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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Polymer in HP XP and FB Printer Ink Series

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 Sustainability, Environment, Water, Population and Communities.

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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Polymer in HP XP and FB Printer Ink Series

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
CPI Graphics Limited (ABN 54 004 081 501)
41-45 Mills Road
BRAESIDE VIC 3195

Hewlett-Packard Australia Pty Limited (ABN 74 004 394 763)
353 Burwood Highway
FOREST HILL VIC 3131

NOTIFICATION CATEGORY
Limited-small volume: Synthetic polymer with NAMW ≥1000 Da.

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, polymer constituents, residual monomers/impurities, use details, import volume, identity of recipients.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: Melting/Boiling Point, Vapour Pressure, Hydrolysis as a Function of pH, Partition Coefficient, Dissociation Constant, Adsorption/Desorption, Particle Size, Flash Point, Flammability Limits, Autoignition Temperature and Explosive Properties

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
HP XP and FB Printer Ink series (containing notified polymer at up to 5%)

MOLECULAR WEIGHT
Mn >1000 Da.

ANALYTICAL DATA
Reference NMR, IR, GPC and UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY >98%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None

ADDITIVES/ADJUVANTS
None

LOSS OF MONOMERS, OTHER REACTANTS, ADDITIVES, IMPURITIES
None

DEGRADATION PRODUCTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Brown liquid

Property	Value	Data Source/Justification
Freezing Point	Not determined	Being a viscous liquid, expected to have a freezing point of <0°C
Boiling Point	Not determined	Expected to decompose at high temperatures before boiling.
Density	1098 kg/m^3 at 25°C	Measured
Vapour Pressure	Not determined	The notified polymer has a high molecular weight and is therefore expected to have very low vapour pressure
Water Solubility	Test 1: < 3.8 ×10^{-3} g/L at 20°C Test 2: < 12 ×10^{-3} g/L at 20°C, pH 4, 7 and 9	Measured Measured
Hydrolysis as a Function of pH	Not determined	Expected to slowly hydrolyse under ambient environmental conditions
Partition Coefficient (n-octanol/water)	Not determined	The notified polymer is an ionic emulsifier and will tend to accumulate at the phase interface of octanol and water and/or form emulsions
Adsorption/Desorption	Not determined	The notified polymer is expected to partition to surfaces from water in the environment based on its surface activity
Dissociation Constant	Not determined	The notified polymer will be ionised in the environmental pH range (4 – 9)
Flash Point	Not determined	Vapour pressure anticipated to be too low for flash point determination.
Flammability	Not determined	Vapour pressure anticipated to be too low for flammability determination.
Autoignition Temperature	Not determined	Expected to decompose before auto-ignition occurs.
Explosive Properties	Not determined	Not expected to be explosive based on structure.

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

Reactivity
The notified polymer is expected to be stable under normal conditions of use.

Dangerous Goods classification
Based on the submitted physical-chemical data in the above table the notified polymer 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 notified polymer.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED POLYMER (100%) OVER NEXT 5 YEARS
The notified polymer will not be manufactured or reformulated in Australia but will be imported as a component of formulated UV-curable printer inks at concentrations up to 5%.

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

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

PORT OF ENTRY
Sydney, Melbourne

TRANSPORTATION AND PACKAGING
The notified polymer will be imported by sea in 5 L plastic bottles packed within cardboard boxes and transported to the notifier's warehouse or directly to customer's for use.

USE
The notified polymer will be used as a component of UV-curable inks for inkjet printing at concentrations up to 5% at industrial sites only.

OPERATION DESCRIPTION
The notified polymer will not be manufactured, reformulated or repackaged in Australia.

The finished inks containing the notified polymer (up to 5%) will be imported in 5 L plastic bottles and transported to the notifiers' storage facilities and then to customers (industrial printing companies) or directly to customers for use.

Ink bottles will be manually connected to the printing machine via an inlet and attached to a flexible tube which supplies the ink head. The ink will be automatically injected from the bottles into the printing machine. Separate ink bottles will be provided for each of the required colours for printing.

While printers are running, printer operators will monitor their operation and keep the substrate (eg vinyl, paper, etc) feeders stocked and attend to substrate jams. Throughout the print run, quality control will be carried out by printer operators.

After printing, the notified polymer will be fixed (UV-cured) with other ink ingredients into the substrate matrix. Printed articles will be used for a variety of advertising purposes.

Any residual ink within printing equipment will be wiped clean using rags and solvents. These rags and dirty solvents are expected to be disposed of by the printing company through licensed waste disposal contractors.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

NUMBER AND CATEGORY OF WORKERS

Category of Worker	*Number*	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and storage	10-20	4-8	200
Printer operators	> 1000	0.5	5
Service technicians	200	8	200
Wholesale printer supplies	> 1000	8	200

EXPOSURE DETAILS
Transport and warehousing
Workers are not expected to be exposed to ink formulations containing the imported notified polymer except in the event of an accident where the packaging is breached.
Wholesale Workers
Wholesale workers will be involved in opening cardboard cartons, removing the plastic bottles and stacking the individual boxes onto shelves. However, exposure to the notified polymer at up to 5% in the inks is not anticipated except in the event of an accident where the packaging is breached.

Printer operators

Printer operators are not expected to be exposed to ink containing the notified polymer at up to 5%, as the process is mainly automated. However, dermal exposure is possible to the notified polymer during the replacement of ink bottles but is expected to be limited due to the use of gloves. Inhalation exposure may occur to aerosols of the notified polymer during the operation of the printers. However, this is expected to be minimised by local exhaust ventilation employed in areas surrounding printing machines.

Service technicians
Service technicians are expected to experience contact with ink containing the notified polymer at up to 5% during printer maintenance and the replacement of ink bottles. The most likely route of exposure is dermal. However, this is expected to be minimized by the use of cotton gloves. Inhalation exposure may also occur but will be minimized through the use of local exhaust ventilation.

Handling of printed substrates
After application to the substrate, the ink containing the notified polymer is UV-cured (fixed) and hence no longer bioavailable. The printed substrates will be handled by workers and displayed for promotion to the public in a variety of different settings. However, exposure is not anticipated for these workers or members of the public who might experience incidental dermal contact with the printed substrates, as the notified polymer will be bound within a print matrix and is not bioavailable.

6.1.2. Public exposure
Neither the printer inks nor the printed materials containing the notified polymer will be sold to the public. Once cured onto the substrate, the notified polymer is expected to remain bound to the substrate print matrix. Thus, public exposure resulting from the use of the notified polymer is expected to be negligible.

6.2. Human health effects assessment

The results from toxicological investigations conducted on the notified polymer 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
Hen's Egg Test – Chorio-allantoic membrane (HET-CAM)	non-irritating

Toxicokinetics
The notified polymer is a high molecular weight (>1000 Da.) salt with a low proportion of low molecular weight species (<5% with molecular weight <1000Da.) and limited water solubility (<5 mg/L). Based on these characteristics, it is not expected to be absorbed following oral, dermal or inhalation exposure.

Acute toxicity
The notified polymer was found to be of low acute oral toxicity with a LD50 >2000 mg/kg bw. No mortalities or signs of systemic toxicity were observed. The treated animals displayed expected weight gains during the study.

Irritation and Sensitisation
The notified polymer was predicted to be non irritating to the eye in a HET-CAM test.

The HET-CAM assay has not yet been validated as a replacement test for the *in vivo* Draize test, however validation of this assay is currently being considered by the US National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods and the Interagency Coordinating Committee on the Validation of Alternative Methods (NICEATM-ICCVAM). The draft ICCVAM recommendations from this validation process were released in April 2009 and recommended that the Hen's Egg Test-Chorioallantoic Membrane (HET-CAM) not to be used for regulatory hazard classification purposes, based on a lack of adequate data (ICCVAM, 2009).
Therefore, the validity of the HET-CAM assay for determining the irritancy potential for substances such as the notified polymer has not yet been confirmed. However, the minimal response in the HET-CAM assay for the notified polymer indicated non-irritancy potential for the notified polymer.

The notified polymer was not tested for its potential to illicit skin sensitisation. It does not contain any known structural alerts for skin sensitisation (Barratt et al. 1994) and is therefore not expected to be a skin sensitiser.

Health hazard classification
Based on the limited data provided, the notified polymer cannot be classified according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
Based on the limited toxicological data provided, the health hazards resulting from exposure to the notified polymer cannot be determined.

Printer operators and service technicians may encounter dermal and possibly inhalation exposure to ink formulations containing the notified polymer at up to 5% during replacement of ink bottles, printer maintenance and cleaning. These workers are expected to wear gloves to minimise dermal exposure and exhaust ventilation is expected to be in use to minimise inhalation exposure. Furthermore, exposure is not anticipated for workers handling printed substrates as the notified polymer will be bound with a print matrix and is not expected to be bioavailable.

Given the low concentration (≤5%) of the notified polymer in ink formulations, the use of PPE and engineering controls to further minimise exposure and the lack of bioavailability of the notified polymer in the printed material, the risk posed to occupational health and safety of workers is not anticipated to be unacceptable.

6.3.2. Public health
Neither the inks containing the notified polymer nor substrates containing the cured ink will be sold to the public. Therefore, the risk to public health is not considered to be unacceptable, based on the low potential for exposure.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The notified polymer will be imported into Australia as a component of a final product in 5 L plastic ink bottles. No manufacturing and reformulation of the notified polymer will take place in Australia. Environmental release of the notified polymer is unlikely to occur during importation, storage and transportation.

RELEASE OF CHEMICAL FROM USE
The ink bottles will be designed to prevent leakage and will not be opened during transport, use, installation or replacement. Therefore, release of ink containing the notified polymer to the environment is not expected under normal conditions of use. If leakage or spillage does occur, the ink will be physically contained with absorbent material and disposed of to landfill. The ink bottles will be contained within the printer until the contents are consumed and then they will be removed and sent for recycling or disposed of to landfill. Approximately 0.1% of the ink containing the notified polymer will remain in spent ink bottles.

RELEASE OF CHEMICAL FROM DISPOSAL
The majority of the notified polymer is expected to be disposed of to landfill and is expected to remain associated with the substrates (e.g. plastic and paper) to which it has been applied. Of the ≤2.5% notified polymer applied to paper, half of this amount is expected to be recycled. During recycling processes, waste paper is repulped using a variety of chemical agents which, amongst other things, enhance detachment of ink from the fibres.

7.1.2 Environmental fate

The majority of the notified polymer will end up in landfill where it is expected to slowly decompose by abiotic and biotic processes to form water and oxides of carbon, nitrogen and phosphorus.

A small fraction of the notified polymer is expected to be released to the sewerage system due to the recycling of paper to which the product containing the notified polymer will be applied. In the waste water treatment processes in sewage treatment plants, most of the notified polymer is expected to partition to sludge or to suspended solids due to its low water solubility and surface activity, where it will be removed for disposal to landfill or used on land for soil remediation (Painter, 1992). Moreover, the notified polymer is expected to be efficiently removed from waste water in waste water treatment plants through adsorption to sludge or by flocculation due to the cationic component of the polymer (Boethling and Nabholz, 1997).

In surface waters, the notified polymer will partition to suspended solids and organic matter. It is not readily biodegradable but is expected to slowly hydrolyse. Based on its relatively high molecular weight and surface activity the notified polymer is not expected to bioaccumulate.

For the details of the environmental fate study refer to Appendix C.

7.1.3 Predicted Environmental Concentration (PEC)

A PEC for discharge of the notified polymer to surface waters has been calculated assuming that half of the paper to which the ink containing the notified polymer is applied will be recycled and will be discharged to sewers nation wide with no removal in sewage treatment plants. The details of this worst case scenario are as follows:

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	3,000	kg/year
Proportion expected to be released to sewer	1.25%	
Annual quantity of chemical released to sewer	37.5	kg/year
Days per year where release occurs	260	days/year
Daily chemical release:	0.14	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:	0.03408	µg/L
PEC - Ocean:	0.00341	µg/L

7.2. Environmental effects assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	LL50 (96 h) > 100 mg/L	Not harmful
Daphnia Toxicity	EL50 (48 h) > 100 mg/L	Not harmful

The notified polymer is not harmful to fish and aquatic invertebrates up to its limit of solubility in water. The notified polymer is therefore not classified for acute aquatic hazard. Although the notified polymer is poorly water soluble and not readily biodegradable, it is not expected to bioaccumulate and therefore is not classified for long-term aquatic hazards under the Globally Harmonised System of Classification and Labelling of Chemicals (United Nations, 2009).

7.2.1 Predicted No-Effect Concentration

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
Fish LL50	> 100	mg/L
Assessment Factor	1000	
PNEC:	> 100	µg/L

An assessment factor of 1000 has been used to derive a PNEC as acute toxicity endpoints are available for the effects of the notified polymer on aquatic species from only two trophic levels.

7.3. Environmental risk assessment

Risk Assessment	PEC µg/L	PNEC µg/L	Q
Q - River	0.03408	> 100	$< 3.41 \times 10^{-4}$
Q - Ocean	0.00341	> 100	$< 3.41 \times 10^{-5}$

The Risk Quotients (Q = PEC/PNEC) for the worst case discharge scenario have been calculated to be << 1 for both river and ocean compartments. This indicates the notified polymer is not expected to pose an unacceptable risk to the aquatic environment based on its reported use pattern.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the limited data provided, the notified polymer cannot be classified according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

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

When used in the proposed manner, the notified polymer 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 polymer is not expected to pose a risk to the environment.

Recommendations

CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following engineering controls to minimise occupational exposure to the notified polymer at ≤5% as imported in HP XP and FB Printer Ink Series:
  - Exhaust ventilation

- Due to the limited toxicological data provided on the notified polymer, the health hazards resulting from exposure to the notified polymer cannot be determined. Therefore, the following PPE are recommended for workers handling ink bottles and/or connecting the ink bottles to printers:
  - Overalls
  - Gloves

  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 polymer/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 polymer should be disposed of to landfill.

Emergency procedures

- Spills or accidental release of the notified polymer 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 polymer, have post-assessment regulatory obligations to notify NICNAS when any of these circumstances change. These obligations apply even when the notified polymer 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(2) of the Act; if
- the function or use of the polymer has changed from a component of UV-curable inks for inkjet printing for commercial use only, or is likely to change significantly;
- the amount of polymer being introduced has increased from 3 tonnes, or is likely to increase, significantly;
- the polymer has begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the polymer 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 products containing the notified polymer 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

Density 1098 kg/m^3 at 25ºC

Method OECD TG 109 Density of Liquids and Solids.
EC Directive 92/69/EEC A.3 Relative Density.
Remarks Test report not provided

Water Solubility (Test 1) < 3.8 ×10^{-3} g/L at 20ºC

Method Following the general procedure of OCED TG 105, approximately 1 g of notified polymer was added to 500 mL of water and stirred at 48 hours at room temperature. After stirring, the sample was kept undisturbed for 24 hours allowing separation of excess polymer from the water phase. The water phase appeared slightly turbid indicating that non-dissolved material remained in the water phase. From the centre volume ('water phase') of each solution, 100 mL of sample was carefully pipetted, avoiding uptake of larger droplets of notified polymer. This was labelled Sample 1. Another sample was prepared as above and centrifuged at 2575 × g to separate small droplets and part of the upper layer was removed by pipette and was labelled Sample 2. Sample 2 was clearer than Sample 1 but was still opaque, indicating an emulsion was present. Both samples were extracted twice with 70 mL of chloroform. The solvent was removed by a rotary evaporator and a known quantity of heptachloro-propane was added as an internal quantification standard for NMR measurement. The extract in heptachloro-propane was re-dissolved in ~5 mL of deutero-chloroform and a 1 mL aliquot of each sample was transferred to NMR tubes and analysed. The measured concentration of the notified polymer extracted to the water phase was 9.9 mg/L in Sample 1 and 3.8 mg/L in Sample 2.
Remarks A water solubility test could not be conducted according to OECD TG 105 due to the tendency of the notified polymer to form emulsions. These results should be treated with caution as an emulsion was present in the samples. Therefore, the water solubility of the notified polymer is expected to be much lower than 3.8 mg/L.
Test Facility Goldschmidt Analytical Laboratory (2004)

Water Solubility (Test 2) < 12 ×10^{-3} g/L at 20ºC

Method Following the general procedure of OCED TG 105, three tests were conducted at pH 4, 7 and 9, and approximately 1 g of notified polymer was added to 500 mL of water and stirred for 48 hours at room temperature. After stirring the samples were kept undisturbed for 24 hours allowing separation of excess polymer from the water phase. In all three tests the water phase appeared turbid indicating that non-dissolved material remained in the water phase. From the centre volume ('water phase') of each solution, 100 mL of sample was carefully pipetted, avoiding uptake of larger droplets of notified polymer. These samples were centrifuged at 2575 × g to separate small droplets and part of the upper layer was removed by pipette. The samples were slightly opaque in the tests indicating an emulsion was present. Each sample was extracted twice with 70 mL of chloroform. The solvent was removed by a rotary evaporator and a known quantity of heptachloro-propane was added as an internal quantification standard for NMR measurement. The extract in heptachloro-propane was re-dissolved in ~5 mL of deutero-chloroform and a 1 mL aliquot was transferred to a NMR tube and analysed. The measured concentration of the notified polymer extracted to the water phase was not strongly pH dependent (12 mg/L at pH 4 and 11 mg/L at pH 7 and 9).
Remarks A water solubility test could not be conducted according to OECD TG 105 due to the tendency of the notified polymer to form emulsions. These results should be treated with caution as an emulsion was present in the samples. Therefore, the water solubility of the notified polymer is expected to be much lower than 12 mg/L.
Test Facility Goldschmidt Analytical Laboratory (2006)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified polymer
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/White Wistar
Vehicle	None
Remarks - Method	The expiry date on the feed given to the animals had been slightly exceeded. The study author's noted that the food storage conditions were cool and dark and they did not expect any adverse effects as a result. This was confirmed by the feed supplier. No other significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	6 females	2000	None

LD50	>2000 mg/kg bw
Signs of Toxicity	None observed
Effects in Organs	None observed
Remarks - Results	No mortalities or adverse effects were observed following dosing with the test substance. The animals displayed expected body weight gains during the study.
CONCLUSION	The notified polymer/polymer is of low toxicity via the oral route.
TEST FACILITY	Stockhausen GmbH (2004a)

B.2. Irritation – eye The Hen's Egg Test – Utilizing the Chorioallantoic Membrane (HET-CAM)

TEST SUBSTANCE	Notified polymer
METHOD	Hen's Egg Test (HET) - Chorioallantoic Membrane (CAM) Test. Modification of that described by Kemper and Luepke (1986).
Species	SPAFAS chicken eggs
Number of eggs	6 fertilised and incubated for the test substance, negative and positive controls
Observation period	Readings taken at 0.5, 2 and 5 mins
Treatment	The eggs were incubated at 37.5 ± 0.5°C and a relative humidity of 62.5% (±7.5%) in an automatic, rotating incubator for 9 days. After 9 days, the shell over the air sack of each egg was removed, wetted with physiological saline at room temperature for approximately 1 minute and the CAM was removed with forceps. A 200 µL solution of the test substance (undiluted) was applied to each CAM and effects of hyperemia, haemorrhage (including minimal haemorrhage) and coagulation were observed over a period of 5 mins and scored according to the maximum scores shown in the following table.

Effect	*Scores at time (min):*		
	0.5	2	5
Vascular injection	5	3	1
Haemorrhage	7	5	3
Coagulation	9	7	5

Each reaction type can be recorded only once for each CAM, therefore the maximum score per CAM is 21. The mean score was determined for all CAM's similarly tested.

Remarks - Method The duration of application with the test substance was not reported. The positive control used in the test was Texapon ASV (sodium magnesium lauryl-myristyl-6-ethoxy-sulfate) diluted to 5% with tap water. The negative control used in the test was tap water.

RESULTS

Test Solution	*Average Irritation score*
Tap water	0.00
Notified polymer	0.5
5% Texapon ASV (sodium magnesium lauryl-myristyl-6-ethoxy-sulfate)	9.7

Remarks - Results Haemorrhage of the CAM 5 minutes following treatment with the notified polymer was observed in one egg. This resulted in an irritation score of 0.5 out of a possible 21. In comparison, a score of 9.7 was reported for the positive control Texapon ASV at 5% concentration which is known to be slightly *irritating in vivo*. Therefore, a score of 0.5 for the notified polymer is predicted to be practically non-irritating.

CONCLUSION Under the conditions of this test, the notified polymer is predicted to be non-irritating to the eye.

TEST FACILITY Stockhausen GmbH (2004b)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified polymer
METHOD	OECD TG 301 C Ready Biodegradability: Modified MITI Test (I)
Inoculum	Aerobic activated sludge comprising samples from a lake, the effluent of a municipal sewage treatment plant and an extract of surface soil
Exposure Period	28 days
Auxiliary Solvent	None reported
Analytical Monitoring	Pressure decrease measured by Sensomat
Remarks - Method	The inoculum deviated from the OECD Guideline (samples should be collected from no fewer than 10 sites) but was not expected to produce a false positive result. The test substance was directly weighed into test vessels and made up to a final concentration of 100 mg/L in inoculated mineral medium. An abiotic control was made up in the same way except the inoculum was excluded and mercury chloride was added at a concentration of 1% (w/v). Test vessels were incubated in the dark at 25ºC. Since there was no reliable Theoretical Oxygen Demand (ThOD) calculation for the test substance available, the ThOD was measured as the Chemical Oxygen Demand in a separate study (Stockhausen GmbH (2005b)). This study was determined in accordance with the "German Standard Procedure" H 41, DIN 38 409, part 41 (December 1980), and was assessed as a reliable study.

RESULTS

Test substance		*Sodium benzoate*	
Day	*% Degradation**	*Day*	*% Degradation**
5	6	5	75
15	12	15	87
28	15	28	88

*Average of three replicates

Remarks - Results	All validity criteria for the test were satisfied except that at the end of the test, the difference between the two most extreme replicates in degradation of the test substance was greater than 20% (30%), however this would not have affected the reliability and final outcome of the test. The reference substance was degraded > 60% by the 10th day, indicating a suitable aerobic activated sludge inoculum was used. The test substance did not reach the pass level of 60% degradation for this test and therefore cannot be classified as readily biodegradable.
CONCLUSION	The notified polymer cannot be classified as readily biodegradable
TEST FACILITY	Stockhausen GmbH (2005a)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified polymer
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Static Test
Species	Zebra Fish (*Danio rerio*, Hamilton Buchanan)
Exposure Period	96 hours

Auxiliary Solvent	None reported
Water Hardness	70 – 90 mg $CaCO_3$/L
Analytical Monitoring	^{1}H NMR
Remarks – Method	A Water Accommodated Fraction (WAF) was prepared by adding 1500 mg notified polymer to 15 L water and stirring for 24 hours by magnetic stirrer at room temperature. The solution was allowed to stand for 2 hours to let undissolved polymer settle out and 10 L of the WAF was transferred to the test aquarium. Similarly treated dilution water served as the control.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*			
Nominal	*Actual*		*24 h*	*48 h*	*72 h*	*96 h*
0	0	10	0	0	0	0
100(WAF)	< 4	10	0	0	0	0

LL50	> 100 mg/L at 96 hours (based on loading rate)
NOEL	100 mg/L at 96 hours (based on loading rate)
Remarks – Results	Regarding the measured concentrations (NMR), the study author reported there was a "different signal form" between the integrated signal (4.0 – 4.2 ppm) in the pure test substance and the integrated signals (4.0 – 4.2 ppm) in all the samples. A lack of specificity is indicated and hence the analytical monitoring procedure has not been satisfactorily validated. Therefore the nominal (loading rates) will be considered. All validity criteria for the fish test were satisfied. There were no signs of toxicity or mortality observed in any of the fish over the course of the test.

CONCLUSION The notified polymer is not harmful to fish up to the limit of its solubility in water.

TEST FACILITY Stockhausen GmbH (2006a)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE Notified polymer

METHOD OECD TG 202 Daphnia sp. Acute Immobilisation Test – Static test

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None reported
Water Hardness	70 – 90 mg $CaCO_3$/L
Analytical Monitoring	^{1}H NMR
Remarks - Method	A Water Accommodated Fraction (WAF) was prepared by adding notified polymer to water to make up a concentration of 100 mg polymer/L and stirring for 24 hours by magnetic stirrer at room temperature. The solution was allowed to stand for 2 hours to let undissolved polymer settle out. For each test run, approximately 30 mL of the WAF was removed by pipette from approximately 3 cm below the surface of the liquid and transferred into a 50 mL beaker, into which 5 daphnia were added.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	0	5	0	0
100 (WAF)	< 5	25	0	0

EL50	> 100 mg/L (WAF) at 48 hours (based on loading rate)
NOEL	> 100 mg/L (WAF) at 48 hours (based on loading rate)
Remarks – Results	Regarding the measured concentrations (NMR), the study author reported there was a "different signal form" between the integrated signal (4.0 – 4.2 ppm) in the pure test substance and the integrated signals (4.0 – 4.2 ppm) in all the samples. A lack of specificity is indicated and hence the analytical monitoring procedure has not been satisfactorily validated. Therefore the nominal (loading rates) will be considered. The toxic response of daphnia to the reference compound $K_2Cr_2O_7$ gave an EC50 (24 h) of 1.3 mg/L which was considered adequate. All validity criteria of the test were satisfied. No effects on the swimming ability of exposed daphnia were observed over the course of the test.
CONCLUSION	The notified polymer is not harmful to aquatic invertebrates up to the limit of solubility in water
TEST FACILITY	Stockhausen GmbH (2006b)

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