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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

INK BH11 M

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, 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 M

MOLECULAR WEIGHT
> 500 Da

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

3. COMPOSITION

DEGREE OF PURITY > 90%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Dark red powder

Property	Value	Data Source/Justification
Melting Point	Decomposed from ~245°C	Measured
Boiling Point	Not determined	Decomposes on melting
Density	1480 kg/m^3 at 20°C	Measured
Vapour Pressure	< 6.3 x 10^{-7} kPa at 25°C	Measured
Water Solubility	140 g/L at 20.0 ± 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 < -4.35 at 20°C, pH ~7	Measured
Surface Tension	72.5 mN/m at 21°C	Measured
Adsorption/Desorption	log K_{oc} < 1.25 at 30°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): 42.6% Respirable fraction (<10 μm): 0.26% Fraction (<5 μm): 0.11%	Measured
Flash Point	Not determined	Not applicable as low volatility solid.
Flammability	Not highly flammable	Measured
Autoignition Temperature	352°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 150 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 140 g/L at 20ºC and partition coefficient (log Pow) of < -4.35 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 red 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 hour after treatment with minimal conjunctival irritation noted at the 24-hour observation period. All treated eyes appeared normal at the 48-hour observation.

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

Repeated Dose Toxicity
The 28-day oral toxicity study for the notified chemical showed no mortality at up to 1000 mg/kg bw/day. The observation of red coloured urine provided evidence that absorption of the notified chemical had occurred. Based on non-adverse histological findings observed at 150 and 1000 mg/kg bw/day, the NOAEL was established as 150 mg/kg bw/day.

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 exposure estimate of ~0.0014 mg/kg bw/day, compared with NOAEL of 150 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 in 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 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) >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 (Alga).	>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/polymer 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 product 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 ~245ºC

Method EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks Substance decomposed without melting from approximately 245 °C at 101.6 kPa.
Test Facility Harlan Laboratories Ltd. (2009a)

Density 1480 kg/m^3 at 20.7 ± 0.5 ºC

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

Vapour Pressure < 6.3 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 140 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, flasks containing test material and double distilled water were shaken at approximately 30°C. After standing for a period of not less than 24 hours the contents of the flasks were centrifuged. An aliquot of the sample solution was analysed by HPLC to determine the concentration of test material.
Remarks The pH of each test solution was measured and was found to range from pH 4.8 to 5.
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 (1 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 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 < -4.35 at 20.8 ± 0.6ºC, pH ~7

Method EC Directive 92/69/EEC A.8 Partition Coefficient. Flask Method. The partition coefficient was determined by 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. The limit of quantification (LOQ) was calculated as three times the baseline noise at the retention time of the test material.
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 is consistent with the high water solubility of the notified chemical indicating a low affinity for the organic phase and organic component of soils, sediment and sludge.
Test Facility Harlan Laboratories Ltd. (2009a)

Surface Tension 72.5 mN/m at 21.8 ± 0.5°C

Method	EC Directive 92/69/EEC A.5 Surface Tension.
Remarks	Concentration: 0.970 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 30°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 Koc range 1.25-5.63).
Remarks	The test was conducted at pH ~7 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	Harlan Laboratories Ltd. (2009a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE

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	Red stained faeces was 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 – Limit Test.
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	5 M	2000	0/5
2	5 F	2000	0/5

LD50	> 2000 mg/kg bw
Signs of Toxicity - Local	Red coloured staining which prevented evaluation of erythema was noted at the treatment sites of all animals during the study. Small superficial scattered scabs were noted at the treatment site of one female, nine to fourteen days after dosing.
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	Red coloured staining was noted at all treated skin sites throughout the study but was reported to not affect evaluation of skin responses. No evidence of skin irritation was noted 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
Observation Period	72 hours
Remarks - Method	No significant protocol deviations

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	0.3	0.3	0.3	2	< 48 hrs	0
Conjunctiva: chemosis	0.3	0.3	0.3	2	< 48 hrs	0
Conjunctiva: discharge	0.3	0.3	0.3	2	< 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	Red coloured staining of the fur was noted around all treated eyes throughout the study. No corneal or iridial effects were noted during the study. Moderate conjunctival irritation was noted in all treated eyes one hour after treatment with minimal conjunctival irritation noted at the 24-hour observation period. All treated eyes appeared normal at the 48-hour 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	Ethanol/distilled water 7:3 v/v
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)	776.27	1.0
5	1011.28	1.30
10	837.08	1.08
25	705.27	0.91
*Positive Control**		
15	no data	9.49

*α-Hexylcinnamaldehyde

Remarks - Results	A stimulation index of less than 3 was recorded for all three tested concentrations indicating that the notified chemical is a non-sensitiser. The positive control gave a stimulation index of 9.49 confirming the sensitivity of the test. There were no mortalities or signs of systemic toxicity noted. Red coloured staining of the ears and fur was noted post-dose on Days 1 to 3 in animals treated with the test material at a concentration of 25% w/w.
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/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 M by oral gavage, daily for 28 days.

Day 0 was regarded as the first day of treatment. Red or light red faeces and/or urine were observed in animals' cages for both sexes, in the bedding, at all the dose levels tested from Day 1, or 2, respectively, as follows:

1000 mg/kg bw/day:
Red faeces were observed for 27/28 days in both the male and female animals, as of Day 1 of treatment period. In addition, red urine occurred for 26/28 days in the male animals, or 22/28 days, in the females.

150 mg/kg bw/day:
Both male and female animals showed red faeces from Day 2, for 26/28 days.

25 mg/kg bw/day:
Light red faeces were observed for 18 to 23 days of 28 treatment days, in the male or female animals, respectively. These changes were ascribed to elimination of INK BH11 M 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 M 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 M on haematology parameters in the male and female animals.

Variations were noted on occasion in the mean values of different parameters, such as, but not limited to, platelets (PLT), basophile (BA%), neutrophile (NE%), monocite (MO%), eosinophil (EO%), or large unstained cells (LUC%) values at all the dose levels, in both sexes. However, these variations were not statistically significant, and evaluation of the mean and individual results in correlation with the control and historical haematology data did not reveal any test-item related cause of the changes noted, and/or no consistent dose or gender-related response was observed. Therefore, these differences observed between the control and treated groups were considered to be incidental which were not related to treatment and generally remained within the historical control ranges, or were with no toxicological significance.

Clinical chemistry evaluation revealed a significantly higher serum total bilirubin concentration in both sexes (males at 150 and 1000 mg/kg bw/day, females at 1000 mg/kg bw/day), exceeding the historical control ranges in the 1000 mg/kg bw/day female group, and with a trend to a dose response.
However, there were no other clinical chemistry or histopathologic alterations that indicated changes in the liver. It is possible that the intense colour of the test item caused a spectral interference in determination of total bilirubin concentration.

Statistically significant changes of aspartate aminotransferase activity (AST) and phosphorus (Phos.) mean values were noted in the treated animals compared to control. AST mean values were lower in male animals at all the dose levels tested, attaining statistical significance at 25 mg/kg bw/day (low dose); however, AST values

were slightly increased in the female animals, with no statistical significance and remained below the upper limit of the historical control range. Phosphorus mean value was statistically higher in the 1000 mg/kg bw/day female animals, but lower than control in the treated male animals.

In summary, most of the observed variations in the clinical chemistry parameters, although they were occasionally statistically significant, were not dose-related, showed no consistent gender response and/or were within the normal historical control ranges. The only variation that may have a toxicological significance at 1000 mg/kg bw/day dose level in both sexes was the apparent total bilirubin increase, which may be due to spectral interference with the analytical method by the discoloured test item in the plasma. In the absence of any additional evidence of liver damage from other measurements of liver function or from liver histopathology, bilirubin changes in correlation with INK BH11 M administration is considered equivocal by the study authors in the conditions of this study.

Effects in Organs

At macroscopic evaluation, red discoloration of the stomach, small intestine (duodenum, jejunum, ileum), colon, cecum and/or rectum was observed in 6/10 and 10/10 animals at 150 and 1000 mg/kg bw/day, respectively. Additionally, red digestive content was present in 8/10 and 10/10 rats at 150 and 1000 mg/kg bw/day, respectively. These findings were considered to be test item-related.

At microscopic evaluation, minimal focal/multifocal intracytoplasmic deposit of red pigment in the mesenteric and/or mandibular lymph nodes was noted in 7/10 animals at 1000 mg/kg bw/day. In addition, minimal focal intracytoplasmic deposit of red pigment in the Peyer's patches of the ileum was recorded in two animals at 150 mg/kg bw/day (303 and 354) and one animal at 1000 mg/kg bw/day (402). 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 M administration daily for 28 days, at up to and including 1000 mg/kg bw/day. Statistically higher kidneys weights relative to body weight were recorded at 1000 mg/kg bw/day in the male animals. In the female animals, the mean absolute brain weights were apparently lower at 25 and 1000 mg/kg bw/day than those recorded in the control animals. As these changes were within the historical range, had low magnitude and were not correlated with pathological findings, they were considered incidental and not related to treatment.

All other examined organ weights (absolute and relative to the body and brain weights) were similar in the control and test item treated groups.

Remarks – Results

INK BH11 M administered daily by oral gavage for 28 days in Wistar rats did not lead to any toxicologically significant clinical 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 red, or light red (faeces at 25 mg/kg bw/day) for up to 27 out of 28 days of treatment, in an apparent dose related manner.

A significantly higher serum total bilirubin concentration was observed in both sexes (males at 150 and 1000 mg/kg bw/day, females at 1000 mg/kg bw/day), exceeding the historical control ranges in the 1000 mg/kg bw/day female group, and with a trend to a dose response. In the absence of any additional evidence of liver damage from other measurements of liver function or from liver histopathology, bilirubin changes may be due to spectral interference with the analytical method by the coloured test item in the plasma, and a correlation with INK BH11 M administration is considered equivocal in the conditions of this study. At necropsy, test item related red discoloration of the stomach, small intestine (duodenum, jejunum, ileum), colon, cecum and/or rectum was observed at 150 and 1000 mg/kg bw/day. Red digestive content was also noted at 150 and 1000 mg/kg bw/day. At microscopic evaluation, minimal focal/multifocal intracytoplasmic deposit of red pigment in the mesenteric and/or mandibular lymph nodes was noted at 1000 mg/kg bw/day. In addition, minimal focal intracytoplasmic deposit of red pigment in the Peyer's patches of the ileum was recorded in animals treated at 150 and 1000 mg/kg bw/day. There were no adverse findings on the organ weights.

In conclusion, based on histological findings, the no observed adverse effect level (NOAEL) for INK BH11 M is considered to be 150 mg/kg bw/day observed at 150 and 1000 mg/kg bw/day.

CONCLUSION

The No Observed (Adverse) Effect Level (NO(A)EL) was established as 150 mg/kg bw/day in this study, based on non-adverse histological findings.

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, 2500, 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 In both tests there was neither an increase in the number of revertant colonies (more than twice as many as that of the negative control) nor a dose related response was observed at any dose levels in any strain of base-pair substitution type or frame-shift type with or without metabolic activation.

The revertant colonies of the positive controls showed an increase of more than twice that of the negative controls indicating the study was performed correctly.

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	312.5, 625, 1250*, 2500*, 5000*	3 hr	20 hr
Test 2	156.25, 312.5*, 625*, 1250*, 2500*, 5000	20 hr	28 hr
Present			
Test 1	312.5, 625, 1250*, 2500*, 5000*	3 hr	20 hr
Test 2	156.25, 312.5*, 625*, 1250*, 2500*, 5000*	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	> 1250			
Test 1		> 5000	> 5000	negative
Test 2		> 1250	> 5000	negative
Present	> 2500			
Test 1		> 5000	> 5000	negative
Test 2		> 2500	> 5000	negative

Remarks - Results	Some cytotoxicity was observed at the high 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. \ The positive controls 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 adbsorbed 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 not reported.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
7	1	7	70
14	1	14	77
21	2	21	78
28	2	28	77

Remarks - Results	The test solutions were red, although there were no noted adverse effects on the reliability of this test. Test substance concentrations were 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 (2009)

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.
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

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: 14-16°C, 5.2-12.7 mg O_2/L, pH 6.2-7.7.

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	962-1096	3 × 10	0	0	0	0	0

*Limit of quantitation (LOQ) was determined to be 6.3 mg/L.

LC50	>1000 mg/L at 96 hours.
NOEC	1000 mg/L at 96 hours.
Remarks – Results	Whilst the temperature range was above the range given in the protocol (by 2°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 was no fish mortality 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.
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
Remarks - Method	After a range-finding test, a limit test at a concentration of 100 mg/L was conducted in accordance with the guidelines above. Four replicates per concentration each had 5 daphnia added. The daphnia were observed for immobilisation every 24 hours over the course of the test. Test conditions were: 20 - 21°C, 8.4-9.6 mg O_2/L, pH 7.6-8.0.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	<LOQ*	4 × 5	0	0
100	100-103	4 × 5	0	0

*Limit of quantitation (LOQ) was determined to be 3.5 mg/L.

EC50	>100 mg/L at 48 hours
NOEC	100 mg/L at 48 hours
Remarks - Results	After 48 hours of exposure, there was no daphnid immobility observed in the test vessels or in the controls, thereby validating the test.
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: 94-109 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 5.3-7.8. 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 red 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 460 nm. Modifications to the guidelines above to increase light intensity and decrease 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 reference material (3,5-dichlorophenol). Test conditions: 21 ± 1°C, pH 7.5-8.6, 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 8.0 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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