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January 2002

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

NT-18

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Director
Chemicals Notification and Assessment

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1. APPLICANT

HP Australia Limited of 31-41 Joseph Street, BLACKBURN VIC 3130 (ABN 74 004 394 763) has submitted a standard notification statement in support of their application for an assessment certificate for NT-18.

2. IDENTITY OF THE CHEMICAL

The chemical name, CAS number, molecular and structural formulae, molecular weight, spectral data, details of the chemical composition and details of exact import volume and customers have been exempted from publication in the Full Public Report and the Summary Report.

3. PHYSICAL AND CHEMICAL PROPERTIES

The following physicochemical data (Huntington, 2001a) refer to the notified chemical.

Appearance at 20°C & 101.3 kPa:	Black powder
Boiling Point: **OECD TG 103**	Decomposes without melting or boiling above 205ºC (See comments below)
Melting Point: **OECD TG102**	>360 ºC
Relative Density: **OECD TG 109**	1.25 at 20ºC
Particle Size: **OECD TG 110**	Size range: 0.876 to 48.6 μm: 1.3% by volume <2 μm 98.7% by volume between 2 and 48.6 μm 24% by weight <10 μm Mass median aerodynamic diameter was 8.94 μm
Vapour Pressure: **OECD TG104**	2.7×10^{-5} Pa at 25°C (See comments below)
Water Solubility:	1.31×10^{-4} g/L at 20ºC

OECD TG 105	(See comments below)
Partition Co-efficient (n-octanol/water): OECD TG 117	log Pow = 5.1 at 20°C (See comments below)
Hydrolysis as a Function of pH:	Not determined (see comments below)
Adsorption/Desorption: Draft OECD TG121	Koc <60 at 20°C
Dissociation Constant: OECD TG112	pK_a = 4.2 at 21°C (see comments below)
Flash Point:	Not applicable for solids
Flammability: EEC Method A.10	Not highly flammable
Auto-ignition Temperature: EEC Method A.16	Does not self ignite
Explosive Properties: EEC method A.14	Not explosive
Reactivity/Stability: EEC Method A.17	Not oxidising

3.1 Comments on Physico-Chemical Properties

Differential scanning calorimetry (20-400°C) exhibited a large exotherm beginning at 205°C which, corresponded to compound decomposition. A small endotherm at 140°C was attributed to volatilisation of residual solvent (water and 2-propanol), which are present in the commercial product.

The vapour pressure was determined using a vapour pressure balance. The measured value (mean of three separate determinations) of 2.7 x 10^{-5} Pa at 25°C is small, but is nevertheless appreciable for an ionic salt of relatively high MW. It is possible that the measured vapour pressure was due to residual 2-propanol and water, both of which are present in small quantities in the commercial product (see below).

The water solubility was determined by the flask method by stirring an excess of the compound with water at 30°C for one, two and three days (duplicate samples for each stirring regime), filtering off undissolved material (0.45 µm filters), and allowing to equilibrate at 20°C for 24 hours prior to analysis by High Performance Liquid Chromatography (HPLC). The saturated solubility was determined as 0.13 mg/L and, since there were no significant differences between the concentration of test material in the solutions prepared through

stirring for one, two or three days, saturation was reached within one day.

Determination of the rate of hydrolysis as a function of pH was attempted for the notified chemical (Huntingdon, 2001b). However, definitive experiments were not performed due to the low water solubility, which precluded accurate solution analysis and tracking of degradation rates due to interfering peaks in the chromatograms, including the solvent blanks. However, hydrolysis of the phenol-iron bonds in the centre of the complex anion is likely under modest pH conditions (pH >4) and this would then lead to formation of polynuclear ferric hydroxide complexes and eventual precipitation of iron hydroxide. While the resultant free organic ligands contain azo and phenolic groups, these are not expected to be susceptible to hydrolysis under the usual environmental conditions where 4< pH <9.

The n-octanol/water partition coefficient was determined using the HPLC method where the retention time of the test compound eluted with a particular solvent on a C8-18 column (highly hydrophobic) is compared with those of a series of standard compounds, with known values for Log Kow, when eluted with the same solvent. In the present case the elution solvent used was tetrahydrofuran (THF)/ammonium acetate buffer (55/54 vol/vol) at pH 5.8, while 6 reference compounds were used with Log Kow ranging between 1.7 (acetophenone) and 6.2 (DDT). The retention time of the notified chemical on the column corresponded to a Log Kow of 5.1, which indicates that the chemical has a high affinity for the oil phase. This is a high value for an ionic compound but is in accord with the significant hydrocarbon content of the complex anion, which will have compatibility with the C8-18 component of the static column phase. It is also likely that the high affinity for the hydrophobic environment would also be enhanced by the formation of neutral ion pair aggregates between the organo-metallic anion and dissolved Na^+ or NH_4^+ ions in the vicinity of the solution-hydrophobic surface interface.

The soil adsorption coefficient Koc was also determined using a method based on the retention time of the compound on an HPLC column. However, the stationary phase of the column used (a cyano column) in this experiment has both hydrophobic and polar components and in this respect is intended to simulate the chemical characteristics of many soils. Since the retention time of the test chemical when eluted with THF/0.02 M ammonium acetate (50/50 v/v) was less than that of the reference monolinuron (Koc =60) when eluted with the same mobile phase, the value of Log Koc for the notified chemical was determined as < 60 indicating that the notified chemical has low affinity for soil, and is also expected to have high mobility in soil (McCall *et al*, 1980).

It is often considered that compounds with high values for Log Kow will also have appreciable soil adsorption coefficients (ie. Koc and Log Koc), and several linear mathematical correlations reflecting this general correlation have been published (European Union, 1996 and Lyman *et al*, 1990). These correlations are based on the affinity of organic compounds for a hydrocarbon environment (reflected by Log Kow) and the affinity of the compound for the organic (humic) component of soils and sediments (Koc), which is often assumed to behave like hydrocarbons. However, while these correlations are generally reasonably sound for non ionic compounds composed primarily of hydrocarbon and organic groups with low polarity, when a substantially organic compound is also ionic (or contains highly polar functional groups), this type of correlation breaks down due to the failure to account for factors such as electrostatic interactions (electric double layer effects) between the ionised phenolic and carboxylic acid residues in humic material and the organic ions, and possible interactions between the organic ions and the ionic charges on the surfaces of

minerals in the soil (eg clay, silica and mica minerals).

In the present case the large organo-metallic anion is negatively charged, and is consequently expected to be electrically incompatible with humic material, which also carries negative charges by virtue of the high concentration of ionised carboxylate residues. Consequently, despite the apparently high value for Log Kow a low value for Koc is in accord with the ionic character of the notified chemical.

The dissociation constant was determined using UV/visible spectroscopy after addition of hydrochloric acid to a solution of the new compound in methanol (approx. 25 mg/L). The pKa of 4.2 at 21°C would correspond to that of the azo substituted para chloro phenol moiety of the chelating ligands (see notes on hydrolysis above).

4. PURITY OF THE CHEMICAL

Purity: 97.03%

Hazardous Impurities:

Chemical name:	2-Propanol
CAS No.:	67-63-0
Weight percentage:	1.05%
Toxic properties:	Classified as R11 (Highly flammable) and R36 (Irritating to eyes) in accordance with the NOHSC Approved Criteria. It is also classified as R67 (Vapours may cause drowsiness and dizziness) in accordance with 67/548/EEC

Non-hazardous Impurities (> 1% by weight):

Chemical name:	Water
Weight percentage:	1.2%
CAS No.:	7732-18-5

Additives/Adjuvants: None

5. USE, VOLUME AND FORMULATION

The notified chemical is intended to be used as an ingredient of a toner for electrophoto-copying machine or electrophoto-graphic printer and will be contained in toner at less than 1%. The new toner will be imported as a customer product into Australia where no reformulation or repackaging will take place. Future manufacturing of the notified chemical or the toner in Australia is not anticipated.

The toner will be sealed up in a plastic bottle (containing 500-2000 g) or cartridge (containing 300-1500 g) outside Australia. A maximum of 10 tonnes per year of notified chemical will be imported over the first 5 years.

The toner bottle or cartridge is designed so that no release of the toner occurs until the shutter or the seal tape is removed. The toner containing the notified chemical consists of binder resin(s) (45-55%), iron oxide (40-50%) and other ingredients. It is formulated as a powder with average particle size from 5μm to 10 μm and distribution from 1 μm to 30 μm.

During copying or printing operation, the toner will be transferred onto the paper and firmly fixed by heat.

6. OCCUPATIONAL EXPOSURE

Waterside, warehouse and transport workers are unlikely to be exposed to the notified chemical unless the packaging is breached.

The toner is mainly used in the offices for copying or printing. Airborne generated dust, including dust toner, around the printer may occur.

Inhalation, ocular or dermal exposure to the toner may occur during toner replacement, particularly in the event of a container leak or spill. More commonly, occasional dermal exposure to toner residues inside the machine may occur during machine servicing or paper feed problems.

Office workers and machine maintenance workers may be intermittently exposed to the notified chemical contained in the plastic toner bottle or cartridge when replacing the spent container, and during repair maintenance and cleaning of printers or photocopiers. Maintenance workers for printers or photocopiers may potentially come in contact with the notified chemical more often than office workers. Exposure is expected to be controlled through the design of the cartridges or bottles and the printing or photocopying machines. Printer or photocopier maintenance personnel often wear cotton or disposable gloves. Pre-packed cartridges or bottles are sealed and worker exposure to the toner is minimised by the use of the replacement procedures recommended by the manufacturer.

Service personnel are trained for handling toner, and maintain a service manual.

Contact with paper printed with toner containing the notified chemical is unlikely to result in dermal exposure, as it will be bound in the structure of the paper.

7. PUBLIC EXPOSURE

The formulated toner containing NT-18 (<1%) will be imported, distributed and supplied in Australia for consumers in sealed particular plastic bottles or cartridges. In the event of an accidental spill, the material will not be cleaned up using a vacuum cleaner as fine powder can form explosive dust-air mixtures. The spilled powder will be lightly sprayed with water to prevent formation of dust, then swept up and carefully transferred to a waster container for disposal. Disposal is subject to federal, state or local laws. In case of large spills, all sources of ignition including sparks and static electricity are to be eliminated. Public exposure during transport is unlikely.

NT-18 is used as an ingredient of toner for electro-photocopying machine or printer. The toner bottle or cartridge is tightly sealed and installed inside of an electro-photocopying machine or a printer machine to supply toner. The potential for public exposure to the notified chemical is expected to be very low when replacing the toner bottle or cartridge. During copying or printing operation, the toner will be transferred onto the paper and firmly fixed by heat. The potential for public exposure thereafter is negligible. The empty cartridges remain closed and are either recycled or reused or sent to landfill in accordance with relevant local regulations.

8. ENVIRONMENTAL EXPOSURE

8.1 Release

There are two principal pathways of release to the notified chemical into the environment- as a residual unused toner and through disposal of the waste paper.

The residual unused toner (ie that remaining in the spent cartridges after most of the material has been used) will be disposed of as office waste and will be either incinerated or disposed of to landfill. During normal use, this should be fairly small, since it is expected that a maximum of 225 g of toner product (ie 15 %) would remain in the spent cartridges, and 100 g (5%) in bottle. However, since the notified chemical constitutes only 1% of the imported toner product, it is estimated that the maximum release of the notified chemical from each spent cartridge would be 2.25 grams, and 1.00 grams from each empty toner bottle. Based on a maximum annual import of 10 tonnes of the notified chemical, and assuming a maximum release of 15%, this equates to a potential maximum release of 1.5 tonnes each year. Most of the emptied toner bottles and cartridges will be disposed of into landfill, but release of the residual toner should occur only after destruction of the integrity of the cartridge. Since the toner would be used throughout Australia, the release will be diffuse and at relatively low levels.

In normal use, the product will be incorporated into a thermo-cured resin (ie the print) and firmly bound to the paper substrate, and hence, would be released to the environment through disposal of the waste paper. The anticipated fate of the material would be associated with that of the paper, and is described below.

8.2 Fate

The notified chemical is not readily biodegradable (Huntingdon, 2001c) and was degraded to a maximum of 11% over 28 days when subjected to a closed bottle test (OECD TG 301D), which involved monitoring the concentration of dissolved oxygen when the chemical (at a nominal concentration of 3 mg/L) was incubated with sewage bacteria. In contrast with this result, a reference compound (sodium benzoate) was 86% degraded after 28 days, which demonstrated the viability of the bacterial culture used in this test. Accordingly, the notified chemical cannot be classed as readily biodegradable, although it is likely that it would slowly degrade after a prolonged period in a landfill.

The majority of the notified chemical will be associated with the print and bound strongly to paper. Waste paper disposal is effected either through high temperature incineration, recycling or deposition into landfill.

High temperature incineration would destroy the chemical with evolution of water and oxides of carbon and nitrogen, while sodium and iron in the chemical would become associated with ash. Similarly, it is expected that during the extensive repulping and bleaching procedures implied by paper recycling, the material would be destroyed chemically through initial hydrolysis of the anion and probably by subsequent oxidation and degradation of the organic ligands. The resultant degradation products would most likely become incorporated into waste sludge. However, given that the compound has a relatively low value for Koc (<60), despite the low water solubility (0.13 mg/L), most of the un-degraded chemical is expected to remain in the aqueous waste. This waste stream would be comprehensively treated prior to discharge, but given that the rate of aerobic biodegradation is slow, it is unlikely that any of the compound remaining dissolved in the water would be degraded during this process, and so this would be released from the pulp mills to receiving waters.

Some waste paper may be disposed of directly to landfill, and although not readily biodegradable, it is anticipated that prolonged residence in an active landfill environment would eventually degrade the notified chemical producing the usual landfill gases while the contained iron and sodium would become associated with soil.

Aqueous leachate from a landfill could conceivably contain low concentrations of non-degraded compound, which would be received into the wider environmental water compartment. Although the chemical is moderately toxic to algae (see Environmental Effects), it would be released at very low concentration, and is not expected to have a large detrimental effect on the environment. The same considerations will apply to effluent discharged (after treatment) from paper recycling facilities.

Although the chemical has a relatively high value for Log Kow (5.3), it also has a modest water solubility (0.13 mg/L) and this, together with the molecular weight (around 900 g/mol) indicates little potential for bioaccumulation (Connell, 1990).

9. EVALUATION OF TOXICOLOGICAL DATA

Toxicological data were collected on the notified chemical using the test chemical ST439 (same as NT-18).

9.1 Summary of Toxicological Investigations

Endpoint & Result	*Assessment Conclusion*
Rat, acute oral LD_{50}= >2000 mg/kg bw	Low toxicity
Rat, acute dermal LD_{50}=> 2000 mg/kg bw	Low toxicity
Rabbit, skin irritation	Non-irritating
Rabbit, eye irritation	Slightly irritating
Guinea pig, skin sensitisation - adjuvant test	No evidence of sensitisation
Rat, oral repeat dose Toxicity – 28 Days.	NOEL[1] 15 mg/kg bw/day NOAEL[2] 1000 mg/kg bw/day
Genotoxicity - bacterial reverse mutation	Non mutagenic
Genotoxicity – *in vitro* chromosomal aberration	Non genotoxic

9.2 Acute Toxicity

9.2.1 Acute Oral Toxicity

TEST SUBSTANCE	Notified Chemical
METHOD	OECD 423 Acute Oral Toxicity – Acute toxic class method
Species/Strain	Rat/ Sprague Dawley
Vehicle	Corn oil
Remarks - Method	

[1] NOEL; No observed effect level
[2] NOAEL; No observed adverse effect level

RESULTS

Group	*Number & Sex Of Animals*	*Dose mg/kg bw*	*Mortality*
1	3 females	2000	None
2	3 males	2000	None

LD50	>2000 mg/kg bw
Signs of Toxicity	None
Effects in Organs	There were no gross pathological changes observed.
Remarks - Results	There were no deaths, no clinical signs of reaction to treatment and no abnormalities revealed at macroscopic examination
CONCLUSION	The notified chemical is of low toxicity via the oral route.
FACILITY (REFERENCE)	Huntingdon Life Sciences Ltd (Huntingdon, 2000a)

9.2.2 Acute Dermal Toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD 402 Acute Dermal Toxicity.
Species/Strain	Rat/ Sprague-Dawley
Vehicle	Corn oil
Type of dressing	Occlusive
Remarks – Method	Observation period was for 15 days; 24 hour exposure; test material formulated at 55.6% w/v in corn oil was administered at 3.6 mL/kg bw.
RESULTS	There were no deaths and no systemic response to treatment following a single dermal application to a group of 10 rats at a dose level of 2000 mg/kg bw.

Group	*Number & Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 males, 5 females	2000	None
2	5 females	2000	None

LD50	>2000 mg/kg bw
Signs of Toxicity	

- Local	Very slight mild irritation (Grade 1 erythema) was seen in seven animals (4 males and 3 females) following removal of the dressing, resolving completely by day 13. A residual black staining from the test substance was noted for all animals and localised spots and/or scabbing in 3 males and one female during the study
- Systemic	Macroscopic examination revealed scabbing on the dose site of one female.
Effects in Organs	None
Remarks – Results	
CONCLUSION	The notified chemical is of low acute dermal toxicity via the dermal route.
FACILITY (REFERENCE)	Huntingdon Life Sciences Ltd (Huntingdon, 2000b)

9.2.3 Acute Inhalation Toxicity

Not submitted

9.2.4 Skin Irritation

TEST SUBSTANCE	Notified Chemical
METHOD	OECD 404 Acute Dermal Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Observation Period	4 days
Vehicle	
Type of Dressing	Semi-occlusive
Remarks – Method	
RESULTS	Application was for 4 hours to intact skin
Remarks – Results	All draize scores were equivalent to zero. No dermal irritation was observed following a single occlusive application of the notified chemical to intact skin for 4 hours.
CONCLUSION	The notified chemical is non-irritating to skin.
FACILITY (REFERENCE)	Huntingdon Life Sciences Ltd (Huntingdon, 2000c)

9.2.5 Eye Irritation

TEST SUBSTANCE	Notified chemical
METHOD	OECD 405 Acute Eye Irritation/Corrosion EPA Health Effects Test Guidelines OPPTS 870.2400
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Observation Period	3 days
Remarks - Method	Single instillation to the rabbit eye

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	1	72 hours	0
Conjunctiva: chemosis	0	0	0			0
Conjunctiva: discharge						
Corneal opacity	0	0	0			0
Iridial inflammation	0	0	0			0

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

Remarks - Results	Transient, hyperaemia of the blood vessels of the conjunctivae was seen in all animals, resolving completely by two days after instillation
CONCLUSION	The notified chemical is slightly irritating to the eyes
FACILITY (REFERENCE)	Huntingdon Life Sciences Ltd (Huntingdon, 2000d)

9.2.6 Skin Sensitisation

TEST SUBSTANCE	Notified chemical
METHOD	OECD 406 Skin Sensitisation – Magnusson and Kligman Maximisation Study
Species/Strain	Guinea pig/Dunkin Harley strain
PRELIMINARY STUDY	Maximum non-irritating concentration: intradermal: 0.1-10% w/v

topical: 10-60% w/v

MAIN STUDY

Number of Animals: Test Group: 10 Control Group: 5

INDUCTION PHASE: Induction Concentration
intradermal: 0.5% w/v
topical: 60% w/v

Signs of Irritation: Slight irritation was seen in test animals at sites receiving intradermal injections of the notified chemical at 0.5% w/v. Assessment of erythema was obscured due to black staining over the dose site in test animals following a topical application with the notified chemical 60% w/v.

CHALLENGE PHASE

1st challenge: topical application: 60% w/v
topical application: 30% w/v

Remarks - Method: For preliminary study, 60% w/v of the test material was the maximum practical concentration that could be prepared and dosed topically, which did not give rise to irritating effects.

RESULTS: No signs of toxicity or ill health was observed in treated animals.

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*			
		1st challenge		*2nd challenge*	
		24 h	*48 h*	*24 h*	*48 h*
Test Group	60%	0/10	0/10	-	-
	30%	0/10	0/10	-	-
Control Group	60%	0/5	0/5	-	-
	30%	0/5	0/5	-	-

Remarks - Results: There was no evidence of reactions indicative of skin sensitisation to the notified chemical.

CONCLUSION: The notified chemical was not a skin sensitiser under the conditions of the test.

FACILITY (REFERENCE): Huntingdon Life Sciences Ltd (Huntingdon, 2001d)

9.3 28-Day Repeat Dose Oral Toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD 407 Repeated Dose 28-Day Oral Toxicity Study in Rodents
Species/Strain	Rat/Sprague-Dawley
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days; Dose regimen: 7 days per week; Post-exposure observation period: none (animals were sacrificed on the 29th day)
Vehicle	Corn oil
Remarks - Method	Functional observational battery and motor activity were performed at regular intervals during the study

Group	*Number & Sex Of Animals*	*Dose mg/kg bw/day*	*Mortality*
Control	5 males, 5 females	0	0
Low dose	5 males, 5 females	15	0
Intermediate dose	5 males, 5 females	150	0
High dose	5 males, 5 females	1000	0

Clinical Observations

There were no treatment –related effects on bodyweight and food consumption. However, food consumption among the female treated groups was slightly superior to that of control during the 4-week treatment period. Dark/black staining of the tail and black pelleted faeces were noted mainly among the high dose level animals. These findings were due to the black colour of the test substance and were not of toxicological significance.

The functional observational battery data for animals treated with the notified chemical at dosages up to 1000 mg/kg/day, did not reveal behavioural changes that were indicative of neurotoxicity.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

There were no treatment related effects on haematological parameters and blood chemistry investigations. The small differences seen in treated males and females were not dose-related and were of no toxicological significance.

Effects on Organs

No treatment related effects were observed on organ weights. A slight decrease in the spleen weights of females receiving 1000 mg/kg/day was noted when compared with control, after adjustment for terminal body weight. However, there were no microscopic changes noted in the spleen.

Remarks –

Dark/black tails and black-pelleted faeces were noted among high dose level animals (1000 mg/kg/day). The macroscopic examination revealed brown tail staining among high dose level animals, a slight pink discoloration of the forestomach among treated animals and a dark discoloration of the gastro-intestinal tract among rats receiving 150 or 1000 mg/kg/day.

In the absence of any observed microscopic changes in these tissues or any other treatment-related changes, these findings were considered to be of no toxicological significance.

CONCLUSION

The NOEL established in this study was 15 mg/kg bw/day based on macroscopic examination, which revealed a discoloration of the gastro-intestinal tract at the next higher dose tested (150 mg/kg bw/day).

The NOAEL established in this study was 1000 mg/kg bw/day (highest dose tested with no treatment-related effects seen).

FACILITY (REFERENCE)	Huntingdon Life Sciences Ltd (Huntingdon, 2000e)

9.4 Genotoxicity

9.4.1 Genotoxicity-Bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD 471 Bacterial Reverse Mutation Test. US EPA OPPTS 870.5100 Bacterial Reverse Mutation Test
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100. *E. coli*: WP2 (pKM101).
Metabolic Activation System	S9 fraction from Aroclor-induced rat liver microsomes
Experimental Design	Preliminary test: Plate incorporation assay Main test: Pre-incubation procedure

Concentration Range in Main Test	a) With metabolic activation: 5, 15, 50, 150, 500, 1500, 5000 µg/plate. b) Without metabolic activation: 50, 150, 500, 1500, 5000 µg/plate.
Vehicle	Dimethyl sulphoxide
Remarks - Method	Three replicates were used for each concentration. Positive controls used were: benzo[a]pyrene and nitrofluorene (*S. typhimurium)* 2-Aminoanthracene and 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Present	No cytotoxicity	Not detected	Not observed	Negative
Absent				

Remarks - Results	No substantial increases in revertant colony numbers over control counts were obtained with any of the tester strains following exposure to the notified chemical at any concentration in either the presence or absence of S9 mix. No visible thinning of the background lawn of non-revertant cells was obtained following exposure to the notified chemical.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
FACILITY (REFERENCE)	Huntingdon Life Sciences Ltd (Huntingdon, 2001e)

9.4.2 Genotoxicity-In Vitro

TEST SUBSTANCE	Notified chemical
METHOD	OECD 473 In vitro Mammalian Chromosomal Aberration Test. US EPA OPPTS 870.5375 *In Vitro* Mammalian Chromosome Aberration Test
Cell Type/Cell Line	Human lymphocytes
Metabolic Activation	Aroclor-induced rat liver microsomes, S9 fraction

System

Vehicle: Dimethyl sulphoxide

Remarks – Method: Positive controls used were mitomycin C and cyclophosphamide.

Duplicate cultures were used for each treatment.

The total number of cells containing aberrations both with and without gaps was calculated.

On dosing at 2% v/v into aqueous tissue culture medium, giving a final concentration of approximately 4940 μg/mL a precipitate was observed. On dosing at 1% v/v into culture medium a precipitate was also observed at concentrations of 312.5 μg/mL and above.

Duplicate cultures were used for each treatment and two cultures were treated with the solvent control.

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Present			
Test 1	2.44, 4.88, 9.77, 19.53, 39.06, 78.13*, 156.25* and 312.5* μg/mL	3 hours	21 hours
Test 2	9.77, 19.53, 39.06, 78.13*, 156.25* and 312.5* μg/mL	3 hours	21 hours
Absent			
Test 1	2.44, 4.88, 9.77, 19.53, 39.06, 78.13*, 156.25* and 312.5* μg/mL	3 hours	21 hours
Test 2	2.44, 4.88, 9.77, 19.53*, 39.06*, 78.13* and 312.5 μg/mL	21 hours continuous treatment	21 hours

* doses used for metaphase analyses

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*		
	Cytotoxicity in Main test	*Precipitation*	*Genotoxic Effect*
Present			
Test 1	MI (mitotic index) = 51% reduction at 312.5 μg/mL	Not observed	Negative
Test 2	MI = 40% reduction at 312.5 μg/mL	Not observed	Negative
Absent			

Test 1	No MI reduction at and below 312.5μg/mL	Not observed	Negative
Test 2	MI= 68% at 312.5 μg/mL	Strong precipitate at 156.25 and 312.5 μg/mL	Negative

Remarks – Results	Due to the presence of strong precipitates in treated cultures (Test 2: 21-hour treatment without S9) at 156.25 and 312.5 μg/mL, metaphase analysis was not scored. Quantitative analysis of polyploidy showed no increase in the number of polyploid metaphase cells when compared to the solvent control. In both the presence and absence of S9 mix, there were no statistically significant increase in the proportion of metaphase figures containing chromosomal aberrations, at any dose level, when compared with the solvent control in either test.
CONCLUSION	The notified chemical was not clastogenic to human lymphocytes treated in vitro under the conditions of the test.
FACILITY (REFERENCE)	Huntingdon Life Sciences Ltd (Huntingdon, 2001f)

9.5 Overall Assessment of Toxicological Data

The notified chemical was of low acute oral and dermal toxicity in rats with an LD_{50}>2000 mg/kg bw/day. A skin irritation test in rabbits showed that the chemical was not irritating. An eye irritation test also in rabbits showed transient conjunctival irritation seen in all animals, resolving completely by two days after instillation. Negative responses were observed in a skin sensitisation study in guinea pigs.

The 28-day repeat dose oral study in rats revealed dark/black staining of the tail and black-pelleted faeces mainly among the high dose level animals. These findings were due to the black colour of the test substance. The macroscopic examination revealed brown tail staining among high dose level animals, a slight pink discoloration of the forestomach among treated animals and a dark discoloration of the gastro-intestinal tract among rats receiving 150 or 1000 mg/kg/day. In the absence of any observed microscopic changes in these tissues or any other treatment-related changes, these findings were considered to be of no toxicological relevance. The NOEL established in this study was 15 mg/kg bw/day and the NOAEL established in this study was 1000 mg/kg bw/day

The notified chemical was non-mutagenic and non-clastogenic in an *in vitro* bacterial reverse mutation assay and chromosome aberration assay, respectively.

The notified chemical would not be classified as a hazardous substance according to *Approved Criteria for Classifying Hazardous Substances* (National Occupational Health and Safety Commission (NOHSC), 1999) in terms of the toxicological data provided.

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

Test	Species	Results (measured)
Acute toxicity to Fish OECD TG 203 (Huntingdon, 2001g)	*Oncorhyncus mykiss* Rainbow trout	96 h LC50 > 9.2 mg/L 96 h NOEC = 9.2 mg/L
Acute toxicity (immobilisation) OECD TG 202 (Huntingdon, 2001h)	*Daphnia magna*	48 h EC50 > 7.4 mg/L 48 h NOEC = 7.4 mg/L
Chronic (Reproduction) Toxicity OECD TG 211 (Huntingdon, 2001i)	*Daphnia magna*	21 d EC50 > 7.4 mg/L 21 d LOEC = 7.4 mg/L 21 d NOEC 2.5 mg/L
Algal Growth Inhibition OECD TG 201 (Huntingdon, 2001j)	*Selenastrum capricornutum*	72 h E_bC50 = 4.6 mg/L 0-72 h E_rC50 > 12 mg/L 72 h NOEC = 0.42 mg/L
Bacterial Respiration Inhibition OECD TG 209) (Huntingdon, 2000f)	Sewage bacteria	Not inhibitory 3 h NOEC = 100 mg/L (suspension)

NOEC - no observable effect concentration
LOEC – lowest observed effect concentration

The ecotoxicity data were included in the submission. These data were generated according to accepted OECD test protocols.

The fish acute toxicity and *Daphnia* immobilisation tests were conducted using static methodologies with a single test concentration of the notified chemical made up as saturated solutions prepared with the aid of a DMSO + 10% Tween 80 co-solvent. In the fish test, the concentration was measured as 9.2 mg/L, while it was determined as 7.4 mg/L in the *Daphnia* test. Ten juvenile rainbow trout were used in the fish test, which was conducted over a 96-hour period, and since no mortality or sub-lethal effects were observed over the test duration, the notified chemical is classified as being non-toxic to this species of fish up to the limit of its water solubility. Similarly in the *Daphnia* immobilisation test, twenty four *Daphnia* were used (4 replicate tests, each using 6 animals), and no statistically significant immobilisation was observed over the 48-hour test period. The notified chemical is classified as being non toxic to *Daphnia* up to the limit of its water solubility.

The notifier submitted a study on the effect on *Daphnia* reproduction of prolonged exposure (21 days) to solutions containing the notified chemical, which was conducted under semi-static conditions (test media renewed 3 times each week). The test media contained

(measured) concentrations of the new compound of 0.26, 0.80, 2.5 and 7.4 mg/L, and were also prepared with the aid of a co-solvent (DMSO + 10% Tween 80) with 7.4 mg/L solution corresponding to saturation. Appropriate solvent controls were used at each concentration using 20 replicates (one *Daphnia*) for each solvent control and 10 replicates (again one *Daphnia*) for each exposure concentration. The number of *Daphnia* progeny and general condition of the animals was monitored over the 21-day test period, with the reproductive output for each exposure compared with that of the corresponding solvent controls. The results were analysed using a standard statistical package (SAS) to provide the data tabulated above. There was a slight reduction in the number of *Daphnia* young produced per adult for exposure at 2.5 mg/L (ie. 5.2 % after 14 days and 9 % after 21 days) and a more significant effect at 7.4 mg/L (ie. 32.3 % after 14 days and 20.1 % after 21 days). According to Mensink *et al* (1995), the notified chemical is classified as being very slightly toxic to this species under conditions of chronic exposure.

The algal growth inhibition test was conducted using measured concentrations of the test substance between 0.42 and 12 mg/L with a solvent control. The test compound was added to the media in a mixed DMSO/Tween 80 solvent. The rate of growth of the algal biomass was monitored over the 72-hour test period and some inhibition of algal growth was observed at all test concentrations. The growth data were analysed using standard statistical techniques to provide the EC50 data in the table above. According to the scale of Mensink *et al* (1995), the notified chemical is classified as being moderately toxic to this species of green algae.

The tests on bacterial respiration inhibition were conducted with suspensions of sewage bacteria in synthetic sewage containing nominal quantities of the test material between 1 and 100 mg/L. Measurements of oxygen respiration rate were begun after 30 minutes of constant aeration; for all nominal levels of the test substance, the rate of O_2 consumption was no different from that of the control (no test compound), which indicates that the notified chemical does not inhibit bacterial respiration at levels of 100 mg/L and less. In contrast to this result, the EC_{50} of the reference substance (3,5-dichlorophenol) was determined as 7.8 mg/L, which established the viability of the sewage bacteria culture used in this test.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

Although the notified chemical is moderately toxic to green algae, it does not exhibit acute toxicity to either fish or *Daphnia* up to the limit of its water solubility, but is slightly toxic to *Daphnia* under conditions of chronic exposure. However, the chemical is not expected to be released to the water compartment except at very low concentrations and in a very diffuse manner. Consequently, use of the chemical as indicated as a component of toners for printers and photocopying is not likely to constitute a hazard to the aquatic compartment.

In the event of accidental spillage or release of the toner, the clean up operation would probably entail disposal to landfill. The long term fate of the majority of the notified chemical is expected to be either through paper recycling, landfill disposal or incineration of waste paper. In the first two cases it is anticipated that the chemical would be destroyed either by a vigorous chemical environment (paper recycling) or through slow biological and abiotic processes. Even in the absence of substantial degradation, the relatively low usage rate and diffuse nature of disposal patterns indicate very slow release into the wider environment, and this at low concentrations. Incineration of the compound would lead to its total destruction with production of water and oxides of carbon and nitrogen while the

sodium and iron components would report to ash.

However, the notified chemical is not expected to have affinity for soil and, if released to soil or sediments, will be mobile in these media and is expected to be slowly leached into the water compartment. The notified chemical is not expected to have potential for bioaccumulation.

The notified chemical is not considered to be a hazard to the environment when used as a component of printer and photocopying toner as indicated.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

Hazard Assessment

Based on the toxicological data provided, the notified chemical would not be acutely toxic via the oral and dermal route. It is not likely to be a skin sensitiser or genotoxic. It is not likely to be a skin irritant but could be a slight eye irritant. The NOAEL established in a 28-day repeat dose oral study in rats was 1000 mg/kg bw/day (highest dose tested with no treatment-related effects).

The notified chemical is not classified as a hazardous substance according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999).

Data on the particle size of the notified chemical show that a large proportion of the particles are within the respirable range (24% by weight<10 μm). Also, the toner is formulated as a powder with average particle size from 5 μm to 10 μm and distribution from 1 μm to 30 μm. The toner is considered a nuisance dust and employers are responsible for maintaining atmospheric levels of toner dust below the NOHSC exposure standard of 10 TWA[3] mg/m^3 (NOHSC, 1995). Australia does not have a national exposure standard for respirable dust, however, the American Conference of Governmental Industrial Hygienists (ACGIH) TLV[4] is 3 TWA mg/m^3 (ACGIH, 2001).

The material safety data sheet (MSDS) for the toner states that it would not be classified as a hazardous substance according to NOHSC Approved Criteria for Classifying Hazardous Substances (NOHSC, 1999).

Occupational Health and Safety

Exposure to toner containing the notified chemical can occur during machine operation, during clearing paper feed problems and machine maintenance. Transport and storage of the toner bottles and cartridges is unlikely to result in worker exposure except in the event of accidental spillage.

From the repeat dose toxicity studies, the NOAEL of 1000 mg/kg bw/day (higher dose tested

[3] TWA; Time Weighted Average
[4] TLV; Threshold Limit Value

with no treatment related effects) was established for NT-18. Assuming 100% dermal absorption, a 70 kg worker would need to be dermally exposed to 70 g NT-18/day or >7 kg toner/day to reach the NOAEL. This is unlikely given the routine conditions of use. The calculations described do not include a safety factor.

The main route of exposure is dermal. While ocular exposure to toner dust may occur, the risk of eye irritation in workers involved in transport, storage, use and disposal of the notified chemical in this application is low.

Printing staff performing additions of toner and replacement of a used toner container (cartridge or bottle) are expected to be exposed infrequently to the notified chemical as the toner container is sealed and loaded directly into a printing machine. Upon application to the paper, the toner is fused to the surface and release is unlikely to occur. Therefore, the risk of adverse health effects to printing personnel is low and no personal protective equipment is required. Nevertheless, due to the small toner particle size, any generation of dust should be avoided.

Service personnel may be exposed to the notified chemical when cleaning printer/copier equipment and replacing copier developer. However, as the notified chemical is not likely to be hazardous and given its low concentration in the toner (<1%), the risk of adverse health effects is low. Cotton or disposable gloves may be worn to prevent skin irritation due to frequent exposure to toners, and workers should avoid any generation of dust when handling the toner.

Spilt residues should be swept up manually or using a dust explosion-proof vacuum cleaner and placed within a waste container.

Workers handling printed paper are not at risk of adverse health effects because the polymer is fixed to the paper and not available for exposure or dermal uptake.

Given these considerations, the chemical will not pose a significant health hazard in the occupational environment.

In the event that the notified chemical will be handled as a raw ingredient at high concentrations, workers should be protected from skin contamination because it has staining properties.

Public Health

There is potential for public exposure to the notified chemical from spillage of the toner during exchange of cartridges in printers and photocopiers. However, given the low toxicity profile of NT-18 and the very low proportion of NT-18 in the toner, the potential for dermal and inhalational exposure of the public to the notified chemical during use of the toner cartridges is considered to be very low.

13. RECOMMENDATIONS

To minimise occupational exposure to NT-18 the following guidelines and precautions should be observed:

- In case manufacturing of the notified chemical or toner is performed in Australia, protective eyewear, clothing and gloves should be worn when handling the notified chemical;
- Generation of dust clouds when handling the toner should be avoided;
- Service operators should wear cotton or disposable gloves when handling the toner (ie when removing spent cartridges or bottles containing the notifed chemical or when servicing printers or photocopiers);
- Spillage of the notified chemical should be avoided. Spillage should be cleaned up promptly with absorbents which should be put into containers for disposal;
- A copy of the appropriate MSDS should be easily accessible to employees.

If products containing the notified chemical are hazardous to health in accordance with the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999), workplace practices and control procedures consistent with State and Territory hazardous Substances regulations must be in operation.

13.1 Secondary notification

The Director of Chemicals Notification and Assessment must be notified in writing within 28 days by the notifier, other importer or manufacturer:

(1) Under Sub-section 64(1) of the Act:

If the conditions of use are varied, then greater exposure of the public may occur. In such circumstances, further information may be required to assess the hazards to public health.

Or

(2) Under Sub-section 64(2) of the Act:
- if any of the circumstances listed in the subsection arise.

The Director will then decide whether secondary notification is required.

14. MATERIAL SAFETY DATA SHEET

The MSDS for the toner containing the notified chemical was provided in a format consistent with the *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC, 1994).

This MSDS was provided by the applicant as part of the notification statement. It is reproduced here as a matter of public record. The accuracy of this information remains the responsibility of the applicant.

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Attachment 1

The Draize Scale (Draize, 1959) for evaluation of skin reactions is as follows:

Erythema Formation	*Rating*	*Oedema Formation*	*Rating*
No erythema	0	No oedema	0
Very slight erythema (barely perceptible)	1	Very slight oedema (barely perceptible)	1
Well-defined erythema	2	Slight oedema (edges of area well-defined by definite raising	2
Moderate to severe erythema	3	Moderate oedema (raised approx. 1 mm)	3
Severe erythema (beet redness)	4	Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

The Draize scale (Draize *et al*., 1944) for evaluation of eye reactions is as follows:

CORNEA

Opacity	*Rating*	*Area of Cornea involved*	*Rating*
No opacity	0 none	25% or less (not zero)	1
Diffuse area, details of iris clearly visible	1 slight	25% to 50%	2
Easily visible translucent areas, details of iris slightly obscure	2 mild	50% to 75%	3
Opalescent areas, no details of iris visible, size of pupil barely discernible	3 moderate	Greater than 75%	4
Opaque, iris invisible	4 severe		

CONJUNCTIVAE

Redness	*Rating*	*Chemosis*	*Rating*	*Discharge*	*Rating*
Vessels normal	0 none	No swelling	0 none	No discharge	0 none
Vessels definitely injected above normal	1 slight	Any swelling above normal	1 slight	Any amount different from normal	1 slight
More diffuse, deeper crimson red with individual vessels not easily discernible	2 mod.	Obvious swelling with partial eversion of lids	2 mild	Discharge with moistening of lids and adjacent hairs	2 mod.
Diffuse beefy red	3 severe	Swelling with lids half-closed	3 mod.	Discharge with moistening of lids and hairs and considerable area around eye	3 severe
		Swelling with lids half-closed to completely closed	4 severe		

IRIS

Values	*Rating*
Normal	0 none
Folds above normal, congestion, swelling, circumcorneal injection, iris reacts to light	1 slight
No reaction to light, haemorrhage, gross destruction	2 severe

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