analysis, provided by the Sponsor, is given in Appendix 3. The test article information and certificate of analysis provided by the Sponsor were considered an adequate description of the characterisation, purity and stability of the test article. Determinations of stability and characteristics of the test article were the responsibility of the Sponsor.

Controls

The negative (vehicle) control group consisted of animals dosed with corn oil using the same dosing regimen and dose volume used for the test article treated animals.

Untreated controls were not included in this study as the vehicle is commonly used in this laboratory and considered to have no mutagenic risk.

The positive control, ethylnitrosurea (ENU: Sigma-Aldrich, Poole, UK) was freshly prepared in water for injection as tabulated below:

Table 3: Positive Control

Dose volume (mL/kg)	Concentration of ENU solution (mg/mL)	Dose of ENU administered (mg/kg)
10.0	10.0	100.0

TEST ARTICLE FORMULATION

Preparation

Dosing preparations were prepared daily for the Range-Finder Experiment and weekly for the Main Experiment. CARDURA™E10P formulations were prepared in corn oil as follows:

The test article was weighed and the required volume of vehicle was added to achieve the final concentration. Formulations were then stirred for a short time at high speed until homogenous.

The following concentrations of CARDURA™E10P were used during this study:

Table 4: CARDURA™E10P Concentrations Tested

Experiment	Concentration of dosing preparation (mg/mL)	Dose administered (mg/kg/day)
Range-Finder	100.0	1000
Main Experiment	25.00 50.00 100.0	250 500 1000

Stability and homogeneity

Stability of CARDURA™E10P in corn oil for up to 7 days at 2-8°C or at room temperature (15-25°C) was confirmed in this study (Formulations Analysis Report).

To ensure homogeneity, dose preparations were stirred continuously (on a magnetic stirrer) immediately before and throughout dosing.

Formulations analysis

Homogeneity and stability assessments were performed on CARDURA™E10P preparations at 25 and 100 mg/mL in corn oil prior to the start of the Main Experiment. Triplicate samples were taken from the top and bottom of each formulation and analysed immediately. The remaining formulations were split into two aliquots: one held at 15-25°C (room temperature) and the other at 2-8°C. These aliquots were sampled (in triplicate from the top and bottom) 24 hours and 7 days after preparation.

Analysis of achieved concentration was performed on each formulation prepared for use in week 1 and week 5 of the Main Experiment. Either duplicate (test article formulations) or single (vehicle control) samples were taken at random on each occasion from the bulk preparations prior to daily aliquoting.

TEST SYSTEM

Species, strain and supplier

A total of 45 out-bred young adult male MutaTMMouse (CD₂-lacZ80/HazfBR) were purchased from Harlan, UK. This random bred mouse strain was designed specifically for the detection of mutations in the *lacZ* gene in a lambda gt10 transgene.

Mice not dosed in this study were transferred to Covance Laboratories Ltd stock.

Specification

Three animals were dosed during the Range-Finder Experiment. They were 6-9 weeks old and weighed 22-24 g on the first day of dosing (see minor deviations from protocol, Appendix 4).

Animal specification for the Mutation Experiment was as follows:

Table 5: Animal Specification

		Main Experiment
	Number of animals used in study	35M
	Weight range on first day of assay (g)	23 – 33
	Approximate age on first day of dosing (weeks)	8 – 13 ^a
M	Male	
a	See minor deviations from protocol, Appendix 4	

Environment

The animals were routinely kept in the following environment except for short periods of time where experimental procedures dictated otherwise. The animals were housed in a room air-conditioned to provide 15-20 air changes/hour. The temperature and relative humidity ranges were 20 to 24°C and 45 to 65%, respectively. Fluorescent lighting was controlled automatically to give a cycle of 12 hours light (0600 to 1800) and 12 hours dark. The study room was used to house animals allocated to other studies.

The animals were housed in groups of three in cages that conformed with the 'Code of practice for the housing and care of animals used in scientific procedures' (Home Office, London, 1989).

Environmental enrichment

In order to enrich both the environment and the welfare of the animals, they were provided with wooden Aspen chew blocks and nesting material.

Diet, water and bedding

Throughout the study the animals had access *ad libitum* to SQC Rat and Mouse Maintenance Diet No 1, Expanded (Special Diets Services Ltd. Witham). Each batch of diet was analysed for specific constituents and contaminants.

Mains water was provided *ad libitum* via water bottles. The water is periodically analysed for specific contaminants.

Bedding was provided on a weekly basis to each cage by use of clean European softwood bedding (Datesand Ltd, Manchester). The bedding was analysed for specific contaminants.

No contaminants were expected to be present in diet, water or bedding at levels that might interfere with achieving the objective of the study. Results of analyses performed on diet, water, bedding and environmental enrichment are held centrally at Covance Laboratories Ltd.

METHODS

Pre-experimental procedures

Acclimatisation and health procedures

All animals were given a clinical inspection for ill health on arrival. They were acclimatised for at least 5 days and a veterinary inspection was performed before the start of dosing to ensure their suitability for study.

Allocation to treatment group

On arrival animals were randomly allocated to cages in groups of three for the Range-Finder animals or groups of seven for the Main Experiment.

Checks were made to ensure the weight variation of Main Experiment animals prior to dosing was minimal and did not exceed $\pm 20\%$ of the mean weight.

Identification of the test system

The animals were individually identified by uniquely numbered subcutaneous electronic transponder. Cages were appropriately identified (using a colour-coded procedure) with study information including study number, study type, start date, number and sex of animals, together with a description of the dose level and proposed time of necropsy.

Experimental observations

Health monitoring

All animals were examined at the beginning and the end (nominal) of the working day to ensure that they were in good health and displayed no signs of overt toxicity. Any animal that showed marked signs of ill health was isolated and euthanised as described below. All decedents were subjected to necropsy and findings were recorded in the raw data but have not been reported.

Post dosing observations

An individual record was maintained of the clinical condition of all Range-Finder Experiment and Main Experiment animals dosed in the study.

Observation times were as follows:

Table 6: Post dosing observation times

Animals	Day	Approximate observation time
Range-Finder Experiment	1 2 to 15	Immediate, 0.5, 1.0, 2.0 and 4.0-6.0 hours post dose Pre-dose, immediate, 0.5, 1.0, 2.0 and 4.0-6.0 hours post dose
Main Experiment	1 2 to 7 8 to 42 43 to 45	Immediate, 1.0, 2.0 and 4.0 hours post dose Pre-dose, immediate, 1.0, 2.0 and 4.0 hours post dose Pre-dose, immediate, 2 hours post dose Once daily

Body weights

Individual body weights were recorded as follows:

Table 7: Body weight recording

Animals	Day body weight was recorded
Range-Finder Experiment	Day 1 (prior to dosing) and Day 8 and 15 (prior to termination) ^a
Main Experiment	Day -1 (at study set-up), Day 1, 8, 15, 22, 29 and 36 (prior to dosing), Day 43, Day 45 (prior to necropsy)
Decedents	Prior to necropsy

^a See minor deviations from protocol (Appendix 4).

Food and water consumption

Food and water consumption were recorded weekly. These were calculated and reported as g food consumed/animal/week or mL water consumed/animal/week respectively.

Animal euthanasia and necropsy

Moribund animals were euthanised by an overdose of sodium pentobarbitone, given via intraperitoneal injection and subsequently ensured by cervical dislocation. Necropsy was performed on all decedents and macro observations recorded to determine possible cause of morbidity/death.

Range-Finder Experiment animals were euthanised by an overdose of sodium pentobarbitone, given via intraperitoneal injection and subsequently ensured by cervical dislocation. No necropsy was performed.

Main Experiment animals were euthanised by an overdose of sodium pentobarbitone, given via intraperitoneal injection and subsequently ensured by cervical dislocation in the same sequence used for dosing.

Tissue samples

No tissues were taken from Range-Finder Experiment animals. The following samples were retained from Mutation Experiment animals.

Table 8: Tissue samples

Group	Tissues for mutation analysis	Reserve samples for possible future mutation analysis
1	L, BM, K, SC (st)	Sp (vd)
2	L, BM, K, SC (st)	Sp (vd)
3	L, BM, K, SC (st)	Sp (vd)
4	L, BM, K, SC (st)	Sp (vd)
5	L, BM, K, SC (st)	Sp (vd)
L	Liver	
BM	Bone marrow (both femurs)	
K	Kidney	
SC (st)	Developing sperm cells from seminiferous tubules	
Sp (vd)	Spermatazoa from the vas deferens	

Liver, kidney and testes weights were recorded. Liver and kidney tissues were rinsed in phosphate buffered saline (PBS) and flash frozen in liquid nitrogen. Frozen samples were moved to a freezer at <-50°C for long term storage.

Spermatazoa were isolated from the cauda epididymis; developing sperm cells were isolated from the seminiferous tubules. For each animal the cauda epididymis and seminiferous tubules were incubated separately in PBS at approximately 37°C for approximately 1 hour after which the tissue was removed and the spermatazoa or developing sperm cell suspensions were stored frozen at <-50°C.

Bone marrow was removed from both femurs by cutting off each end to expose the bone marrow and repeatedly flushing with approximately 2 mL of PBS. These samples were briefly held at 2-8°C prior to isolation of the cells by centrifugation. The supernatant was removed and the cell pellets were stored frozen at <-50°C.

DNA extraction

DNA from the liver, kidney and bone marrow was extracted using the Stratagene RecoverEase™ system (Stratagene Ltd., Cambridge, UK) according to the manufacturer's instructions. Prior to extraction the tissues were homogenised as follows:

A slice of liver was excised from the frozen tissue, thawed and homogenised in 5 mL lysis buffer using a Wheaton Douce homogeniser.

One half of each kidney was sliced off, thawed and homogenised in 5 mL lysis buffer using a Wheaton Douce homogeniser.

The frozen pellet of bone marrow cells was thawed and resuspended in 8 mL of lysis buffer. No homogenisation was required.

DNA from developing sperm cells was extracted using the following phenol:chloroform method. 5mL lysis buffer containing 100 mM DTT was added to the thawed developing sperm cells, followed by the addition of 0.5 mL 10% SDS, 0.25 mL proteinase K (20 mg/mL) and 60 µL of RNase A (10 mg/mL). The cells were then incubated at 37°C for 30 minutes and then transferred to 50°C for 2.5 hours. The cellular digest was then gently mixed with an equal volume of phenol:chloroform:isoamyl alcohol (ratio 25:24:1) for approximately 10 minutes. The aqueous and organic phases were separated by centrifugation and the aqueous phase retained. 1.2 volumes of 8 M potassium acetate were added followed by approximately 6 mL of chloroform:isoamyl alcohol (24:1) and gently mixed for approximately 10 minutes. The aqueous phase was isolated by centrifugation and the DNA precipitated with absolute ethanol. The DNA was removed, washed in 70% (v/v) ethanol and air-dried, prior to dissolution in TE-4 buffer.

Isolated genomic DNA from all tissues was stored at 2-8°C until required for packaging.

Culturing of bacteria for transfection

The proprietary strain of *E. coli* C *lac*⁻ *galE*⁻ Kan^r (*galE*⁻ Amp^r) was developed and supplied by J Gossen and J Vijg, Ingeny BV, Leiden, The Netherlands.

An overnight (approximately 16 hours) culture of *E. coli* C *lac*⁻ *galE*⁻, inoculated from a frozen stock, was grown in a shaking incubator at 37±1°C in 10 mL of Luria broth (LB) containing maltose (0.2% w/v), Kanamycin (50 µg/mL) and Ampicillin (50 µg/mL). An aliquot (1 mL) of this overnight culture was inoculated into fresh LB medium (98 mL) together with 1 mL of 20% w/v maltose and incubated with shaking at 37±1°C for approximately 4-5 hours. The cells were centrifuged (approximately 2020 x 'g') for 10 minutes and resuspended in approximately 80 mL of LB medium containing 10 mM magnesium sulphate. The cell suspension was stored on ice until used for phage adsorption.

DNA packaging into phage heads

For each tissue/animal, approximately 5 µl of DNA solution (ideally containing about 7.5 µg of DNA for maximum packaging efficiency) were mixed with Stratagene Transpack packaging reagents (Stratagene Ltd., Cambridge, UK) according to the manufacturer's instructions. Following the necessary incubation and mixing procedures, the resulting mixture contained DNA packaged into phage heads ready for transfection.

Transfection of host bacteria

The assay involved the scoring of titration plates to determine the total number of plaque-forming units (pfu), and selection plates using the positive selection method to determine the numbers of mutants.

Titration plates

For the titration plates, packaged DNA (10 µL) was diluted with SM buffer (190 µL) and 10 µL of this dilution adsorbed to 500 µL suspension of *E. coli* C *lac⁻ galE⁻ Kan^r (galE⁻ Amp^r)* for approximately 20-30 minutes at room temperature. After adsorption, the phage/bacteria was suspended in 12 mL 1:3 LB:NaCl, 0.75% w/v agar (top agar) containing 10 mM magnesium sulphate and plated onto petri dishes (14 cm diameter) containing 12 mL 1:3 LB:NaCl, 1.5% w/v agar (bottom agar). Once the agar had gelled, the plates were inverted and incubated overnight at 37±1°C.

Positive selection plates

The remaining packaged DNA was divided approximately equally into 3 tubes and incubated at room temperature with bacterial suspension (500 µL/tube) for approximately 20-30 minutes, suspended in top agar (as above) containing 10 mM magnesium sulphate and 0.3% w/v phenyl-galactose (P-gal), poured onto plates containing bottom agar (as above) and incubated overnight at 37±1°C.

Scoring of plates

Following overnight incubation, plates were either scored immediately or stored at 2-8°C and scored as soon as possible.

The number of clear plaques on each plate were counted manually and recorded in the raw data. This data was used to determine the number of plaque forming units (pfu) per reaction.

Packaging and transfection controls

Tissues were processed and analysed using a 'block' design, where DNA samples from the negative and positive control groups and each treatment group were processed together. As data sets for each tissue were nearing completion it became impractical to always have animals from each group in any one set of reactions, however, tissue specific positive control DNA were always included in each set, to confirm the success of each packaging and plating occasion. Data from a packaging occasion were only accepted if the concurrent positive control DNA packaging reaction yielded a characteristic elevated mutant frequency compared to historical negative control mutant frequencies.

Analysis of results

Treatment of data

The unit of analysis was the animal.

The total number of mutant plaques and the total (titre) number of pfu are reported for the liver, kidney, bone marrow and developing sperm cells for each animal. The mutant frequency (MF) was calculated for each animal and, from these figures, the mean MF per treatment group. The data obtained with the liver, kidney and bone marrow in the treatment groups were compared to the relevant concurrent control data. No historical control data were available for the developing sperm cells.

Statistical analysis of MF per tissue was performed as follows:

Groups 1, 2, 3, 4 were analysed using one-way analysis of variance (ANOVA). An overall dose response test was performed along with Dunnett's test for pairwise comparisons of each treated group with the vehicle control. All tests were performed with a one-sided risk for increasing response. Levene's test for equality of variances across the groups was also performed and in all cases showed no evidence of heterogeneity ($P > 0.01$).

The positive control (Group 5) was compared with vehicle control (Group 1) using a two-sample t-test. The test was interpreted with a one-sided risk for increasing response. Levene's test for equality of variances between the groups was also performed. Where this showed evidence of heterogeneity ($P \leq 0.01$), the data were rank-transformed prior to analysis.

Acceptance criteria

Acceptance criteria for individual packaging reactions

For every packaging reaction the data were accepted as valid and used for mutation assessment if the following criteria were met:

1. The concurrent positive control DNA packaging reaction yielded an elevated mutant frequency compared to historical vehicle control data.
2. The pfu for each sample on any packaging occasion exceeded 30,000, although in the event of a poorly packaging sample data were accepted if the pfu fell between 10,000 and 30,000.
3. Unless pfu numbers were low (<30,000), at least 1 mutant plaque should be present in each sample. Where low pfu numbers or low MF were obtained, nil mutant counts may be accepted, if this result was not considered markedly different to that obtained on other packaging occasions.

Acceptance criteria for the assay

The assay was considered valid if all the following criteria were met:

1. The group mean vehicle control MF was comparable with the laboratory's historical vehicle control ranges (where available), and
2. At least five animals out of each group were available for analysis, and
3. A total of at least 200,000 pfu from at least three packaging occasions were obtained per tissue per animal.

Acceptance of data that do not meet these criteria are discussed in the Results section.

Evaluation criteria

For valid data, the test article was considered positive, i.e. capable of inducing mutation, if a statistically significant increase in the MF occurred at one or more dose levels in at least one of the tissues examined.

The test article was considered negative in this assay if no statistically significant increase in MF occurred in any of the tissues examined.

The biological relevance of any positive findings were considered in the context of the laboratory's background control historical database.

Computer Systems

The major computer systems used on this study were as follows:

Table 9: Computer Systems

Activity	Computer System
Scheduling	CMS (Covance Management System)
Formulations	Talisman
Formulation analysis	Empower
In-life data collection	Connex
Data generation and collation	Costar / Mutamouse.xls
Report generation	Microsoft Office / Adobe Acrobat

Version numbers of the systems are held on file at Covance.

RESULTS

Range-Finder Experiment

Individual animal body weights are presented in Table 14. No loss of body weight was observed over the dosing period (Table 16).

No clinical signs of toxicity were observed during the 15 day dosing period.

As 1000 mg/kg/day was the maximum recommended dose level for this study (OECD, 2011), this dose level was selected as the maximum dose for the Main Experiment. Two further doses, of 250 and 500 mg/kg/day (equivalent to 25 and 50% of the maximum dose) were also tested in the Main Experiment.

Mutation Experiment

Body weights

Individual animal body weights are presented in Table 17. No loss of body weight or reduced body weight gain was observed over the dosing period (Table 19).

Food and water consumption

Food and water consumptions are presented in Table 20 and Table 21 respectively. No treatment-related effects compared to the vehicle control were observed.

Clinical signs

No clinical signs of toxicity were observed in any animal following treatments with vehicle, CARDURA™E10P (at 250, 500 or 1000 mg/kg/day) or the positive control (ENU, 100 mg/kg/day).

Two animals from Group 2 (250 mg/kg/day CARDURA™E10P, animals 8 and 14) were found dead or moribund (respectively) prior to dosing on Day 9. Necropsy examination of animal 8 identified a rupture in the oesophagus that was considered to be a dosing-related trauma. Although similar effects were not observed in animal 14, the euthanasia method used may have prevented identification of a small rupture and therefore it is considered that dosing-related trauma may have been the cause of this mortality.

Validity of study

The majority of data from any individual packaging reaction providing less than 30,000 pfu were excluded from analysis (due to a possible increase in the variability of the calculated mutant frequencies) and have not been reported. For two packaging occasions data from <30,000 pfu but >10,000 pfu were accepted for analysis. For animal 29, kidney, the observed mutant frequency (MF) demonstrated a clear positive response and was consistent with all other packaging occasions. For animal 1, bone marrow, all reactions gave low pfu counts and the data from this reaction were highly comparable with all other reaction data. Consequently, acceptance of these data was considered justified.

For the majority of animals data were generated for at least 200,000 pfu per tissue, generated from at least three independent packaging reactions. No data were obtained for the following tissues: animals 8 and 14 (died prior to the scheduled sample time), animals 18, 20, 21 and 31 bone marrow (DNA failed to package), animals 25 and 35 developing sperm cells (no DNA was obtained from the collected cells) and animal 6 developing sperm cells (DNA failed to package).

For each set of data accepted for mutation assessment, concurrent packaging of at least one positive control DNA sample yielded a characteristic elevated mutant frequency compared to historical negative control mutant frequencies; this confirming the correct functioning of the packaging reactions on each occasion.

With just one exception, at least 1 million pfu were obtained per group, per tissue from a minimum of five animals. The exception was the bone marrow for Group 2, for which only 903,785 pfu were obtained from a total of four animals. The data obtained for this group showed low variability between animals, and given that a clear mutagenic response was observed at higher doses in bone marrow, the limited amount of data available for this group is considered to have no adverse effect on data interpretation.

Group mean vehicle control mutant frequency data for liver, kidney and bone marrow were comparable with the laboratory's historical data and the developing sperm cell vehicle control data were considered comparable with published literature (Lambert *et al.*, 2005).

The study was therefore accepted as valid.

Analysis of mutation data

Following administration of 250, 500 or 1000 mg/kg/day of CARDURA™E10P the following group mutant frequency data were obtained:

Liver analysis

A statistically significant increase in mutant frequency (MF) was observed in the liver following dosing with CARDURA™E10P at 1000 mg/kg/day (Table 10). This MF was approximately 3.1-fold greater than the concurrent mean control value. Although lower doses of CARDURA™E10P did not induce a significant increase in MF, an increase in group mean MF compared to the concurrent vehicle control was observed and a significant linear trend was also observed.

Qualitative analysis of individual animal MF showed that the majority of animals dosed at 500 and 1000 mg/kg/day had MF that exceeded the concurrent vehicle controls (Table 22) and also the mean and median values of the laboratory's historical control data (Appendix 1).

The data confirmed that CARDURA™E10P did induce mutation in the liver of treated animals.

Table 10: Group summary – Liver

Group	Treatment	Dose (mg/kg/day)	Group Mean MF (x 10 ⁻⁶ ± SD)
1	Vehicle control	0	49.85 ± 18.91
2	CARDURA™E10P	250	68.07 ± 23.42
3	CARDURA™E10P	500	116.33 ± 51.26
4	CARDURA™E10P	1000	155.56 ± 139.89 *
5	ENU	100	561.13 ± 230.91 *** A,S DR***

* P≤0.05

*** P≤0.001

A ANOVA, dose response and Dunnett's (Groups 2, 3 and 4 vs Group 1)

S two-sample t-test (Group 5 vs group 1)

DR Significant dose response test

Kidney analysis

Statistically significant increases in mutant frequency (MF) were observed in the kidney following dosing with CARDURA™E10P at all dose levels (Table 11). A significant linear trend was also observed.

Qualitative analysis of individual animal MF showed that the majority of animals dosed at 25, 500 and 1000 mg/kg/day had MF that exceeded the concurrent

vehicle controls (Table 23) and also the median value of the laboratory's historical control data (Appendix 1).

The data confirmed that CARDURA™E10P did induce mutation in the kidney of treated animals.

Table 11: Group summary – Kidney

Group	Treatment	Dose (mg/kg/day)	Group Mean MF (x 10 ⁻⁶ ± SD)
1	Vehicle control	0	52.66 ± 22.19
2	CARDURA™E10P	250	104.81 ± 26.01 **
3	CARDURA™E10P	500	123.69 ± 17.45 ***
4	CARDURA™E10P	1000	114.00 ± 25.57 ***
5	ENU	100	739.23 ± 139.98 *** A,SR DR***

** P≤0.01

*** P≤0.001

A ANOVA, dose response and Dunnett's (Groups 2, 3 and 4 vs Group 1)

S two-sample t-test (Group 5 vs group 1)

R Rank transformed data

DR Significant dose response test

Bone marrow analysis

Statistically significant increases in mutant frequency (MF) were observed in the bone marrow following dosing with CARDURA™E10P at 500 and 1000 mg/kg/day (Table 12). No increase in MF was observed following dose administration of CARDURA™E10P at 250 mg/kg/day, however, a significant linear trend was observed.

Qualitative analysis of individual animal MF showed that the majority of animals dosed at 500 and 1000 mg/kg/day had MF that exceeded the concurrent vehicle controls (Table 24) and also the mean and median values of the laboratory's historical control data (Appendix 1).

The data confirmed that CARDURA™E10P did induce mutation in the bone marrow of treated animals.

Table 12: Group summary – Bone marrow

Group	Treatment	Dose (mg/kg/day)	Group Mean MF (x 10 ⁻⁶ ± SD)
1	Vehicle control	0	41.21 ± 9.44
2	CARDURA™E10P	250	43.86 ± 10.98
3	CARDURA™E10P	500	76.41 ± 14.89 **
4	CARDURA™E10P	1000	118.62 ± 19.80 ***
5	ENU	100	510.18 ± 346.39 *** AR,S DR***

** P≤0.01

*** P≤0.001

A ANOVA, dose response and Dunnett's (Groups 2, 3 and 4 vs Group 1)

S two-sample t-test (Group 5 vs group 1)

R Rank transformed data

DR Significant dose response test

Developing sperm cell analysis

No statistically significant increases in mutant frequency (MF) were observed in the developing sperm cells following dosing with CARDURA™E10P at 250, 500 or 1000 mg/kg/day (Table 13) and no significant linear trend was observed. Qualitative analysis of individual animal MF showed that all animals dosed with CARDURA™E10P had MF that were comparable with the concurrent vehicle controls (Table 25).

The data confirmed that CARDURA™E10P did not induce mutation in the developing sperm cells. It should be noted that as mature sperm were not analysed, the effects of CARDURA™E10P on the late stages of sperm development were not examined and therefore no conclusions can be drawn on whether or not CARDURA™E10P is a germ cell mutagen.

Table 13: Group summary – Developing sperm cells from seminiferous tubules

Group	Treatment	Dose (mg/kg/day)	Group Mean MF (x 10 ⁻⁶ ± SD)
1	Vehicle control	0	27.83 ± 8.19
2	CARDURA™E10P	250	30.94 ± 12.26
3	CARDURA™E10P	500	30.29 ± 7.02
4	CARDURA™E10P	1000	26.13 ± 11.54
5	ENU	100	796.99 ± 165.10 *** A, SR

*** P≤0.001

A ANOVA, dose response and Dunnett's (Groups 2, 3 and 4 vs Group 1)

S two-sample t-test (Group 5 vs group 1)

R Rank transformed data

Formulations analysis

Results of the formulations analysis are presented in Formulations Analysis Report. The formulations were confirmed to be homogenous (CV<6%) and stability assessments confirmed there was no change (ie <10% difference) in achieved concentrations for samples stored at 15-25°C or 2-8°C for 24 hours or 7 days compared to samples analysed immediately after preparation.

Formulations used for week 1 and week 5 dose administrations in the Main Experiment were shown to be within $100 \pm 10\%$ of nominal and therefore acceptable for use.

No test article was detected in vehicle control samples.

CONCLUSION

It was concluded that CARDURA™E10P induced mutation in the *lacZ* transgene in the liver, kidney and bone marrow of male Muta™Mice that had been dosed daily for 42 days at up to 1000 mg/kg/day (the maximum recommended dose for this study). No evidence of mutation was observed in the developing sperm cells.

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TABLES

Body weight data

Table 14: Range-Finder individual body weights

		Test Article	CARDURA TM E10P		
		Group	1		
		Level (mg/kg/day)	1000		
Group/ Sex	Animal Number	Individual body weights (g) for Day:			
		1	8	15	
1M	1	22.7	24.7	23.8	
1M	2	23.3	24.3	25.0	
1M	3	22.3	22.6	23.3	

Table 15: Range-Finder group mean body weights

		Test Article	CARDURA TM E10P	
		Group	1	
		Level (mg/kg/day)	1000	
Day		Mean body weights (g) for Group: 1M		
1	Mean	22.8		
	SD	0.50		
8	Mean	23.9		
	SD	1.12		
15	Mean	24.0		
	SD	0.87		

Table 16: Range-Finder individual body weight gain

Test Article	CARDURA™E10P
Group	1
Level (mg/kg/day)	1000

Day		Mean body weight gain (g) for Group: 1M
1-8	Mean	1.1
	SD	0.85
8-15	Mean	0.2
	SD	0.92

Table 17: Main Experiment individual body weights: Days -1 to 29

		Test Article	Vehicle		CARDURA™E10P		ENU	
		Group	1	2	3	4	5	
		Level (mg/kg/day)	0	250	500	1000	100	
Group/ Sex	Animal Number	-1	Individual body weights (g) for Day:					29
			1	8	15	22		
1M	1	23.3	23.6	24.6	25.4	25.7	25.8	
1M	2	26.4	27.0	27.5	27.0	27.5	27.4	
1M	3	24.5	25.7	25.9	25.4	25.5	25.3	
1M	4	28.6	29.0	29.5	29.8	30.7	31.8	
1M	5	24.8	25.5	26.4	25.4	26.7	27.1	
1M	6	23.1	23.5	23.9	24.2	24.1	24.3	
1M	7	27.6	28.3	28.2	27.9	29.1	29.8	
2M	8	24.6	24.7	25.7	NT	NT	NT	
2M	9	28.6	29.6	30.7	30.4	30.1	30.3	
2M	10	32.4	32.8	34.6	34.7	34.6	34.2	
2M	11	24.8	25.4	25.9	25.9	26.4	26.0	
2M	12	23.4	24.0	25.4	24.2	24.6	25.0	
2M	13	23.9	25.0	24.9	24.2	24.5	24.5	
2M	14	22.1	23.7	24.3	NT	NT	NT	
3M	15	27.5	28.4	29.6	30.3	31.1	31.8	
3M	16	25.4	26.7	27.3	27.2	27.9	28.5	
3M	17	26.0	26.8	27.4	27.0	27.4	27.5	
3M	18	25.4	26.0	27.2	26.2	27.2	28.1	
3M	19	23.5	24.5	24.7	24.6	24.9	24.4	
3M	20	27.1	27.7	28.4	28.2	28.9	28.8	
3M	21	26.4	26.7	28.2	27.8	27.9	28.0	
4M	22	24.8	25.9	27.1	27.4	27.2	26.7	
4M	23	23.9	24.6	26.1	26.1	26.9	27.2	
4M	24	24.6	25.9	27.3	27.5	27.7	27.8	
4M	25	27.7	28.3	30.1	30.1	30.1	29.3	
4M	26	24.0	24.5	26.3	26.4	27.1	27.7	
4M	27	23.5	24.7	25.4	25.4	25.7	25.4	
4M	28	25.9	26.9	28.7	28.3	29.0	28.3	
5M	29	27.2	27.6	23.4	24.4	25.5	24.5	
5M	30	28.2	28.0	24.4	25.0	26.5	24.5	
5M	31	27.1	27.6	24.9	25.3	26.0	25.2	
5M	32	26.7	27.9	23.7	24.1	25.3	24.2	
5M	33	25.1	26.1	23.9	24.7	25.6	23.7	
5M	34	26.3	26.8	23.6	24.1	25.3	23.8	
5M	35	27.8	28.4	24.3	24.2	25.4	23.6	

NT = Not taken (animal died)

Table 17 continued: Main Experiment individual body weights: Days 36 to 45

Test Article	Vehicle	CARDURA TM E10P			ENU
Group	1	2	3	4	5
Level (mg/kg/day)	0	250	500	1000	100
Group/ Sex	Animal Number	Individual body weights (g)			for Day:
		36	43	45	
1M	1	26.2	27.6	27.3	
1M	2	28.4	29.5	29.7	
1M	3	26.1	26.9	26.9	
1M	4	32.6	34.0	33.9	
1M	5	28.5	29.8	30.1	
1M	6	24.3	23.4	24.8	
1M	7	30.5	31.7	31.5	
2M	8	-	-	-	
2M	9	29.6	30.5	30.1	
2M	10	34.2	34.3	34.0	
2M	11	26.8	27.2	27.1	
2M	12	25.1	26.8	27.5	
2M	13	25.6	26.1	26.4	
2M	14	-	-	-	
3M	15	32.4	32.9	33.0	
3M	16	28.9	29.9	29.6	
3M	17	26.6	27.6	27.7	
3M	18	28.2	30.0	30.1	
3M	19	24.5	25.7	25.5	
3M	20	28.9	29.7	29.3	
3M	21	28.5	29.0	28.5	
4M	22	26.7	27.6	27.5	
4M	23	27.9	29.2	28.7	
4M	24	27.1	28.0	27.7	
4M	25	29.8	30.7	30.7	
4M	26	27.8	28.4	28.6	
4M	27	25.8	25.9	25.5	
4M	28	28.5	29.2	29.3	
5M	29	25.4	27.0	26.8	
5M	30	25.6	27.0	27.5	
5M	31	26.0	26.7	26.9	
5M	32	25.3	27.3	27.4	
5M	33	25.4	26.0	26.5	
5M	34	24.5	25.8	26.0	
5M	35	24.8	26.0	26.4	

Table 18: Main Experiment group mean body weights

		Test Article Group Level (mg/kg/day)	Vehicle 1 0	CARDURA™E10P 2 250		3 500		4 1000		ENJ 5 100
Day		1M	Mean body weights (g) for Group:					5M		
			2M	3M	4M					
-1	Mean	25.5	25.7	25.9	24.9	26.9				
	SD	2.12	3.58	1.32	1.46	1.02				
1	Mean	26.1	26.5	26.7	25.8	27.5				
	SD	2.14	3.42	1.24	1.40	0.78				
8	Mean	26.6	27.4	27.5	27.3	24.0				
	SD	1.98	3.82	1.51	1.63	0.53				
15	Mean	26.4	27.9	27.3	27.3	24.5				
	SD	1.91	4.58	1.76	1.57	0.47				
22	Mean	27.0	28.0	27.9	27.7	25.7				
	SD	2.26	4.31	1.87	1.45	0.44				
29	Mean	27.4	28.0	28.2	27.5	24.2				
	SD	2.64	4.15	2.17	1.23	0.57				
36	Mean	28.1	28.3	28.3	27.7	25.3				
	SD	2.84	3.75	2.41	1.29	0.50				
43	Mean	29.0	29.0	29.3	28.4	26.5				
	SD	3.44	3.42	2.23	1.51	0.60				
45	Mean	29.2	29.0	29.1	28.3	26.8				
	SD	3.07	3.12	2.30	1.63	0.54				

Table 19: Main Experiment group mean body weight gain

		Test Article Group Level (mg/kg/day)	Vehicle 1 0	CARDURA™E10P 2 250		3 500		4 1000		ENJ 5 100
Day		1M	Mean body weight gain (g) for Group:					5M		
			2M	3M	4M					
-1-1	Mean	0.6	0.8	0.8	0.9	0.6				
	SD	0.30	0.50	0.32	0.31	0.45				
1-8	Mean	0.5	0.9	0.9	1.5	-3.5				
	SD	0.38	0.63	0.45	0.41	0.79				
8-15	Mean	-0.1	-0.4	-0.2	0.0	0.5				
	SD	0.62	0.54	0.51	0.22	0.35				
15-22	Mean	0.6	0.2	0.6	0.4	1.1				
	SD	0.54	0.34	0.31	0.39	0.25				
22-29	Mean	0.3	-0.0	0.3	-0.2	-1.4				
	SD	0.46	0.36	0.50	0.53	0.48				
29-36	Mean	0.7	0.3	0.1	0.2	1.1				
	SD	0.44	0.71	0.50	0.45	0.33				
36-43	Mean	0.9	0.7	1.0	0.8	1.3				
	SD	0.82	0.62	0.45	0.37	0.49				
43-45	Mean	0.2	0.0	-0.2	-0.1	0.2				
	SD	0.58	0.46	0.26	0.26	0.25				

Food and water consumption data

Table 20: Main Experiment group mean weekly food consumption

Test Article		Vehicle	CARDURA TM ELOP		ENU	
Group		1	2	3	4	5
Level (mg/kg/day)		0	250	500	1000	100
Week	Mean food consumption (g/animal/week) for Group:					
		1M	2M	3M	4M	5M
1	Mean	23.6	23.6	23.2	24.9	14.8
	SD	1.08	1.41	1.44	0.39	3.26
2	Mean	21.8	22.5	20.6	24.7	23.7
	SD	1.87	1.48	2.43	0.95	0.50
3	Mean	21.6	22.7	21.8	25.5	25.3
	SD	2.30	4.38	1.10	2.12	0.94
4	Mean	22.1	22.4	21.8	24.8	19.5
	SD	2.82	2.36	1.21	1.75	0.68
5	Mean	32.9	33.0	31.8	31.7	32.0
	SD	0.73	2.61	2.31	1.19	0.97
6	Mean	23.8	25.1	24.4	28.0	25.7
	SD	2.63	1.28	0.80	2.07	1.35

Table 21: Main Experiment group mean weekly water consumption

Test Article		Vehicle	CARDURA TM ELOP		ENU	
Group		1	2	3	4	5
Level (mg/kg/day)		0	250	500	1000	100
Week	Mean water consumption (g/animal/week) for Group:					
		1M	2M	3M	4M	5M
1	Mean	25.5	24.1	24.7	26.2	13.7
	SD	2.46	1.25	1.09	1.18	1.50
2	Mean	23.9	24.6	22.1	27.2	22.7
	SD	2.77	5.11	1.18	1.89	1.28
3	Mean	24.2	25.0	25.0	27.1	24.1
	SD	3.88	4.70	1.91	5.95	1.22
4	Mean	22.4	25.3	26.4	30.9	19.0
	SD	2.68	4.08	1.84	3.72	1.71
5	Mean	24.1	25.8	25.1	28.0	24.5
	SD	5.14	3.42	1.44	2.42	3.34
6	Mean	25.5	28.3	29.0	32.4	25.9
	SD	2.93	5.24	3.79	4.05	3.08

Mutant frequency (MF) data

Table 22: CARDURA™E10P: Liver mutant frequencies

Group / Treatment (mg/kg/day)	Animal	pfu	Mutants	Animal MF (x10 ⁻⁶)	N	Group MF (x10 ⁻⁶)	SD
1 / Vehicle (0)	1	932,466	29	31.43	3	49.85	18.91
	2	1,034,517	56	54.71	4		
	3	208,104	4	19.43	3		
	4	667,000	46	69.70	3		
	5	685,009	42	61.96	3		
	6	699,016	31	44.82	3		
	7	377,522	25	66.92	3		
2 / CARDURA™E10P (250)	8	ND	-	-	-	68.07	23.42
	9	571,619	55	97.24	3		
	10	1,206,603	93	77.89	3		
	11	833,750	60	72.73	3		
	12	1,637,485	56	34.56	3		
	13	697,682	40	57.94	3		
	14	ND	-	-	-		
3 / CARDURA™E10P (500)	15	854,427	80	94.62	3	116.33	51.26
	16	535,601	33	62.27	3		
	17	507,587	50	99.55	3		
	18	443,555	35	79.74	3		
	19	283,475	55	196.08	3		
	20	1,297,982	130	101.22	5		
	21	944,472	169	180.83	5		
4 / CARDURA™E10P (1000)	22	951,809	436	462.93	4	155.56	139.89
	23	741,037	36	49.10	3		
	24	1,064,532	172	163.29	4		
	25	1,496,748	141	95.20	5		
	26	872,436	98	113.52	3		
	27	701,684	82	118.10	3		
	28	953,810	87	92.18	3		
5 / ENU (100)	29	966,483	506	529.10	4	561.13	230.91
	30	231,449	134	585.10	3		
	31	504,252	371	743.55	4		
	32	871,769	84	97.38	3		
	33	1,698,849	897	533.60	5		
	34	1,384,025	1119	817.09	8		
	35	1,588,794	978	622.09	5		

pfu Plaque forming units

MF Mutant frequency

SD Standard deviation

N Number of packaging occasions (from which animal MF is derived)

ND No data obtained

Table 23: CARDURA™E10P: Kidney mutant frequencies

Group / Treatment (mg/kg/day)	Animal	pfu	Mutants	Animal MF (x10 ⁻⁶)	N	Group MF (x10 ⁻⁶)	SD
1 / Vehicle (0)	1	488,911	17	35.14	3	52.66	22.19
	2	839,086	51	61.43	3		
	3	659,663	32	49.02	3		
	4	436,218	42	97.30	3		
	5	436,885	13	30.07	3		
	6	890,445	44	49.94	3		
	7	905,786	41	45.74	3		
2 / CARDURA™E10P (250)	8	ND	-	-	-	104.81	26.01
	9	621,644	77	125.18	3		
	10	990,495	79	80.60	3		
	11	1,123,228	89	80.08	3		
	12	1,406,703	140	100.58	3		
	13	690,345	94	137.61	3		
	14	ND	-	-	-		
3 / CARDURA™E10P (500)	15	721,027	95	133.15	3	123.69	17.45
	16	1,057,862	109	104.13	3		
	17	907,120	117	130.35	3		
	18	807,070	75	93.91	3		
	19	908,454	118	131.27	3		
	20	530,932	74	140.86	3		
	21	443,555	58	132.15	3		
4 / CARDURA™E10P (1000)	22	1,331,332	117	88.81	3	114.00	25.57
	23	747,040	109	147.46	4		
	24	701,017	79	113.89	3		
	25	563,615	54	96.83	3		
	26	1,260,630	112	89.79	4		
	27	725,696	80	111.41	4		
	28	350,842	52	149.79	3		
5 / ENU (100)	29	955,144	507	536.44	3	739.23	139.98
	30	850,425	603	716.58	3		
	31	1,416,041	884	630.90	4		
	32	336,835	308	924.09	3		
	33	635,651	419	666.16	3		
	34	1,085,209	918	854.89	4		
	35	534,267	447	845.53	4		

pfu Plaque forming units
MF Mutant frequency
SD Standard deviation
N Number of packaging occasions (from which animal MF is derived)
ND No data obtained

Table 24: CARDURA™E10P: Bone marrow mutant frequencies

Group / Treatment (mg/kg/day)	Animal	pfu	Mutants	Animal MF (x10 ⁻⁶)	N	Group MF (x10 ⁻⁶)	SD
1 / Vehicle (0)	1	212,773	9	42.75	6	41.21	9.44
	2	268,801	10	37.60	3		
	3	210,105	11	52.91	3		
	4	237,452	12	51.07	5		
	5	561,614	15	26.99	3		
	6	772,386	25	32.71	3		
	7	591,629	26	44.41	3		
2 / CARDURA™E10P (250)	8	ND	-	-	-	43.86	10.98
	9	263,465	8	30.69	4		
	10	262,798	14	53.84	4		
	11	274,804	13	47.81	6		
	12	210,105	7	33.67	5		
	13	227,447	12	53.32	5		
	14	ND	-	-	-		
3 / CARDURA™E10P (500)	15	238,786	14	59.25	3	76.41	14.89
	16	230,115	16	70.27	5		
	17	226,780	21	93.58	5		
	18	FTP	-	-	-		
	19	208,104	17	82.56	4		
	20	FTP	-	-	-		
	21	FTP	-	-	-		
4 / CARDURA™E10P (1000)	22	450,892	47	105.34	3	118.62	19.80
	23	363,515	42	116.76	3		
	24	266,133	34	129.11	3		
	25	325,496	48	149.03	3		
	26	356,178	46	130.52	4		
	27	289,478	32	111.72	3		
	28	230,115	20	87.83	3		
5 / ENU (100)	29	259,463	242	942.59	4	510.18	346.39
	30	276,805	264	963.86	4		
	31	FTP	-	-	-		
	32	256,128	95	374.84	5		
	33	473,570	115	245.41	6		
	34	384,859	95	249.46	4		
	35	223,445	63	284.94	3		

pfu Plaque forming units
MF Mutant frequency
SD Standard deviation
N Number of packaging occasions (from which animal MF is derived)
ND No data obtained
FTP DNA failed to package

Table 25: CARDURA™E10P: Developing sperm cells mutant frequencies

Group / Treatment (mg/kg/day)	Animal	pfu	Mutants	Animal MF (x10 ⁻⁶)	N	Group MF (x10 ⁻⁶)	SD
1 / Vehicle (0)	1	562,281	23	41.34	4	27.83	8.19
	2	628,314	12	19.30	4		
	3	725,696	23	32.03	4		
	4	350,842	9	25.92	4		
	5	300,817	6	20.16	4		
	6	FTP	-	-	-		
	7	536,935	15	28.23	3		
2 / CARDURA™E10P (250)	8	ND	-	-	-	30.94	12.26
	9	553,610	10	18.25	5		
	10	785,059	20	25.75	4		
	11	287,477	12	42.19	4		
	12	933,133	21	22.74	4		
	13	574,287	26	45.75	4		
	14	ND	-	-	-		
3 / CARDURA™E10P (500)	15	609,638	25	41.44	4	30.29	7.02
	16	552,943	10	18.28	4		
	17	332,166	11	33.47	4		
	18	398,866	12	30.40	4		
	19	539,603	17	31.84	4		
	20	739,036	22	30.08	4		
	21	572,286	15	26.49	4		
4 / CARDURA™E10P (1000)	22	542,271	14	26.09	4	26.13	11.54
	23	555,611	12	21.83	3		
	24	499,583	24	48.55	4		
	25	FE	-	-	-		
	26	534,267	13	24.59	4		
	27	751,042	12	16.15	4		
	28	619,643	12	19.57	4		
5 / ENU (100)	29	631,649	450	719.98	4	796.99	165.10
	30	606,303	648	1080.11	4		
	31	474,237	326	694.71	3		
	32	526,930	465	891.83	3		
	33	1,376,021	1051	771.90	6		
	34	1,408,704	869	623.42	6		
	35	FE	-	-	-		

pfu Plaque forming units
MF Mutant frequency
SD Standard deviation
N Number of packaging occasions (from which animal MF is derived)
ND No data obtained
FTP DNA failed to package
FE Failed DNA extraction

APPENDICES

Appendix 1

Historical vehicle control data ranges for: Muta™Mouse mutant frequency

Table 26: Muta™ Mouse historical control, male liver data

	Mutant frequency
Number of Studies	8
N	90
Mean	63.17
SD	36.47
Median	55.86
Observed Range	7.71 to 231.49
95% Reference Range	15.25 to 165.40

Calculated in April 2008 by CLEH Statistics from studies started between March 1998 and August 2003.

Table 27: Muta™ Mouse historical control, male kidney data

	Mutant frequency
Number of Studies	1
N	5
Mean	142.09
SD	220.01
Median	50.73
Observed Range	24.26 to 535.12
95% Reference Range	24.26 to 535.12

Calculated in April 2008 by CLEH Statistics from studies started in June 2000.

Table 28: Muta™ Mouse historical control, male bone marrow data

	Mutant frequency
Number of Studies	6
N	70
Mean	41.83
SD	27.23
Median	38.33
Observed Range	10.43 to 185.94
95% Reference Range	10.56 to 114.42

Calculated in April 2008 by CLEH Statistics from studies started between January 1996 and March 2002.

Appendix 2 Calculation of mutant frequency and titre

Total plaques

The total number of plaques is estimated by scoring the titre plates manually. Each plaque is marked on the base and recorded on a tally counter if necessary. The plates may be checked by a second person to ensure no plaques were unmarked.

Titre

Titration involved diluting a 10 µL aliquot to 200 µL and plating 10 µL of this onto each of three petri dishes. The total number of plaques (T) is the sum total of all three plate counts. This total number of plaques (T) were present in 1.5 µL of the original reaction and 1.5 µL represents 0.15% of 1000 µL (total reaction volume).

Total titre, or number of pfu in original reaction

$$\frac{100}{0.15} \times T = 667 \times T$$

Mutant frequency

The total volume available for selection plating is 10 µL less than the original reaction mix, which is 990 µL. The total number of pfu available must be corrected:

$$667 \times T \times \frac{990}{1000} = 660 \times T$$

Finally MF is calculated by dividing the number of mutants on the three selection plates (M) by the total number of pfu available for selection.

$$MF = \frac{M}{660 \times T}$$

The final figure is usually multiplied by 10^6 and presented as mutants per 10^6 pfu.

**Appendix 3
Certificate of analysis**



Momentive Specialty Chemicals B.V.
Seattleweg 17
3195 ND PERNIS - ROTTERDAM
THE NETHERLANDS

CERTIFICATE OF ANALYSIS

Central Dispensary Covance Laborato
Mark Wrightson
Otley Road Harrogate 1
NORTH YORKSHIRE
HG3 1PY
GREAT BRITAIN

Date	08.03.2012
Purchase order item	Online Nr. 8966
Purchase order date	08.03.2012
Delivery item	83490333 000010
Delivery date	26.03.2012
Order item	2979033 000010
Order date	08.03.2012
Customer number	100100
Shipping date	08.03.2012

Certified that the whole of the supplies detailed hereon have been inspected and tested and, unless otherwise stated, conform in all respects with the contract/purchase order requirements.

Material:
906766 CARDE10P CARDURA(TM)Glycidyl Ester E 10 P DRUM 200 KG
Batch PH1H0558 / Quantity 2 KG
Manufactured date 30.08.2011

Characteristic	Unit	Value	Lower Limit	Upper Limit	Test Method
Color, Pt-Co		29		35	ASTM D1209
Water	% (m)	0.0		0.1	ASTM D4672
Epoxy Group Content	mmol/kg	4129	4100	4250	SMS 2026
Appearance CFFSM	-	Passes			VISUAL

This document is computer generated and therefore requires no signature.
*** End ***

If you have any questions regarding this certification or delivery please call your local account representative or order center. This is to confirm the accuracy of our information provided by the quality assurance department which is independent from production.

Appendix 4
Minor deviations from protocol

Protocol section	Subject	Deviation
Test System	Age of animals	Due to limited animal supply, some animals used in both the Range-Finder Experiment and Main Experiment were outside of protocol specification. Range-finder animals were 6-9 weeks old rather than 8-10 weeks old. Although some animals were slightly below the specified age, their purposes was solely for dose determination and therefore this deviation had no effect on study integrity or data interpretation. The age range of Main Experiment animals was 8-13 weeks instead of 8-10 weeks old. The 13 week old animals were confirmed to the positive control group, whilst the remaining 11-12 week old animals were randomly assigned throughout the vehicle and treatment groups. The animals used were considered to be young adults and therefore acceptable for mutant frequency assessment. Whilst mutant frequency is affected by age, this has generally only been seen in animals that exceed 4-6 months old (Lambert <i>et al.</i> , 2005). This deviation is therefore considered to have no adverse effect on study integrity or data evaluation.
Methods	Body weights	In error the final body weight reading in the Range-Finder Experiment was taken on day 8 and not Day 7. As this is used solely for dose setting purposes and is considered to be equivalent to weekly readings (as per the Main Experiment) this deviation has no adverse effect on study integrity.

CONTRIBUTORY REPORT

Formulations Analysis Report

The following report consists of 8 pages.

(included in the total pagination indicated on the title page of the main report)

Final Formulations Analysis Contributory Report

Study Title:	Induction of <i>lacZ</i> - mutations in the liver, kidney, bone marrow and spermatazoa of treated Muta™ Mice
Test article	CARDURA™E10P
Covance Study Number:	8259749
Author	P Cox
Version	Final
Report Issued:	February 2013
Page Number:	1 of 8

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CONTRIBUTING SCIENTIST STATEMENT

I, the undersigned, hereby declare that the work described in this analysis summary was performed by me or under my supervision and that the findings provide a true and accurate record of the results obtained.

P. J. Cox

P Cox BSc MRSC CChem
Contributing Scientist - Formulations Analysis
Covance Laboratories Ltd

6 Feb 2013

Date

1 SUMMARY

Test article CARDURA™E10P, was formulated in corn oil by Central Dispensary for treatment during the study.

Formulations received 9 May 2012, were analysed to determine achieved concentration, homogeneity and 1 and 7 day stability at both room temperature and refrigerated conditions. Formulations were to be considered homogeneous if the coefficient of variation (CV) of the results is $\leq 6.0\%$. In addition, all the homogeneity results should be within $\pm 10\%$ of the mean. The target range for preparation of liquid formulations is 90 to 110% of nominal (mean result). Results were within these criteria. No significant degradation ($>10\%$) was observed over the storage period at either temperature.

Formulations received 6 June and 11 July 2012 were analysed to determine achieved concentration. The target range for preparation of liquid formulations is 90 to 110% of nominal. Results were within these criteria.

Test article was not detected in the control samples.

The analytical procedure was validated in this study.

2 PROCEDURES

2.1 Linearity of response

A set of standard solutions over the approximate concentration range 33.3 to 200 µg/mL was submitted to HPLC. A calibration graph was constructed for the standard solutions. The lowest calibration standard was diluted by a factor of 4. This solution was used to determine a limit of detection (LOD).

2.2 Precision and accuracy

The precision and accuracy of the analytical procedure was determined as follows:

- Sets of six standards at concentrations 33.33 and 200.0 µg/mL were prepared from a stock solution.
- 2 validation formulations at 5 and 100 mg/mL were prepared. Sets of six samples were extracted and diluted to 4.985 µg/mL.
- A blank extraction was also performed.
- A six point, calibration line was constructed from a different stock solution from the one above.
- All solutions were submitted to HPLC.
- The concentration of test chemical sets was calculated from their HPLC responses using the calibration line.
- The mean and standard deviations of these observations were used to determine the relative precision (coefficient of variation) and accuracy of the assay.

$$\text{precision \%} = \frac{\text{standard deviation}}{\text{mean}} \times 100$$

$$\text{accuracy \%} = \frac{\text{mean calculated test article concentration}}{\text{theoretical test article concentration}} \times 100$$

2.3 Achieved Concentration, Homogeneity and Stability

Samples were removed in triplicate from the top and bottom of each formulation. These were analysed for test article concentration to determine homogeneity and achieved concentration. Following sampling the formulations were split into two aliquots, one stored at room temperature (15-30°C) and one stored refrigerated (2-8°C). Following storage these aliquots were analysed as above to determine 1 and 7 day stability.

2.4 Achieved Concentration

Duplicate samples for analysis were analysed from each formulation, except for the control samples where a single analysis was performed. These were analysed for test article concentration to determine achieved concentration.

2.5 Analytical procedure

The analytical procedure Covance 8259749-01F, issued 20 March 2012, was used to determine achieved concentration.

3 DATA EVALUATION AND MAJOR COMPUTER SYSTEMS

Activity	Computer System
Scheduling	CMS (Covance Management System)
Data collection and processing	Empower2 data capture system, Build number 2154*
Report production	Microsoft word and Adobe Acrobat

* Statistical data presented in this report are generated using this software.

4 RESULTS

4.1 Linearity of response

A set of standard solutions was submitted to HPLC. The concentration/response curve was straight with an intercept approaching zero. The correlation coefficient (r) was 0.9972. A minimum value of 0.9950 is considered acceptable. Linearity of response was therefore considered to be acceptable. An injection at concentration 8.326 µg/mL had a signal to noise ratio >26 and was considered acceptable as the LOD.

4.2 Precision and Accuracy

	Expected concentration (µg/mL)			
	33.33	4.985*	4.985 [#]	200.0
Precision %	2.11	1.84	3.80	2.57
Accuracy %	99	100	96	100

* extracted and diluted from 5 mg/mL formulation

[#] extracted and diluted from 100 mg/mL formulation

Precision is normally expected to be less than 5% for this type of test and accuracy is regarded as acceptable if it is between 95% and 105% (85% to 115% for extractions). The results were within these limits. The method was therefore accepted in terms of precision and accuracy. There was no significant detector response from control extraction thereby confirming selectivity of the method.

4.3 Achieved Concentration, Homogeneity and Stability

Conc. (mg/mL)	Storage (°C)	Time (Days)	Result as % Nominal						Mean (%)	CV (%)
			Top					Bottom		
25	15-30	0	103	97	98	100	97	94	98	3.08
25	15-30	1	103	105	104	104	103	103	104	0.81
25	15-30	7	98	101	103	102	101	103	101	1.78
25	2-8	1	103	103	101	102	101	101	102	0.88
25	2-8	7	105	103	102	103	103	103	103	0.89
100	15-30	0	96	92	90	91	89	87	91	3.34
100	15-30	1	104	103	100	99	99	98	101	2.53
100	15-30	7	105	107	104	104	104	100	104	2.07
100	2-8	1	100	99	99	98	98	97	98	0.79
100	2-8	7	99	98	97	101	100	100	99	1.42

Formulations were to be considered homogeneous if the coefficient of variation (CV) of the results is ≤ 6.0%. In addition all the homogeneity results should be within ± 10% of the mean. The target range for preparation of liquid formulations is 90 to

110% of nominal (mean result). Results were within these criteria. No significant degradation (>10%) was observed over the storage period at either temperature.

4.4 Achieved Concentration

4.4.1 6 June 2012:

Conc (mg/mL)	Results as % nominal concentration	
25	99	102
50	97	91
100	94	93

4.4.2 11 July 2012:

Conc (mg/mL)	Results as % nominal concentration	
25	106	105
50	91	91
100	108	104

The target range for preparation of liquid formulations is 90 to 110% of nominal. Results were within these criteria.

Test article was not found in the control samples.

ORIGIN ID 000A (614) 225-4462
MAILROOM MANAGER
HEXION SPECIALTY CHEMICALS
180 EAST BROAD ST
28TH FLOOR
COLUMBUS, OH 43215
UNITED STATES US

SHIP DATE 22FEB13
ACTWGT 0.8 LB
CAD 104799454/WSXI2600

BILL SENDER

Part # 156148-434 R17805/7840/19815

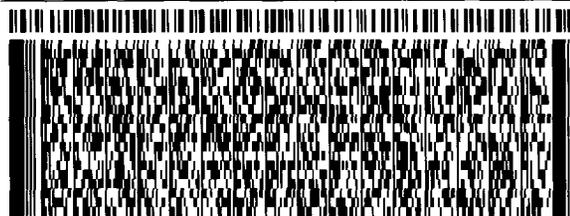
TO SECTION 8(E)
USEPA ROOM 6428
1200 PENNSYLVANIA AVE NW
ARIEL RIOS BUILDING
WASHINGTON DC 20460

(614) 225-3367

REF:

INV
PO:

DEPT



FedEx
Express



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TRK# 7948 1780 5910
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TUE - 26 FEB A1
** 2DAY **

SA RDVA

20460
DC-US DCA

