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September 2010

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

INK BH11 C

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (the Act) and Regulations. This legislation is an Act of the Commonwealth of Australia. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is administered by the Department of Health and Ageing, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of the Environment, Water, Heritage and the Arts.

For the purposes of subsection 78(1) of the Act, this Full Public Report may be inspected at our NICNAS office by appointment only at Level 7, 260 Elizabeth Street, Surry Hills NSW 2010.

This Full Public Report is also available for viewing and downloading from the NICNAS website or available on request, free of charge, by contacting NICNAS. For requests and enquiries please contact the NICNAS Administration Coordinator at:

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Director
NICNAS

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Brother International (Aust) Pty Ltd (17 001 393 835)
Suite 1, Level 3, Building A
11 Talavera Road
MACQUARIE PARK NSW 2113

NOTIFICATION CATEGORY
Limited-small volume: Chemical other than polymer (1 tonne or less per year).

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: chemical name, other names, CAS number, molecular and structural formulae, molecular weight, use details, import volume and identity of recipients.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
No variation to the schedule of data requirements is claimed.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
Japan (2009)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
INK BH11 C

MOLECULAR WEIGHT
> 500 Da

ANALYTICAL DATA
Reference NMR, IR, HPLC, UV spectra and Karl Fischer titration data were provided.

3. COMPOSITION

DEGREE OF PURITY > 95%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Blue powder

Property	Value	Data Source/Justification
Melting Point	Decomposed from ~330°C	Measured
Boiling Point	Not determined	Decomposes on melting.
Density	1750 kg/m^3 at 21°C	Measured
Vapour Pressure	< 1.3 x 10^{-7} kPa at 25°C	Measured
Water Solubility	201 g/L to 220 g/L at 20 ± 0.5°C	Measured
Hydrolysis as a Function of pH	$t_{½}$ >1 year at 25°C, pH 4–9	Measured
Partition Coefficient (n-octanol/water)	log Pow < -2.83 at 22.7 ± 1.0°C	Measured
Surface Tension	72.0 mN/m at 21.6 ± 0.5°C	Measured
Adsorption/Desorption	log K_{oc} < 1.25 at 40°C	Measured
Dissociation Constant	Not determined	The notified chemical is a salt and is

		expected to be ionised under environmental conditions
Particle Size	Inhalable fraction (<100 μm): 25.2% Respirable fraction (<10 μm): 0.48% Fraction (< 5 μm): 0.15%	Measured
Flash Point	Not determined	Not applicable as low volatility solid.
Flammability	Not highly flammable	Measured
Autoignition Temperature	> 400°C	Measured
Explosive Properties	Not expected to be explosive	The structural formula contains no explosophores.

DISCUSSION OF PROPERTIES
For full details of tests on physical and chemical properties, refer to Appendix A.

Reactivity
The notified chemical is predicted to be stable under normal conditions of use.

Dangerous Goods classification
Based on the submitted physical-chemical data in the above table the notified chemical is not classified according to the Australian Dangerous Goods Code (NTC, 2007). However the data above do not address all Dangerous Goods endpoints. Therefore consideration of all endpoints should be undertaken before a final decision on the Dangerous Goods classification is made by the introducer of the chemical.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported only as a component of ink, which has already been incorporated into cartridges (< 5% concentration).

MAXIMUM INTRODUCTION VOLUME OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	< 1	< 1	< 1	< 1	< 1

PORT OF ENTRY
Sydney

TRANSPORTATION AND PACKAGING
The notified chemical will be imported as a component of ink-jet printer ink contained in 12 mL plastic cartridges. The cartridges will be packed in plastic bags which in turn will be packaged in cardboard boxes. Boxes of these cartridges will be transported by road to storage, retail and end-use sites.

USE
The notified chemical will be used as a dye component of imported inkjet printer inks (< 5%).

The inks will be used by office workers and the public for routine but varied colour printing operations in home and small office scenarios. Sealed ink cartridges containing the notified chemical will be used as necessary to replace spent cartridges in inkjet printers.

OPERATION DESCRIPTION
No reformulation or repackaging of the notified chemical will occur in Australia. The products containing the notified chemical will be delivered to the end-user in the same form in which they are imported. The cartridges will be installed or replaced into the inkjet printer by office workers, service technicians or consumers.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

Exposure to the notified chemical during the importation, transport and storage of the printer cartridges is not expected, except in the unlikely event of an accident where the cartridge and its packaging may be breached.

Both office workers and service technicians may be exposed to the notified chemical in inks (< 5% concentration) while changing printer cartridges, and service technicians may additionally be exposed during printer maintenance. Dermal exposure to small quantities of the notified chemical may occur if the print heads are touched while replacing the cartridges. In addition, dermal and possibly ocular exposure could occur when handling faulty or ruptured cartridges. Exposure during handling and cleaning or printer components is likely to be limited to the fingertips. Therefore, the exposure of these workers is expected to be minimal and infrequent.

Dermal exposure of workers may also occur when handling printed media before the ink is adequately dried, especially when printing on non-absorbent materials. Dermal exposure of office workers to the notified chemical from dried inks on printed paper is expected to be minimal, as the dye will be largely bound to the paper within the matrix of the dried ink.

The extent of dermal exposure of workers to wet ink on paper or other non-absorbent substrate has been estimated by the notifier. One kilogram of pure dye would be expected to produce several million sheets of A4 coloured text or graphics. Under worst-case conditions, each piece of A4 paper could be assumed to incorporate a maximum of 1 mg of notified chemical. Based on a 50% transfer on contact when handling printed paper or other substrate (assuming partially dry ink), and the relative contact area of fingertips and paper size:
Area of contact with finger ends (four fingers on one hand) = 8 cm^2
A4 sized paper = ~600 cm^2
% Removal = (8/600) × 0.5 × 100 = < 1%
∴ Exposure to fingertips per event = < 1% of 1 mg = < 0.01 mg per event.

For extensive contact with wet ink on paper or other substrate (i.e. > 10 events per day) the daily systemic exposure (assuming no washing between events) for a 70 kg person using 100% dermal absorption, would be:
Daily exposure = (< 0.01 (mg/event)×10) ÷ 70 = ~0.0014 mg/kg bw/day.

6.1.2. Public exposure

The exposure of the public to the notified chemical in inkjet printer inks is expected to be identical, or of a lesser extent, than that experienced by office workers using the same ink.

6.2. Human health effects assessment

The results from toxicological investigations conducted on the notified chemical are summarised in the table below. Details of these studies can be found in Appendix B.

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2000 mg/kg bw; low toxicity
Rabbit, skin irritation	non-irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – Local lymph node assay	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOAEL 1000 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro chromosomal aberration	non genotoxic

Toxicokinetics

There are no toxicokinetic data on the notified chemical. The notified chemical has a molecular weight > 500 Da and a water solubility of 201 g/L to 220 g/L at 20ºC and partition coefficient (log Pow) of < -2.83 at 20ºC. The moderately high molecular weight and hydrophilicity of the notified chemical suggest that dermal absorption is unlikely, however there may be potential for absorption across the GI tract. This is supported by the

observation of blue coloured urine in animals in the repeated dose 28-day oral toxicity study in rats.

Acute toxicity
The notified chemical is of low acute dermal and oral toxicity (LD50 >2000 mg/kg bw) based on studies conducted in rats.

No acute inhalation toxicity study was conducted using the notified chemical.

Irritation and Sensitisation
Based on studies conducted in rabbits the notified chemical is not irritating to skin but slightly irritating to eyes. Conjunctival irritation was noted in all treated eyes one and 24 hours after treatment with minimal conjunctival irritation noted at the 48-hour observation. Given blue coloured staining of the lower half of the cornea and on the nictitating membrane was noted in two treated eyes at the 24-hour observation and in all treated eyes at the 48 and 72-hour observations, additional observations were made on Days 7, 14 and 21 to assess the reversibility of the ocular effects. Although the effects had not cleared in all treated animals at the 14-day observation, all treated eyes were normal at the 21-day observation.

In a LLNA study, the notified chemical showed no evidence of a skin sensitisation potential.

Repeated Dose Toxicity
Oral administration of the test material to rats for a period of 28 consecutive days at dose levels of 25, 150 and 1000 mg/kg/day resulted in no adverse treatment related effects at any dose level despite evidence (i.e. blue coloured urine) that absorption of the notified chemical had occurred. A NOAEL of 1000 mg/kg bw/day was established based on non-adverse histology findings observed at this dose level.

Mutagenicity
The notified chemical was not mutagenic in a bacterial reverse mutation assay and not clastogenic to Chinese hamster lung cells *in vitro*.

Summary
The notified chemical is of low acute oral and dermal toxicity. It is not irritating to skin but may be slightly irritating to eyes. It is not a skin sensitiser and not mutagenic. Given the notified chemical can cross the GI tract, there may be some potential for systemic toxicity via the oral route.

Health hazard classification
Based on the provided data the notified chemical is not classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
Dermal and ocular exposure of workers to the notified chemical should occur infrequently and be of small amounts, given the containment of the notified chemical within ink cartridges. The notified chemical, present in inks at concentrations < 5%, is not likely to be toxic at the highest levels of probable exposure (worst-case systemic exposure estimate of ~0.0014 mg/kg bw/day, compared with NOAEL of 1000 mg/kg bw/day).

Given that exposure of workers to the notified chemical is expected to be low, the OHS risk is not considered to be unacceptable.

6.3.2. Public health
The exposure and hazard of the notified chemical to the members of the public during the use of inkjet printers are expected to be identical or similar to that experienced by office workers. Therefore, the risk of the notified chemical to the health of the public is assessed to be low. The unlikely but potential public exposure through accidents during importation, transportation or storage is considered as negligible.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical is an ink dyestuff and will be imported into Australia as a component of ink in ready-to-use sealed printing cartridges for home or office inkjet printers. No manufacturing, reformulation or repackaging of the notified chemical will take place in Australia. Environmental release of the notified chemical is unlikely to occur during importation, storage and transportation as containers are designed to minimise release. In the event of an accidental spill the ink containing the notified chemical will be absorbed with inert material and disposed of to landfill.

RELEASE OF CHEMICAL FROM USE

The notified chemical will be contained in ink cartridges and it is expected that < 1% of the annual import volume of the notified chemical may be spilt. If leakage or spillage does occur, the ink will be physically contained with absorbent material and disposed of to landfill. The ink cartridges will be contained within the printer until the contents are consumed. The empty cartridges, estimated to contain < 1% of the annual import volume of notified chemical, will be removed and disposed of to landfill or sent for recycling.

RELEASE OF CHEMICAL FROM DISPOSAL

Most of the notified chemical will be bound to printed paper and, once the ink has dried, will be contained in an inert matrix. It is assumed that 50% of the waste paper will end up in landfill and the rest will undergo paper recycling processes. During recycling processes, waste paper is repulped using a variety of chemical agents which, amongst other things, enhance detachment of ink from the fibres. The notified chemical may partition to the supernatant water, due to its high water solubility, which is released to the sewer. Notified chemical in the sludge generated during the recycling process will be sent to landfill for disposal.

7.1.2 Environmental fate

For the details of the environmental fate studies please refer to Appendix C.

The majority of the notified chemical will be bound to paper, of which half is assumed to be recycled. During the paper recycling process, waste paper is repulped using a variety of alkaline, dispersing and wetting agents, water emulsifiable organic solvents and bleaches. Trade sources estimate the washing process will recover 30-60% of the total amount of ink and, therefore, at least 30% of the notified chemical in the recycled paper will be disposed of with sludge to landfill. However, due to the high water solubility of the notified chemical, a greater proportion can be expected to remain in the aqueous phase released to the sewer. The notified chemical is not readily biodegradable, however, due to its low log Pow and its high water solubility, its potential for bioaccumulation is expected to be low in exposed aquatic organisms.

In landfill, notified chemical in sludge may leach, due to its high water solubility, although potential cationic functional groups on the notified chemical may result in sorption to negatively charged sites on sediments and soils. The notified chemical is likely to remain in the ink matrix bound to paper that is disposed of to landfill. The notified chemical is expected to slowly degrade through biotic and abiotic processes to form water, oxides of carbon, nitrogen and sulphur, and inorganic salts.

7.1.3 Predicted Environmental Concentration (PEC)

A predicted environmental concentration (PEC) worst case scenario has been calculated on the assumptions that 50% of the annual import of the notified chemical is released to the sewer as de-inking aqueous wastes from paper recycling over 260 days/year, with no removal of the notified chemical by sewage treatment plant (STP) processes.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	<1,000	kg/year
Proportion expected to be released to sewer	50 %	
Annual quantity of chemical released to sewer	<500	kg/year
Days per year where release occurs	260	days/year
Daily chemical release:	1.92	kg/day
Water use	200.0	L/person/day
Population of Australia (Millions)	21.161	million
Removal within STP	0%	
Daily effluent production:	4,232	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	<0.45	µg/L
PEC - Ocean:	<0.05	µg/L

STP effluent re-use for irrigation occurs throughout Australia. The agricultural irrigation application rate is assumed to be 1000 L/m^2/year (10 ML/ha/year). The notified chemical in this volume is assumed to infiltrate and accumulate in the top 10 cm of soil (density 1500 kg/m^3). Using these assumptions, irrigation with a concentration of 0.454 µg/L may potentially result in a soil concentration of approximately 3.029 µg/kg. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated irrigation, the concentration of notified chemical in the applied soil in 5 and 10 years may be approximately 15.15 µg /kg and 30.29 µg /kg, respectively.

7.2. Environmental effects assessment

The results from ecotoxicological investigations conducted on the notified chemical are summarised in the table below. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	LC50 (96 h) >1000 mg/L	Not harmful to fish
Daphnia Toxicity	EC50 (48 h) >100 mg/L	Not harmful to aquatic invertebrates
Algal Toxicity	E_rC50 (72 h) >100 mg/L	Not harmful to algae
Inhibition of Bacterial Respiration	IC50 (3 h) >1000 mg/L	Not harmful to microbial respiration

Under the Globally Harmonised System of Classification and Labelling of Chemicals (United Nations, 2009) the notified chemical is not harmful to fish, aquatic invertebrates, algae or microbial respiration.

7.2.1 Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) was calculated with the minimum toxicity for daphnia and algae (>100 mg/L), and an assessment factor of 100, as the endpoints for three trophic levels are available.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (algae and daphnia).	>100	mg/L
Assessment Factor	100	
PNEC:	>1,000	µg/L

7.3. Environmental risk assessment

Based on the above PEC and PNEC values, the following Risk Quotient (Q) has been calculated:

Risk Assessment	*PEC µg/L*	*PNEC µg/L*	*Q*
Q - River:	0.45	>1000	<0.001
Q - Ocean:	0.05	>1000	<0.0001

The concentration of the notified chemical in surface waters is expected to be very low based on the reported use pattern and the maximum import volume. It is not expected to bioaccumulate, based on its high water solubility and low partition coefficient. As the risk quotients are well below 1, the notified chemical is not expected to pose a risk to the aquatic environment.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the provided data the notified chemical is not classified as hazardous according to the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)].

Human health risk assessment
Under the conditions of the occupational settings described, the notified chemical is not considered to pose an unacceptable risk to the health of workers.

When used in the proposed manner, the notified chemical is not considered to pose an unacceptable risk to public health.

Environmental risk assessment
On the basis of the PEC/PNEC ratio and the reported use pattern, the notified chemical is not expected to pose a risk to the environment.

Recommendations

CONTROL MEASURES
Occupational Health and Safety

- No specific engineering controls, work practices or personal protective equipment are required for the safe use of the notified chemical itself, however, these should be selected on the basis of all ingredients in the formulation.

  Guidance in selection of personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

- A copy of the MSDS should be easily accessible to employees.

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)] workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by physical containment, collection and subsequent safe disposal.

Regulatory Obligations

Secondary Notification
This risk assessment is based on the information available at the time of notification. The Director may call for the reassessment of the chemical under secondary notification provisions based on changes in certain circumstances. Under Section 64 of the *Industrial Chemicals (Notification and Assessment) Act (1989)* the notifier, as well as any other importer or manufacturer of the notified chemical, have post-assessment regulatory obligations to notify NICNAS when any of these circumstances change. These obligations apply even when the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS).

Therefore, the Director of NICNAS must be notified in writing within 28 days by the notifier, other importer or manufacturer:

(1) Under Section 64(1) of the Act; if
- the importation volume exceeds one tonne per annum notified chemical;

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a component (< 5%) in inkjet printer inks in sealed cartridges, or is likely to change significantly;
- the amount of chemical being introduced has increased from one tonne per annum, or is likely to increase, significantly;
- the chemical has begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

The Director will then decide whether a reassessment (i.e. a secondary notification and assessment) is required.

Material Safety Data Sheet
The MSDS of the notified chemical and products containing the notified chemical provided by the notifier were reviewed by NICNAS. The accuracy of the information on the MSDS remains the responsibility of the applicant.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point Decomposed from 330ºC

Method EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks Substance decomposed without melting from approximately 330 °C at 101.8 kPa.
Test Facility Harlan Laboratories Ltd. (2009a)

Density 1750 kg/m^3 at 21.2 ± 0.5 ºC

Method EC Directive 92/69/EEC A.3 Relative Density.
Remarks
Test Facility Harlan Laboratories Ltd. (2009a)

Vapour Pressure < 1.3 x 10^{-7} kPa at 25ºC

Method EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks Vapour pressure was determined using a vapour pressure balance.
Test Facility Harlan Laboratories Ltd. (2009b)

Water Solubility 201 g/L to 220 g/L at 20.0 ± 0.5ºC

Method Modification of EC Directive 92/69/EEC A.6 Water Solubility.
Flask method. After a preliminary test, a series of solutions were prepared in double distilled water (16.1% to 26.0%) and shaken at approximately 30°C for 72 hours. After standing for a period of 26 hours the contents of the flasks were visually inspected.
Remarks The standard A6 method was not applicable to this test material due to the highly viscous samples produced at high concentration levels. It was therefore not possible to prepare samples at five times the saturation level as recommended in the guideline. Thus, the water solubility was estimated based on visual inspection.
The sample solutions were dark blue. A minor amount of undissolved test material was observed in the concentration range 16.1% to 20.1%. The undissolved test material was considered to be due to impurities as increasing the test substance concentration did not significantly affect the appearance of the samples. Test solutions at concentrations ≥22.0% contained excess material and were more viscous than the samples at lower concentrations.
The pH of each test solution was measured and was found to range from pH 7.2 to 7.3.
Test Facility Harlan Laboratories Ltd. (2009a)

Hydrolysis as a Function of pH $t_{½}$ >1 year at 25°C, pH 4–9

Method EC Directive 92/69/EEC C.7 Degradation: Abiotic Degradation: Hydrolysis as a Function of pH. Test concentrations (0.5 g/L) at pH 4, 7, and 9 were maintained at 50°C. After 5 days, the concentrations were determined by HPLC.

pH	*T (°C)*	*$t_{½}$*
4	25	>1 year
7	25	>1 year
9	25	>1 year

Remarks The C7 test is not applicable to certain mixtures. However, in this case, as the chromatographic profiles for the samples were extremely similar to those for the respective standards, the results were considered valid.
The solutions were shielded from light whilst maintained at the test temperature. At 24 hours the sample at pH 9 was measured to contain ~86% of the mean initial concentration, however this result was reported to be an outlier as it was out of agreement with other time point samples.
Less than 10% hydrolysis was observed after 5 days at 50°C at pH 4, 7, and 9. Therefore, the test material is considered stable with a half life greater than 1 year at 25°C.
Test Facility Harlan Laboratories Ltd. (2009a)

Partition Coefficient (n-octanol/water) log Pow < -2.83 at 22.7 ± 1.0°C

Method EC Directive 92/69/EEC A.8 Partition Coefficient. Flask Method.
The partition coefficient was determined by comparing the solubility of the test material in n-octanol and water. Flasks containing test material, n-octanol and water were shaken and, after separation, the concentration of the test material in each phase was determined by HPLC.

Remarks No test material was detected in any of the organic samples and were therefore considered to be lower than the limit of detection (LOD, 15.6 mg/L).
The test was conducted at pH ~7 and the notified chemical exists in an ionised form at this pH. Therefore, the results should be treated with caution. However, the low log Pow of the notified chemical is consistent with the high water solubility indicating a low affinity for the organic phase and organic component of soils, sediment and sludge.

Test Facility Harlan Laboratories Ltd. (2009a)

Surface Tension 72.0 mN/m at 21.6 ± 0.5°C

Method EC Directive 92/69/EEC A.5 Surface Tension.

Remarks Concentration: 0.986 g/L
Test was performed using the ISO 304 ring method.
The surface tension result was not corrected using the Harkin-Jordan correction table as this is not applicable to the apparatus used. Once calibrated, the balance and ring assembly used in this test give a direct reading for surface tension that is within the required accuracy (± 0.5 mN/m); this is as a result of the recued ring dimensions. This deviation has been considered not to have affected the integrity of the study.
The test material is considered not to be a surface-active material.

Test Facility Harlan Laboratories Ltd. (2009a)

Adsorption/Desorption – screening test log K_{oc} < 1.25 at 40°C

Method EC Directive 200/59/EC C.19 Estimation of the Adsorption Coefficient (K_{oc}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC). The log K_{oc} for the notified chemical was determined by comparison of its retention time to a calibration curve of known standards (log K_{oc} range 1.25-5.63).

Remarks The test was conducted at pH ~7.1 and the notified chemical exists in an ionised form at this pH. The low log K_{oc} is consistent with the notified chemical's high water solubility and low log Pow. The notified chemical is expected to be mobile in soil, although, as it has potentially cationic functional groups, it may sorb to negatively charged sites in soils and sediments.

Test Facility

Particle Size

Method OECD TG 110 Particle Size Distribution/Fibre Length and Diameter Distributions.

Range (μm)	*Mass (%)*
< 100 (inhalable fraction)	25.2
< 10 (thoracic fraction)	0.477
< 5.5 (respirable fraction)	0.148

Remarks Too few particles were of a size less than 10.0 μm to allow accurate assessment of mass median aerodynamic diameter.
Representative sampling was ensured by rolling the sample container for approximately 10 minutes and sampling from top, middle and bottom prior to definitive testing.
The inhalable fraction is defined as the mass fraction of particles which can be inhaled by nose or mouth, the thoracic fraction is defined as the mass fraction of particles that passes the larynx and the respirable fraction is defined as the mass fraction of particles that

reaches the alveoli.
Test Facility Harlan Laboratories Ltd. (2009a)

Flammability Not determined

Method EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks The flammability limits were not determined, as the test substance could not be ignited with a flame under the conditions of the test. The notified chemical was not flammable under the conditions of the test.
Test Facility Harlan Laboratories Ltd. (2009b)

Autoignition Temperature > 400ºC

Method 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
Remarks No self ignition of the test substance was observed under the conditions of the test.
Test Facility Harlan Laboratories Ltd. (2009b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 420 Acute Oral Toxicity – Fixed Dose Method.
Species/Strain	Rat/Wistar (HsdRccHan)
Vehicle	Distilled water
Remarks - Method	No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	1F	2000	0/1
2	4F	2000	0/4

LD50	> 2000 mg/kg bw
Signs of Toxicity	There were no mortalities or signs of systemic toxicity.
Effects in Organs	No abnormalities were noted at necropsy
Remarks - Results	Black stained faeces and blue stained urine were noted in all animals during the study. All animals showed expected gains in bodyweight over the observation period.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Harlan Laboratories Ltd (2009c)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity.
Species/Strain	Rat/Wistar (HsdRccHan)
Vehicle	Moistened with distilled water
Type of dressing	Semi-occlusive.
Remarks - Method	No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5F	2000	0/5
2	5M	2000	0/5

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	Blue coloured staining was noted at the treatment sites of all animals during the study but it was reported this did not affect the evaluation of dermal responses. Physical damage, caused by the attempted removal of adhered test material, and resulting in glossy skin, small superficial scattered scabs, and a hardened light brown coloured scab was noted during the study. No other evidence of dermal irritation was noted.
Signs of Toxicity - Systemic	There were no mortalities or signs of systemic toxicity.
Effects in Organs	No abnormalities were noted at necropsy.
Remarks - Results	All animals showed expected gains in bodyweight over the study period.

CONCLUSION The notified chemical is of low toxicity via the dermal route.

TEST FACILITY Harlan Laboratories Ltd (2009d)

B.3. Irritation – skin

TEST SUBSTANCE Notified chemical

METHOD OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain Rabbit/New Zealand White
Number of Animals 3
Vehicle Moistened with distilled water
Observation Period 72 hours
Type of Dressing Semi-occlusive.
Remarks - Method No significant protocol deviations

RESULTS

Remarks - Results Blue coloured staining was noted at all treated skin sites throughout the study but did not affect the evaluation of skin responses. No evidence of skin irritation was noted during the study. All animals showed expected gain in bodyweight during the study.

CONCLUSION The notified chemical is non-irritating to the skin.

TEST FACILITY Harlan Laboratories Ltd (2009e)

B.4. Irritation – eye

TEST SUBSTANCE Notified chemical

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain Rabbit/New Zealand White
Number of Animals 3 males
Observation Period 21 days
Remarks - Method One animal was initially treated as a pilot. Additional observations were made on Days 7, 14 and 21 to assess the reversibility of the ocular effects.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	1.0	1.0	1.0	2.0	< 72 hrs	0
Conjunctiva: chemosis	0.7	0.3	0.7	2.0	< 72 hrs	0
Conjunctiva: discharge	0.3	0.3	0.3	2.0	< 48 hrs	0
Corneal opacity	0	0	0	0	0	0
Iridial inflammation	0	0	0	0	0	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.

Remarks - Results Blue coloured staining of the fur was noted around all treated eyes throughout the study.
Blue coloured staining of the lower half of the cornea and on the nictitating membrane was noted in two treated eyes at the 24-hour observation, in all treated eyes at the 48-, 72-hour and 7-day observations and persisted in two treated eyes at the 14-day observation. The staining did not affect the evaluation of the ocular effects.
No corneal or iridial effects were noted during the study.

Moderate conjunctival irritation was noted in all treated eyes 1 and 24 hours after treatment with minimal conjunctival irritation noted at the 48-hour observation.
One treated eye appeared normal at the 14-day observation and the remaining treated eyes appeared normal at the 21-day observation.

CONCLUSION The notified chemical is slightly irritating to the eye.

TEST FACILITY Harlan Laboratories Ltd (2009f)

B.5. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE Notified chemical

METHOD OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain Mouse/Female CBA/CaOlaHsd
Vehicle 1% Pluronic acid L92 in distilled water
Remarks - Method No significant protocol deviations

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	672.95	1.0
5	643.88	0.96
10	358.66	0.53
25	685.86	1.02
Positive Control[*]		
1	no data	0.96
5	no data	4.29
10	no data	13.15

[*]2,4-dinitrobenzenesulfonic acid

Remarks - Results
The positive control gave a satisfactory response.
A stimulation index of less than 3 was recorded for the three concentrations of the test material (25%, 10% and 5% w/w in 1% pluronic L92 in distilled water).
There were no deaths. No signs of systemic toxicity were noted in the test or control animals during the test. Blue coloured staining of the ears and fur was noted, post dose on Days 1 to 3, in animals treated with the test material at concentrations of 25% or 10% w/w.
Bodyweight changes of the test animals between Day 1 and Day 6 were comparable to those observed in the corresponding control group animals over the same period.

CONCLUSION There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical.

TEST FACILITY Harlan Laboratories Ltd (2009g)

B.6. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
Species/Strain	Rat/Wistar CRL㉿WI)BR
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: none
Vehicle	Sterile water
Remarks - Method	No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5M/5F	0	0/10
low dose	5M/5F	25	0/10
mid dose	5M/5F	150	0/10
high dose	5M/5F	1000	0/10

Mortality and Time to Death

No mortality was observed during the treatment period.

Clinical Observations

No toxicologically significant systemic clinical changes were noted following administration of INK BH11 C by oral gavage, daily for 28 days.

Day 0 was regarded as the first day of treatment. Blue faeces were observed in animals' cages for both sexes, in the bedding, at all the dose levels tested (25, 150 and 1000 mg/kg bw/day), as of Day 1. At 1000 mg/kg bw/day, both the male and female animals eliminated apparently increased volumes of blue urine in the cage bedding for 27 out of 28 treatment days, commencing on Day 1. These changes were ascribed to elimination of INK BH11 C or its metabolites through faeces and/or urine; moreover, in the absence of any clinical pathology alterations, they were not considered adverse effects.

There were no toxicologically significant changes in the animal behaviour, general physical condition, in the reactions to different type of stimuli, grip strength or motor activity in the control or treated groups, at the evaluation performed towards the end of the treatment period.

No adverse effects were noted on the mean body weight and body weight gain values in the treated groups compared to control animals following daily administration of INK BH11 C at dose levels of up to and including 1000 mg/kg bw/day.

There were no test item related differences in the mean daily food consumption in any test item treated groups (25, 150, or 1000 mg/kg bw/day, male or female) when compared to the control.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

There were no differences that were considered toxicologically significant between the control and test item-treated groups, or any adverse effects of INK BH11 C on haematology parameters in the male and female animals.

There were no relevant changes in the examined clinical chemistry parameters that could be attributed to INK BH11 C administration.

Effects in Organs

At macroscopic evaluation, blue discoloration of the digestive content, stomach, small intestine (jejunum, ileum), colon, cecum, kidneys, mesenteric, mediastinal, cervical and/or mandibular lymph nodes were observed in 1/10, 9/10 and 10/10 animals from the Low, Mid and High Dose, respectively. These findings were considered

to be test item-related.

Additionally, blue discoloration of the lungs was recorded in two Mid Dose (305 and 355) and one high dose (455) rats, associated with blue tracheal mass in females 455 and 355.

At microscopic evaluation, test item-related minimal focal/multifocal intracytoplasmic deposit of blue pigment in the jejunum, ileum, cecum, colon, mesenteric and/or cervical lymph nodes was observed in all high dose animals. These findings corresponded with the gross observations.

Minimal multifocal accumulation of blue pigmented macrophages in the lungs noted in one high dose female (455) and two mid dose rats (305 and 355) occasionally associated with mixed cell infiltrate suggest possible oesophageal reflux of the test item in these animals and correlated with macroscopic changes. No perforation was seen histologically

All other macroscopic and microscopic changes were regarded as incidental or procedure-related.

There were no toxicologically significant changes in organ weight values noted after INK BH11 C administration daily for 28 days, at up to and including 1000 mg/kg bw/day.

Remarks – Results

INK BH11 C administered daily by oral gavage for 28 days in Wistar rats did not lead to any toxicologically significant adverse effects at dose levels of 25, 150 or 1000 mg/kg bw/day.

The faeces of all animals administered up to, and including, 1000 mg/kg bw/day, and the urine of the high dose animals (1000 mg/kg bw/day) were coloured blue for 27 out of 28 days of treatment. At necropsy, blue discoloration of the digestive content, stomach, small intestine (jejunum, ileum), colon, cecum, kidneys, mesenteric, mediastinal, cervical and/or mandibular lymph nodes were observed in all the animals, with an apparent dose response. Test item-related minimal focal/multifocal intracytoplasmic deposit of blue pigment in the jejunum, ileum, cecum, colon, mesenteric and/or cervical lymph nodes were observed microscopically at 1000 mg/kg bw/day. There were no adverse findings on the organ weights.

In conclusion, in the conditions of this study, the no observed adverse effect level (NOAEL) for INK BH11 C is considered 1000 mg/kg bw/day based on no adverse findings at this dose level.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as 1000 mg/kg bw/day in this study, based on the non-adverse histology changes observed at this dose level.

TEST FACILITY LAB Research Ltd. (2009a)

B.7. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test.
	Pre incubation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100
	E. coli: WP2uvrA
Metabolic Activation System	S9 mix from Phenobarbital/5,6-benzoflavone induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 0, 313, 625, 1250, 2500, 5000 µg/plate
	b) Without metabolic activation: 0, 313, 625, 1250, 5000 µg/plate
Vehicle	Water
Remarks - Method	No significant protocol deviations

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	> 5000			
Test 1		> 5000	> 5000	negative
Test 2		> 5000	> 5000	negative
Present	> 5000			
Test 1		> 5000	> 5000	negative
Test 2		> 5000	> 5000	negative

Remarks - Results	No cytotoxicity or precipitation was observed at any concentration. No substantial increases in the number of revertant colonies were seen in any strain either in the presence or absence of metabolic activation. The negative controls were within normal limits and the positive controls (2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide, sodium azide, 2-methoxy-6-chloro-9-[3-(2-chloroethyl)aminopropylamino]acridine.2HCl, 2-aminoanthracene, benzo[a]pyrene) demonstrated the sensitivity of the test system.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	BML, INC (2008)

B.8. Genotoxicity – in vitro

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
Species/Strain	Chinese hamster
Cell Type/Cell Line	Lung cell/V79 cell line
Metabolic Activation System	S9 mix from phenobarbital/β-naphthoflavone induced rat liver
Vehicle	DME (Dulbecco's Modified Eagles's)
Remarks - Method	No significant protocol deviations

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0, 9.76, 19.53, 39.06, 78.12*, 156.25*, 312.5*, 625*, 1250	3 hrs	20 hr

Test 2	0, 9.76, 19.53*, 39.06*, 78.12*, 156.25, 312.5, 625, 1250	3 hrs	28 hr
Test 3	0, 9.76, 19.53*, 39.06*, 78.12*, 156.25, 312.5, 625, 1250	20 hr	28 hr
Present			
Test 1	0, 9.76, 19.53, 39.06, 78.12, 156.25*, 312.5*, 625*, 1250	3 hr	20 hr
Test 2	0, 9.76, 19.53*, 39.06*, 78.12*, 156.25*, 312.5*, 625*, 1250	3 hr	28 hr
Test 3	0, 9.76, 19.53*, 39.06*, 78.12*, 156.25*, 312.5*, 625*, 1250	3 hr	28 hr

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent	> 625			
Test 1		> 625	> 1250.00	negative
Test 2		> 625	> 1250.00	negative
Test 3		> 625	> 1250.00	negative
Present	> 625			
Test 1		> 625	> 1250.00	negative
Test 2		> 78.12	> 1250.00	negative
Test 3		> 78.12	> 1250.00	negative

Remarks - Results

Some cytotoxicity was seen at the highest dose levels. No precipitation was observed at any concentration.

No increase in the frequency of chromosomal aberrations or polyploidy was observed at any concentration used in the presence or absence of metabolic activation.

Appropriate positive controls (ethylmethane sulphonate and nitrosodimethylamine) were used and gave large increases in chromosome aberrations, indicating the sensitivity of the test system.

CONCLUSION

The notified chemical was not clastogenic to CHL/V79 cell line treated in vitro under the conditions of the test.

TEST FACILITY

LAB Research Ltd (2009b)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 C Ready Biodegradability: Modified MITI Test (I)
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Shimadzu total organic carbon (TOC) analyser for the determination of the dissolved organic carbon; closed system oxygen consumption measuring apparatus for the determination of the biological oxygen demand (BOD); high performance liquid chromatograph (HPLC) for determination of the test substance concentration.
Remarks - Method	The oxygen uptake of the test substance in mineral medium, innoculated with unadapted micro-organisms, was measured over a period of 28 days in a darkened enclosed respirometer. Evolved CO_2 was absorbed by lime. Biodegradation is expressed as the percentage oxygen uptake, corrected for blank uptake, of the theoretical uptake (ThOD). Test conditions were: 25 ± 1°C, pH 5.8-7.2.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
7	0	7	72
14	0	14	77
21	1	21	77
28	2	28	77

Remarks - Results	The test solutions were blue. There were no noted adverse effects on the reliability of this test. Test substance concentrations was found not to decline over the duration of the test as determined by HPLC. The pass level (60% of ThOD) was not reached by the test substance within a ten day window, or over the test period, thus it is not considered to be readily biodegradable. The percentage degradation of the reference substance (aniline) surpassed the pass level by day 7, thereby validating the test.
CONCLUSION	The notified chemical is not readily biodegradable
TEST FACILITY	CERI (2009a)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Semi-static. EC Directive 92/69/EEC C.1 Acute Toxicity for Fish – Semi-static.
Species	Rainbow trout (*Onchorhynchus mykiss*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	120 mg $CaCO_3$/L
Analytical Monitoring	The test substance concentration was determined by UV/VIS

	spectrophotometry; limit of quantitation (LOQ) was determined to be 25.0 mg/L.
Remarks – Method	Following a range-finding test, a limit test at a concentration of 1000 mg/L (in triplicate) was conducted according to the guidelines above. The fish, 10 per test vessel, were observed for mortality and sublethal responses every 24 hours. Test conditions were: 14-15°C, 5.2-12.8mg O_2/L, pH 7.1-7.7.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
0	<LOQ	2 × 10	0	0	0	0	0
1000	763-1250	3 × 10	0	0	0	0	0

LC50	>1000 mg/L at 96 hours.
NOEC	1000 mg/L at 96 hours.
Remarks – Results	The measured concentration of 76% of the nominal concentration was reported to be due to a sampling error and/or analytical variation and was the result of a frozen duplicate sample (original sample value lost to instrument error). Given that the overall mean of the measured test concentration was 97 % of nominal, it was reportedly justifiable to estimate the LC50 in terms of nominal test concentrations. Blue staining of fins, mouths and bodies of the fish was observed at the test concentration of 1000 mg/L. This effect was reported to be due to the strong blue colour of the test substance and was considered a physical effect and not a toxicological effect of the test substance. Whilst the temperature range was above the range given in the protocol (by 1°C), and the oxygen concentration in the control vessel was observed to have an air saturation value of less than 60%, this is not considered to affect the validity or integrity of the test as no adverse effects in the fish were observed. After 96 hours of exposure, there were no fish mortalities or sublethal effects in the test vessels or controls, thereby validating the test.
CONCLUSION	The notified chemical is not harmful to fish
TEST FACILITY	Harlan Laboratories Ltd. (2009h)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 *Daphnia* sp. Acute Immobilisation Test - Static. EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia - Static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	The test substance concentration was determined by UV/VIS spectrophotometry; limit of quantitation was determined to be 0.10 mg/L.
Remarks - Method	After a range-finding test, a definitive test at concentrations 1.0, 1.8, 3.2, 5.6, 10, 18, 32, 56 and 100 mg/L (in duplicate) was conducted in accordance with the guidelines above. The daphnia were observed for immobilisation every 24 hours over the course of the test. Test conditions were: 20-21°C, 8.7-8.9 mg O_2/L, pH 7.9-8.0.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	<LOQ	2 × 10	0	0
1.0	0.869-0.908	2 × 10	1	1
1.8	1.57-1.69	2 × 10	0	0
3.2	2.75-3.03	2 × 10	0	0
5.6	4.85-5.43	2 × 10	1	2
10	8.90-9.70	2 × 10	0	1
18	15.9-17.3	2 × 10	0	0
32	28.0-31.7	2 × 10	0	1
56	49.4-55.3	2 × 10	0	0
100	98.4-108	2 × 10	0	0

EC50	>100 mg/L at 48 hours
NOEC	100 mg/L at 48 hours
Remarks - Results	Immobilisation was observed in the range-finding test at concentrations of 10 mg/L and 100 mg/L. In the definitive test there was no significant immobilisation in the 20 daphnids exposed to the test concentration of 100 mg/L for a period of 48 hours. Given the difference in immobilisation observed in the range finding test and the definitive test, a second range finding test was conducted to confirm the results of the definitive test. In the second range finding test, there was no immobilisation in daphnids exposed to the test substance at concentrations of 1.0, 10 and 100 mg/L for a period of 48 hours (10 daphnids per duplicate). Based on this result, the immobilisation observed initially was considered to be due to natural causes and not toxicity associated with the test substance.

CONCLUSION The notified chemical is not harmful to aquatic invertebrates

TEST FACILITY Harlan Laboratories Ltd. (2009i)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD Modified OECD TG 201 Alga, Growth Inhibition Test.
Modified EC Directive 92/69/EEC C.3 Algal Inhibition Test.

Species	*Desmodesmus subspicatus*
Exposure Period	72 hours
Concentration Range	Nominal: 100 mg/L Actual: 96.6-111 mg/L
Auxiliary Solvent	None
Water Hardness	0.15 mmol Ca^{2+} and Mg^{2+}
Analytical Monitoring	Cell densities were determined by a Coulter Multisizer Particle Counter
Remarks - Method	After a range-finding test, a limit test at a concentration of 100 mg/L was conducted. Algae was exposed to the test material (six replicates) and observed for growth inhibition. A control group was run in parallel. Due to the coloured nature of the test solutions the test was modified as recommended (EC, 2006) to minimise the effects of light adsorption by the test material at the wavelengths required for photosynthetic growth by increasing light intensity and decreasing test volume. Test conditions were: 24 ± 1°C, continuous illumination, pH 7.7-7.8. A Student's t-test incorporating Bartlett's test for homogeneity of variance was used for statistical analysis.

RESULTS

Biomass		*Growth*	
E_bC_{50} *mg/L at 72 h*	*NOEC mg/L*	E_rC_{50} *mg/L at 72 h*	*NOEC mg/L*
>100	100	>100	100

Remarks - Results: The test cultures were observed to be blue solutions. Spectrophotometer measurements taken at the wavelength required for photosynthesis (460 and 665 nm) showed that the most significant absorption of the test solutions occurred at 665 nm. Modifications to the guidelines above to increase the light intensity and decrease the sample volume overcame this absorption effect.
All cultures were inspected microscopically at 72 hours, and no abnormalities were detected. The cell concentration in the control cultures increased by a factor of 20, thereby validating the test.

CONCLUSION: The notified chemical is not harmful to algae

TEST FACILITY: Harlan Laboratories Ltd. (2009j)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 209 Activated Sludge, Respiration Inhibition Test.
EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test

Inoculum: Activated sewage sludge
Exposure Period: 3 hours
Concentration Range: Nominal: 1000 mg/L
Remarks – Method: Following a preliminary range-finding test, activated sewage sludge was exposed to an aqueous solution of the test material at a concentration of 1000 mg/L (in triplicate) with the addition of a synthetic sewage as a respiratory substrate. The rate of respiration was determined by comparison to data for the control and a reference material (3,5-dichlorophenol). Test conditions: 21 ± 1°C, pH 8.0-8.5, 140 mg $CaCO_3$/L.

RESULTS
IC50: >1000 mg/L
NOEC: 1000 mg/L
Remarks – Results: Test cultures were observed to be strongly coloured solutions with no undissolved test material present.
The 3 h IC50 of 7.3 mg/L for the reference substance (3,5-dichlorophenol) was found to be within the expected normal range of 5 to 30 mg/L. The respiration rates in the two control flasks were within 15% of each other, thereby validating the test.

CONCLUSION: The notified chemical is not harmful to microbial respiration

TEST FACILITY: Harlan Laboratories Ltd. (2009k)

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