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September 2012

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Pyridine, 2-chloro-6-(trichloromethyl)-

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 Public Report may be inspected at our NICNAS office by appointment only at Level 7, 260 Elizabeth Street, Surry Hills NSW 2010.

This 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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SUMMARY

The following details will be published in the NICNAS *Chemical Gazette:*

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS SUBSTANCE	INTRODUCTION VOLUME	USE
STD/1412	Dow AgroSciences Australia Ltd	Pyridine, 2-chloro-6-(trichloromethyl)-	Yes	≤100 tonnes per annum	Component of fertiliser

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the table below.

Hazard classification	*Hazard statement*
Acute toxicity (Category 4)	H302 – Harmful if swallowed
Eye irritation (Category 2A)	H319 – Causes serious eye irritation
Skin sensitisation (Category 1)	H317 – May cause an allergic skin reaction
Carcinogenicity (Category 2)	H351 – Suspected of causing cancer

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) with the following risk phrases:

R22 Harmful if swallowed
R36 Irritating to eyes
R40 Limited evidence of a carcinogenic effect
R43 May cause sensitisation by skin contact

The environmental hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS)* is presented below. Environmental classification under the GHS is not mandated in Australia and carries no legal status but is presented for information purposes.

Hazard classification	*Hazard statement*
Aquatic Environment (Acute Category 3)	H401 – Toxic to aquatic life
Aquatic Environment (Chronic Category 3)	H412 – Harmful to aquatic life with long lasting effects

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

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

Environmental risk assessment
On the basis of the risk quotients and the reported use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

The notified chemical should be classified as follows:
Acute toxicity (Category 4): H302 – Harmful if swallowed
Eye irritation (Category 2A): H319 – Causes serious eye irritation
Skin sensitisation (Category 1): H317 – May cause an allergic skin reaction
Carcinogenicity (Category 2): H351 – Suspected of causing cancer

The following should be used for products/mixtures containing the notified chemical*:
Conc. ≥25%: H302, H319, H317, H351
≥10% Conc. <25%: H319, H317, H351
≥1% Conc. <10%: H317, H351

*if applicable, noting that available data on a microencapsulation formulation containing <20% notified chemical suggests that the statements H302, H319 and H317 do not apply to that formulation.

The Delegate (and/or the Advisory Committee on Chemicals Scheduling) should consider the notified chemical for listing on the SUSMP.

Food Standards Australia New Zealand (FSANZ) should consider whether any additional control measures are required based on the potential for dietary exposure to the notified chemical in microencapsulating formulation, and/or its metabolites.

Health Surveillance

As the notified chemical is a skin sensitiser, employers should carry out health surveillance for any worker who has been identified in the workplace risk assessment as having a significant risk of skin sensitisation.

(Material) Safety Data Sheet and product label

The (M)SDS and label for products containing the notified chemical should contain:
Hazard classifications from either the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS)* or the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), as recommended above
Reference to control measures, as recommended below

CONTROL MEASURES
Occupational Health and Safety

A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical:
Use in ventilated areas [the Safe Work Australia exposure standard for the notified chemical (Safe Work Australia, 2011) should be observed: 10 mg/m^3 (TWA); 20 mg/m^3 (STEL)]
Avoid contact with skin and eyes
Avoid inhalation of vapours and aerosols
Wash hands after use

A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical:

<u>During mixing/loading tasks</u>:
Impervious gloves
Coveralls
Respiratory protection (if ventilation is inadequate)
Goggles (if appropriate, based on the hazard classification of the product containing the notified chemical)

<u>During spray application (if worker is an open cabin)</u>:
Impervious gloves

Coveralls
Respiratory protection (if significant inhalation exposure to the notified chemical is anticipated)

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

A copy of the (M)SDS should be easily accessible to employees.

If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS)*, as adopted for industrial chemicals in Australia, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation should be in operation.

Environment

The following control measures should be implemented by users to minimise environmental exposure during use of the notified chemical:
The notified chemical should not be applied to agricultural soils at a rate greater than 500 grams of notified chemical per hectare per application.
The notified chemical should not be applied to agricultural soils at a rate greater than 1000 grams of notified chemical per hectare per annum.

Disposal

Undiluted products containing the notified chemical should be disposed of according to State and Territory legislative requirements. Containers should be triple-rinsed before disposal and the rinsings added to the spray tank. Containers that are not recycled should be disposed of according to local State and Territory legislative requirements.

Emergency procedures

Spills or accidental release of the notified chemical should be handled by 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
residues of the notified chemical are detected in food producing crops;
products containing the notified chemical are intended to be used by members of the public;
products containing the notified chemical are intended to be used in domestic settings;
the notified chemical is imported in solid form;
additional information becomes available that suggests residues of the notified chemical and/or its metabolites are detected in crops treated with the notified chemical as microcapsules;
products containing the notified chemical are intended to be applied by equipment other than low ground boom.

or

(2) Under Section 64(2) of the Act; if

the function or use of the chemical has changed from a component of fertiliser, or is likely to change significantly;
the amount of chemical being introduced has increased from 100 tonnes 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 and/or its metabolites 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 (M)SDS of the product containing the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the (M)SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Dow AgroSciences Australia Ltd (ABN 24 003 771 659)
20 Rodborough Road
Frenchs Forest NSW 2086

NOTIFICATION CATEGORY
Standard: Chemical other than polymer (more than 1 tonne per year).

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: other names, analytical data, degree of purity and identity of impurities.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: particle size, autoignition temperature

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None.

NOTIFICATION IN OTHER COUNTRIES
USA

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Nitrapyrin

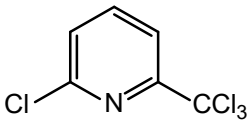
CAS NUMBER
1929-82-4

CHEMICAL NAME
Pyridine, 2-chloro-6-(trichloromethyl)-

OTHER NAME(S)
N-Serve TG
N-Serve TG Nitrogen Stabilizer
eNtrench* Nitrogen Stabiliser (containing the notified chemical at ≤22%)

MOLECULAR FORMULA
$C_6H_3Cl_4N$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
231 Da

ANALYTICAL DATA
Reference UV-Vis, GC-FID and GC-MS spectra were provided.

3. COMPOSITION

DEGREE OF PURITY ≥85%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 ºC AND 101.3 kPa: White waxy solid

Property	Value	Data Source/Justification
Melting Point/Freezing Point	63.6 °C	Measured
Density	1550.4 kg/m^3 at 20 ºC	Measured
Vapour Pressure	4.3 x 10^{-4} kPa at 25 ºC	Measured
Water Solubility	0.072 g/L at 25 °C	Measured
Hydrolysis as a Function of pH	$t_{½}$ = 7.73 d, pH 5 $t_{½}$ = 9.70 d, pH 7 (HEPES buffer) $t_{½}$ = 7.19 d, pH 7 (TRIS buffer) $t_{½}$ = 5.37 d, pH 9	US EPA (2004a)
Partition Coefficient (n-octanol/water)	log Pow = 3.32 at 35 °C	Measured
Surface Tension	71.7 mN/m at 21 ºC	Measured
Adsorption/Desorption	log K_{oc} = 2.40-2.56	Measured
Dissociation Constant	Not determined	Not expected to ionise under environmental conditions based on the pK_a of 0.49 for an analogue, 2-chloropyridine (Linnel, 1960)
Particle Size	Not determined	Notified chemical is a waxy solid
Flammability	Not flammable	Measured
Autoignition Temperature	Not determined	Not expected to autoignite under normal conditions, based on low flammability
Explosive Properties	No indication of explosive properties	Measured
Oxidising Properties	No indication of oxidising properties	Measured
Stability Testing	Stable at normal and elevated temperatures and in the presence of metals and metal ions	Measured
Oxidation/Reduction: Chemical Incompatibility	No indication of chemical incompatibility	Measured

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

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

Physical hazard classification
Based on the submitted physico-chemical data depicted in the above table, the notified chemical is not recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported as a component of a formulated product at ≤22% concentration.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	30-100	30-100	30-100	30-100	30-100

PORT OF ENTRY
Sydney, Melbourne, Brisbane, Adelaide and Perth.

IDENTITY OF MANUFACTURER/RECIPIENTS
Dow AgroSciences Australia Ltd

TRANSPORTATION AND PACKAGING
The formulated product containing the notified chemical (at ≤22%) will be imported in 2 L plastic jerry cans on pallets. The pallets will be transported from the port of entry to the notifier's warehouse, and then distributed to retail outlets by road or rail.

USE
The notified chemical (at ≤22% concentration) is intended to be used as a nitrogen stabiliser for use on crops and pastures. The notified chemical acts by delaying nitrification of ammonia and urea nitrogen fertilisers through the inhibition of soil bacteria.

The notified chemical will be introduced (and used) in a microencapsulated form (suspension capsules), which is designed to decrease the loss of the notified chemical through volatilisation during use. The capsules are within the respirable range (<10 μm).

OPERATION DESCRIPTION
At end-use sites, the imported product containing the notified chemical (at ≤22%) will be mixed with other products (e.g. fertiliser or pesticides) and then applied to crops or pastures. The majority of the notified chemical is expected to be applied by low boom spray.

The product containing the notified chemical will be applied to deliver 500 g/ha of the notified chemical, with a maximum intended application rate of 1000 g/ha of the notified chemical per year. The diluted product will be applied at 20-100 L per hectare.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport	4	12
Storage	4	12
End-users	8	140

EXPOSURE DETAILS
Transport and Storage
Transport and storage workers will only come into contact with the notified chemical (at ≤22% concentration) in the unlikely event of an accident.

End-use
The imported product containing the notified chemical will be mixed with water and/or other fertilisers or pesticides and then applied to fields pre- or post-planting. Farmers and their employees may be exposed to the notified chemical (at ≤22% concentration) via the dermal, ocular and inhalation routes, during mixing/loading of the solutions containing the notified chemical and during (spray) application of the end-use products to crops and pastures. Farmers may also be exposed to the notified chemical when cleaning and maintaining spray equipment, and during accidental spills. Exposure is expected to be minimised by the use of personal protective equipment (PPE), such as coveralls, impervious gloves, goggles and respiratory protection.

The end-use product is intended to be applied to deliver the notified chemical at up to 500 g/ha by low boom spray application. While the notified chemical is only expected to be applied to crops and pastures twice per year, contractors may apply the chemical on a more frequent basis. The US EPA Pesticide Handlers Exposure Database (PHED) Surrogate Exposure Guide (1998) was used to estimate exposure to the notified chemical for a single worker mixing/loading the notified chemical and applying the diluted spray by ground boom with an open cabin. For the purposes of the exposure estimate, the maximum area to be treated per day is assumed to be 150 ha (this equates to a maximum amount of 75 kg of the notified chemical handled per day). It is noted that workers may, on occasion, treat areas greater than 150 ha in a single day, however, they are not expected

to do so on a regular basis.

Assumptions

Application rate:	500 g/hectare
Maximum area treated per day:	150 ha/day
Maximum amount notified chemical handled per day:	75 kg/day
Dermal absorption:	44%
Personal protective equipment:	Gloves, second layer of clothing over normal clothing

The PHED estimate for systemic exposure using the above assumptions for workers mixing/loading and applying the notified chemical is 0.027 mg/kg bw/day. The PHED exposure scenarios for mixing/loading and ground boom are based on workers using liquid pesticides. As the notified chemical is intended to be used in an encapsulated form, this is likely to represent a worst case exposure scenario.

Ground boom application is expected to deliver the spray containing the notified chemical close to the ground, thus decreasing the likelihood of spray drift. Therefore, offsite exposure to the notified chemical is likely to be low.

6.1.2. Public Exposure

The product containing the notified chemical is not intended to be sold to the general public. Therefore, direct public exposure to the notified chemical is only possible through bystander exposure. As the notified chemical is only intended to be applied to crops and pastures in commercial/large-scale settings (and only up to twice/year), exposure of bystanders is expected to be limited.

The public may potentially be exposed to dietary residues of the notified chemical and/or metabolites of the chemical, resulting from plant uptake when the notified chemical is applied on food producing crops. The available information (US EPA, 2005; Dow, 2007a) suggests that residues of the notified chemical have not been detected in plants, but that the metabolite, 6-chloropicolinic acid (6-CPA), has been detected in plants. There is no information available on the potential for the notified chemical, when present as a microencapsulated formulation, and/or its metabolite, 6-CPA, to be taken up by plants. However, given the available data for the notified chemical in free form, this cannot be ruled out.

6.2. Human Health Effects Assessment

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

Endpoint	*Result and Assessment Conclusion*
Toxicokinetics and metabolism	
Pharmacokinetic/Toxicokinetic studies	Rapid absorption in rats with tmax=2 hours. Excretion was 90% after 24 hours and 99% after 72 hours, primarily in the urine, with smaller amounts (11-13%) in the faeces. No observable differences when administered at 1 or 60 mg/kg bw/day. Excreted as 6-CPA or 6-CPA glycine conjugate. Similar absorption and excretion in mice.
Dermal absorption, *in vivo*	44% absorption when applied at 100 g/L concentration.
Acute toxicity - notified chemical	
Various species, acute oral toxicity	LD50 = 1072 mg/kg bw (rat); harmful LD50 = 713 mg/kg bw (mouse and rabbit); harmful LD50 <252 mg/kg bw (guinea pig); toxic
Rabbit, acute dermal toxicity	LD50 = 848 mg/kg bw; harmful
Rabbit, acute dermal toxicity	LD50 >2000 mg/kg bw/day; low toxicity
Rat, acute inhalation toxicity	LC50 >0.03 mg/L/4 hour
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	irritating
Rabbit, eye irritation	irritating
Guinea pig, skin sensitisation – adjuvant test	evidence of sensitisation
Acute toxicity – microencapsulation formulation containing <20% notified chemical	

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 >5000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 >5000 mg/kg bw; low toxicity
Rat, acute inhalation toxicity	LC50 >3.51 mg/L/4 hour; low toxicity
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	slightly irritating
Mouse, skin sensitisation – local lymph node assay	no evidence of sensitisation
Short-term and subchronic toxicity	
Rat, repeat dose dermal toxicity – 28 days	NOAEL = 500 mg/kg bw/day
Rat, repeat dose oral (dietary) toxicity – 3 months	NOAEL = 10 mg/kg bw/day
Mouse, repeat dose oral (dietary) toxicity – 3 months	LOAEL = 200 mg/kg bw/day
Chronic/carcinogenicity	
Dog, chronic oral (dietary) toxicity – 12 months	NOAEL = 3 mg/kg bw/day
Rat, chronic/carcinogenicity oral (dietary) toxicity – 24 months	NOAEL = 5 mg/kg bw/day (chronic) NOAEL ≥60 mg/kg bw/day (carcinogenicity)
Mouse, chronic/carcinogenicity oral (dietary) toxicity – 24 months	NOAEL = 25 mg/kg bw/day (chronic)
Mouse, chronic/carcinogenicity oral (dietary) toxicity – 24 months	LOAEL = 125 mg/kg bw/day (chronic and carcinogenicity)
Mutagenicity and genotoxicity	
Mutagenicity – bacterial reverse mutation	non mutagenic
Mutagenicity – bacterial reverse mutation	non mutagenic
Mutagenicity – bacterial reverse mutation	weakly mutagenic
Genotoxicity – *in vitro* forward mutation assay	non mutagenic
Genotoxicity – *in vivo* unscheduled DNA synthesis	non genotoxic
Genotoxicity – *in vivo* micronucleus study	non clastogenic
Developmental and reproductive toxicity	
Developmental toxicity – range finding (F344 rat)	NOAEL = 15 mg/kg bw/day (maternal)
Developmental toxicity (F344 rat)	NOAEL = 15 mg/kg bw/day (maternal) NOAEL ≥50 mg/kg bw (foetal)
Developmental toxicity – range finding (SD rat)	LOAEL = 50 mg/kg bw/day (maternal)
Developmental toxicity (SD rat)	NOAEL = 15 mg/kg bw/day (maternal) NOAEL = 50 mg/kg bw/day (foetal)
Developmental toxicity (rabbit)	NOAEL = 10 mg/kg bw/day (maternal) NOAEL = 10 mg/kg bw/day (foetal)
Two-generation reproductive toxicity (rat)	NOAEL = 5 mg/kg bw/day (parental) NOAEL = 20 mg/kg bw/day (maternal) NOAEL ≥75 mg/kg bw/day (reproductive) NOAEL = 20 mg/kg bw/day (foetal)

Toxicokinetics, metabolism and distribution.
The notified chemical was rapidly absorbed (t_{max}=2 hours) in rats administered 1 or 60 mg/kg bw. Excretion was also rapid with approximately 90% excreted after 24 hours and 99% excreted after 72 hours. The urine was the primary route of excretion accounting for around 80-85% of the total excretion, as either 6-CPA or the glycine conjugate of 6-CPA. The faeces accounted for approximately 11-13% of excretion. Less than 1% of the notified chemical remained in the carcass at 72 hours. There were no observable differences in absorption or excretion when the notified chemical was repeatedly administered for two weeks at 1 mg/kg bw/day.

Absorption and excretion was similar in a gavage study in mice (25 or 250 mg/kg bw) with almost all the notified chemical excreted by 72 hours, mostly in urine. The main observable difference between mice and rats was that rats excrete a higher proportion of the unconjugated 6-CPA in urine compared to mice that excrete more of the conjugated glycine form.

In an *in vivo* dermal absorption study in rats, dermal absorption was approximately 44% when administered to the skin at a concentration of 100 g/L.

Acute toxicity (notified chemical).
A number of acute oral toxicity studies were conducted with the notified chemical in rats (LD50 = 1072 mg/kg bw), mice (LD50 = 713 mg/kg bw), rabbits (LD50 = 713 mg/kg bw) and guinea pigs (LD50 <252 mg/kg bw).

The rat is the preferred species for evaluation of acute toxicity by the oral route. Based on the weight of evidence, the notified chemical is considered to be harmful by the oral route.

The notified chemical was harmful by the dermal route (LD50 = 848 mg/kg bw) when applied to the skin of rabbits in a solvent vehicle. In the same study, less severe toxicity was observed when it was applied as a solid (LD50 = 2830 mg/kg bw). In addition, in another (more recent) acute dermal toxicity study in rats, the notified chemical was of low toxicity when applied as a solid (that was moistened with water to enhance skin contact; LD50 >2000 mg/kg bw). While the potential for the notified chemical to induce harmful effects following skin contact cannot be ruled out (based on the study results when administered in a vehicle), based on the information available (including the level of detail that was provided in the study reports), the notified chemical is considered to be of low toxicity via the dermal route for classification purposes.

The acute inhalation study that was conducted on the notified chemical only tested a vapour concentration of ~0.03 mg/L. Based on the low concentration of the notified chemical tested in this study, a conclusion on the potential for acute inhalation toxicity (and relevant hazard classification) of the notified chemical cannot be drawn. However, it is noted that the notified chemical has an airborne exposure standard: TWA = 10 mg/m^3, STEL = 20 mg/m^3 (Safe Work Australia, 2011).

Acute toxicity (microencapsulated formulation containing <20% notified chemical).
The microencapsulated formulation containing the notified chemical at <20% concentration was of low acute oral (LD50 >5000 mg/kg bw), dermal (LD50 >5000 mg/kg bw) and inhalation (LC50 >3.51 mg/L/4 hour) toxicity in rats.

Irritation and sensitisation (notified chemical).
The notified chemical was a slight skin irritant in rabbits, in a repeated application study (2 weeks; 5 days/week).

In an eye irritation study in rabbits, moderate (reversible) effects were noted in treated eyes following administration of the notified chemical as a solid and in solution. In a second (more recent) study in rabbits, the notified chemical was found to be an eye irritant following administration of the solid substance (with the eyes remaining unwashed). The irritation effects were noted to have reversed within the 21 day observation period.

The notified chemical (at 10% induction and challenge concentrations) was determined to be a skin sensitiser in guinea pigs (adjuvant test), with skin reactions noted in 3/10 animals at 24 and 48 hours following challenge.

Irritation and sensitisation (microencapsulated formulation <20% notified chemical).
The microencapsulated formulation containing the notified chemical at <20% concentration was a slight skin and eye irritant in rabbits. No evidence of skin sensitisation was observed in an LLNA study with the microencapsulated formulation.

Repeated Dose Toxicity (short-term, sub chronic).
In a 21-day dermal study, rabbits (5/sex/dose) were administered the notified chemical at 0, 100, 500 or 1000 mg/kg bw/day. There were statistically significant increases in absolute and relative liver weights in 1000 mg/kg bw/day males and females and there was evidence of a dose response in both sexes. These findings were not accompanied by associated clinical pathology or histopathological findings but are considered to be treatment related toxicologically significant effects. The NOAEL for systemic toxicity was 500 mg/kg bw/day based on increased liver weights at 1000 mg/kg bw/day. Slight to well defined erythema and oedema were observed in all treated groups, indicating dermal irritation.

In a subchronic oral study, rats (10/sex/dose) were administered dietary doses of the notified chemical at 0, 10, 40 or 120 mg/kg bw/day. Liver and kidney toxicity was observed, mostly at the high dose, but some effects were observed at the middle dose (increased weights and histopathological findings). The NOAEL was established as 10 mg/kg bw/day in this study, based on increased liver weights, nephrosis and tubule degeneration/regeneration in males treated at 40 mg/kg bw/day and above, and brown pigment in the convoluted tubule in females treated at 40 mg/kg bw/day and above.

In a subchronic oral study, mice were administered dietary doses of the notified chemical up to 800 mg/kg bw/day. Mortalities were observed in all 600 mg/kg bw/day (10 males and 10 females) and 800 mg/kg bw/day (10 females) groups. The females treated at 800 mg/kg bw/day died mostly within the first week of treatment. The males and females treated at 600 mg/kg bw/day died between one to two months of treatment. Male groups

that completed 3 months treatment were administered 0, 200, 300 or 400 mg/kg bw/day (10/dose), and females were administered 0, 200 or 400 mg/kg bw/day (10/dose). There were numerous treatment related haematology changes in males and females treated at 400 mg/kg bw/day. Additionally there were increases in aspartate aminotransferase in males treated at 400 mg/kg bw/day, and increases in alanine aminotransferase in males and females treated at 400 mg/kg bw/day and in males treated at 300 mg/kg bw/day. There were dose related increases in liver weights in all treatment groups, accompanied by dose related hypertrophy. Other histopathological findings were in the liver and were observed at 300 mg/kg bw/day and above. Liver toxicity was evident from this study and a LOAEL was established as 200 mg/kg bw/day based on effects observed at the lowest dose.

Repeated Dose Toxicity (chronic/carcinogenicity).
In a chronic study, beagle dogs (4/sex/dose) were administered the notified chemical in the diet at 0, 0.5, 3 or 15 mg/kg bw/day for 12 months. There were treatment related increases of alkaline phosphatase (ALP) at 15 mg/kg bw/day, which was consistent with increased liver weights and slight hepatocellular hypertrophy at this treatment level. Cholesterol levels were also increased in males and females treated at 15 mg/kg bw/day but the relevance of this effect is unclear. The NOAEL was established as 3 mg/kg bw/day, based on these effects.

In a combined chronic/carcinogenicity study, rats were administered the notified chemical in the diet at 0, 5, 20 or 60 mg/kg bw/day for 12 (10/sex/dose) or 24 months (50/sex/dose). There were decreases in body weight gain in females treated at 60 mg/kg bw/day, and in males treated at 20 mg/kg bw/day and above, at 24 months. Kidney and liver toxicity were the main effects, with increased kidney and liver weights at 60 mg/kg bw/day, and associated histopathological findings (hepatocellular hypertrophy and vacuolation in the liver, and protein droplet nephropathy and proteinaceous casts in the kidneys). In males, minor liver effects were also observed at 20 mg/kg bw/day. The NOAEL was established as 5 mg/kg bw/day based on decreased body weights in males treated at 20 mg/kg bw/day. There was also evidence of carcinogenicity in male rats only, based on increased incidence of kidney tumours in 60 mg/kg bw/day males (3/50 tubular adenoma and 3/50 tubular adenocarcinoma with no observations in controls), however these are considered to have resulted from chemically-induced $\alpha_{2\mu}$-globulin accumulation, which is a mechanism that is not relevant for human cancer assessment (US EPA, 2012a).

In a carcinogenicity study, mice were administered the notified chemical in the diet at 0, 5, 25 or 75 mg/kg bw/day for 12 (10/sex/dose) or 24 months (50/sex/dose). The effects observed in this study (increased kidney and liver weights at the high dose) were considered to be treatment related toxicologically significant effects. However, there were no associated pathological findings. Additionally, there were no changes in body weight gain in any of the treated groups. Based on the lack of effects observed, the highest dose tested is not considered to have elicited sufficient toxicity to adequately determine the carcinogenic properties of the notified chemical. Therefore, while the NOAEL was established as 25 mg/kg bw/day, based on increased kidney and liver weights, the dosing may not be sufficient to adequately determine the carcinogenic potential of the notified chemical in mice.

In a carcinogenicity study, mice were administered the notified chemical in the diet at 0, 125 or 250 mg/kg bw/day for 12 (10/sex/dose) or 24 months (50/sex/dose). There were clear indications of toxicity at both dosage levels and in both sexes, including decreased body weight gains and histopathological effects in the liver, stomach, duodenum and jejunum. The study provided evidence of carcinogenic effect at both dose levels. There were dose related statistically significant increases in the incidence of benign papilloma in the nonglandular mucosa of the stomach in both sexes treated at 125 and 250 mg/kg bw/day, and increased incidence of squamous cell carcinomas in high dose males and females, relative to controls. There were non-statistically significant increases in the number of epididymal sarcomas in males treated at 125 and 250 mg/kg bw/day. Increases of lacrimal (Harderian) gland adenomas were statistically significant in females treated at 125 and 250 mg/kg bw/day, but the study authors considered the increased incidence to be not related to treatment. There were statistically significant increases in the incidences of hepatocellular adenomas in males treated at 250 mg/kg bw/day, and in females treated at 125 and 250 mg/kg bw/day, with a clear dose-response relationship in both sexes. Hepatocellular carcinomas were increased compared to controls in males and females treated at 250 mg/kg bw/day but the incidence was not statistically significant, although the study authors considered these tumours to be treatment related. The LOAEL was established as 125 mg/kg bw/day in this study (the lowest dose tested) based on effects at 125 and 250 mg/kg bw/day in both sexes.

Further discussion of the carcinogenic potential of the notified chemical is provided below.

Mutagenicity.

The notified chemical was tested in a number of mutagenicity assays. Negative results were obtained in two separate bacterial reverse mutation tests, in an *in vitro* mammalian cell gene mutation test, in an *in vivo* unscheduled DNA synthesis study and in an *in vivo* micronucleus study in mice. Another mutagenicity study (Zeiger *et al.*, 1988) indicated that the notified chemical may be mutagenic to bacteria in the presence of metabolic activation. A discussion on the available genotoxicity information was provided (Zeiger, 2010). Based on the available data, the notified chemical is not considered to be mutagenic *in vivo*.

Developmental toxicity.
In a developmental range-finding study, mated female F344 rats were administered the notified chemical by gavage at 0, 15, 50 or 100 mg/kg bw/day (9-10/dose) on gestation days (GD) 6 to 15. The maternal NOAEL was established as 15 mg/kg bw/day in this study, based on increased liver weights and liver vacuolation consistent with fatty changes. In the main developmental toxicity study, mated female rats were administered the notified chemical by gavage at 0, 5, 15 or 50 mg/kg bw/day (29-30/dose) on GD 6 to 15. The maternal NOAEL was established as 15 mg/kg bw/day based on hepatocellular vacuolation at 50 mg/kg bw/day. There was no evidence of foetal toxicity in either study.

In a developmental toxicity range-finding study, mated females SD rats (10/dose) were administered the notified chemical at 0, 50, 100 or 200 mg/kg bw/day by gavage on GD 6-15. Due to excessive toxicity, the high dose was terminated early. The maternal LOAEL was established at 50 mg/kg bw/day, based on increased relative liver and kidney weights. There were body weight gain decreases at 100 mg/kg bw/day. There were minimal indications of an effect on reproductive performance at 100 mg/kg bw/day (increased number of resorptions, resorptions per litter and resorption/implant ratio) but the toxicological significance is considered minimal based on the small magnitude. In the main study, mated female rats were administered the notified chemical by gavage at 0, 15, 50 or 120 mg/kg bw/day (28/dose) on GD 6 to 15. The maternal NOAEL was established as 15 mg/kg bw/day based on decreased body weight gains. The NOAEL for foetal toxicity was established as 50 mg/kg bw/day, based on ossification variations observed at the high dose. The test substance did elicit foetal toxicity in the absence of maternal toxicity under the conditions of the study.

In a developmental toxicity study, mated rabbits (25-27/dose) were administered the notified chemical by gavage on GD 6-18 at 0, 3, 10 or 30 mg/kg bw/day. The maternal NOAEL was established as 10 mg/kg bw/day based on decreased body weight gains and increased liver weights. The foetal NOAEL was 10 mg/kg bw/day based on an increased incidence of crooked hyoid at 30 mg/kg bw/day. The test substance was not teratogenic under the conditions of the study, as foetal toxicity was only observed at maternally toxic doses.

Reproductive toxicity.
In a 2-generation reproductive toxicity study, parental (P) generation male and female rats were administered the notified chemical in the diet before mating, during mating, gestation and lactation, and after weaning at 0, 5, 20 or 75 mg/kg bw/day (30/sex/dose). The liver and kidney were the main indicators of systemic toxicity in males and females, in addition to decreases in body weight gain. Foetal toxicity was noted at 75 mg/kg bw/day, based on decreased pup birth weight and liver effects. The parental NOAEL was established as 5 mg/kg bw/day, based on increased kidney and liver weights, and hepatocellular hypertrophy in 20 mg/kg bw/day males and above. The maternal NOAEL was established as 20 mg/kg bw/day, based on increased kidney and liver weights, hepatocellular hypertrophy and vacuolation, and decreased body weight gain at 75 mg/kg bw/day. The reproductive NOAEL was established as ≥75 mg/kg bw/day, as there were no treatment related effects in reproductive performance. The foetal NOAEL was established as 20 mg/kg bw based on decreased pup birth weights and liver effects at 75 mg/kg bw/day. Under the conditions of the study, the notified chemical was not a reproductive toxicant.

Metabolite toxicity.
In a carcinogenicity study, mice were administered 6-CPA in the diet at 0, 100, 300 or 900 mg/kg bw/day for 6 (10/sex/dose), 12 (10/sex/dose) or 24 months (50/sex/dose). The NOAEL was established as 300 mg/kg bw/day, based on decreased body weights, and decreased vacuolation in the proximal convoluted tubule in males treated at 900 mg/kg bw/day. A marginal increase of liver carcinomas in females treated at 900 mg/kg bw/day was not considered to be treatment related, thus the test material was not carcinogenic under the conditions of the study.

In a chronic study, beagle dogs were administered 6-CPA in the diet at 0, 200, 600 or 2000 ppm (equivalent to 0, 5, 16 or 56 mg/kg bw/day) for 12 (1/sex/dose) or 24 months (3/sex/dose). While the study details were limited, the NOAEL was established as ≥56 mg/kg bw/day, based on the absence of any treatment related effects.

A chronic reference dose (RfD; equivalent to an acceptable daily intake) of 0.03 mg/kg bw/day was used for dietary risk assessment of nitrapyrin and 6-chloropicolinic acid (US EPA, 2004b). The chronic RfD was based on the NOAEL of 3 mg/kg bw/day from the chronic dog study with nitrapyrin and using a 100-fold uncertainty factor.

Mode-of-action studies
In a mode-of-action study, liver sections from a 2-week dietary study in mice at 0, 200 or 400 mg/kg bw/day were analysed for hepatocellular proliferation and apoptosis. Proliferating cell nuclear antigen (PCNA) was used as an endogenous marker of hepatocellular proliferation and *in situ* end labelling (ISEL) was used as a marker of hepatocellular apoptosis. The study indicated that a significant amount of hepatocellular proliferation occurred in males treated at 200 and 400 mg/kg bw/day and in females treated at 400 mg/kg bw/day. There were no significant increases in the apoptotic index between treated and control groups.

In a mode-of-action study, liver sections were analysed from male mice exposed to the notified chemical in the diet for 7 and 14 days (with an additional group exposed for 14 days with a 21 day recovery period) at 0, 75, 250 or 400 mg/kg bw/day (6-9/dose). Hepatocellular proliferation was investigated by incorporation of 5-Bromo-2'-deoxyuridine as a surrogate marker, and gene expression analysis was conducted. Absolute and relative liver weights were increased in animals treated at 250 and 400 mg/kg bw/day for 7 and 14 days, but not in the recovery groups. Treatment related histopathological changes were observed in the liver (vacuolisation, hypertrophy, inclusion bodies, mitotic alteration and necrosis) at 250 and 400 mg/kg bw/day, and in the duodenum (hypertrophy and vacuolisation) at all treatment levels, but recovery was observed as there were no histopathological findings in the recovery groups. Increased liver proliferation levels were observed at 250 and 400 mg/kg bw/day with recovery, indicating an association with the increased liver weights and the histopathological findings. The most notable change in gene expression data occurred in the constitutive androstane receptor gene, with a dose-related increase in activation. Activity analysis using 7-pentoxy-resorufin O-deethlyation (PROD) suggested a lack of associated enzyme activity. The study authors proposed that the mode-of-action involves activation of the CAR nuclear receptor followed by hepatocellular proliferation. The study authors also suggest that the detected endpoints in this study show dose-responses, thus further supporting a threshold-based mechanism of carcinogenic activity.

In a mode-of-action study, retrospective analysis of liver sections from a 3-month study in mice was conducted to examine liver proliferation in mice treated at 0, 200 and 400 mg/kg bw/day. Ki-67 immunohistochemistry was used to quantify the presence of proliferative hepatocytes. Increased proliferative responses were noted in both dose groups and in both sexes, although the response was greater in males than in the corresponding dose in females.

Carcinogenicity discussion
As discussed above, carcinogenic effects (in the liver, stomach, epididymis and Harderian gland) were noted in mice treated with the notified chemical at 125 and 250 mg/kg bw/day. In 2005, the notified chemical was classified by the US EPA as "Likely to be carcinogenic to humans", based on the increased incidence of tumours in mice (noting that the forestomach tumours were not considered directly relevant to human health risk assessment due to physiological differences between mice and humans and the Harderian gland tumours were not considered to be response to treatment). In addition, based on the data available at the time, the possibility that there could be a mutagenic basis for the oncogenic effects could not be dismissed. Recently, the carcinogenic potential of the notified chemical and mode of action for mouse liver tumours was re-evaluated (US EPA, 2012a), with the classification of notified chemical revised to "Suggestive evidence of carcinogenic potential". Further discussion of the basis of this re-classification is provided in the following paragraphs.

The epididymal sarcomas that were observed in mice administered the notified chemical at 125 (2/50) and 250 mg/kg bw/day (4/50), but not in the concurrent control group (Dow, 1997a), were originally considered to be treatment related by the US EPA and formed part of the basis for carcinogenicity classification. Epididymal tumours were also observed in another 2-year carcinogenicity study in mice treated with the notified chemical at 25-75 mg/kg bw/day (at 75 mg/kg bw/day; 1/50 Leydig cell tumour) and in control animals (2/50 Leydig cell tumour and 1/50 histiocytic sarcoma). Samples from these studies were recently re-evaluated by a pathology working group by immunohistochemical staining using Mac-2 and F4/80 antibodies (EPL, 2010). The tumours that were originally classified as Leydig cell tumours by the original study pathologists (Dow, 1990), were subsequently classified by the pathology working group as histiocytic sarcomas (*i.e.,* 3/50 in controls and 1/50 in the 75 mg/kg bw/day group). Similarly, the working group confirmed that the epididymal sarcomas that were observed in the mice treated at 125 and 250 mg/kg bw/day were histiocytic sarcomas. The US EPA agreed with

the findings of the working group, and considered that when the data from the two studies are combined, the occurrence of the epididymal histiocytic sarcomas was not related to the notified chemical (US EPA, 2012a).

Regarding the treatment-related increased incidences of liver tumours in mice administered the notified chemical at 125 and 250 mg/kg bw/day, mechanistic data (as discussed above) were submitted to the US EPA to support the proposed mode of action of tumour formation [*i.e.,* induction through activation of the constitutive androstane nuclear receptor (CAR)]. The US EPA considered that cell proliferation and PROD induction data did not support the proposed mode of action for the induction of liver tumours and that the tumours cannot be excluded from being relevant for human cancer hazard assessment. However, it was considered that evidence for the reversibility of the key events had been provided, indicating that a threshold exists for the induction of the liver tumours (US EPA, 2012a).

Based on the available information, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia, as a Category 2 carcinogen (full details of the health hazard classification of the notified chemical are provided in the following section).

Health hazard classification

Based on the available information, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System for the Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the table below.

Hazard classification	*Hazard statement*
Acute toxicity (Category 4)	H302 – Harmful if swallowed
Eye irritation (Category 2A)	H319 – Causes serious eye irritation
Skin sensitisation (Category 1)	H317 – May cause an allergic skin reaction
Carcinogenicity (Category 2)	H351 – Suspected of causing cancer

Based on the available information, the notified chemical is recommended for hazard classification as according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) with the following risk phrases:

R22 Harmful if swallowed
R36 Irritating to eyes
R40 Limited evidence of a carcinogenic effect
R43 May cause sensitisation by skin contact

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

The notified chemical will be handled by workers at ≤22% concentration. The main risks to the health of workers using the notified chemical are acute toxicity (via the oral and potentially inhalation routes), eye irritation, skin sensitisation and repeated-dose toxicity/carcinogenicity.

While the notified chemical is considered to be harmful to human health (acute oral toxicity), ingestion of significant quantities is unlikely under the occupational settings described. While the data provided were not considered adequate to assess the acute inhalation toxicity potential of the notified chemical, it is noted that the notified chemical has an inhalation exposure standard (TWA = 10 mg/m^3, STEL = 20 mg/m^3). Therefore, mixing/loading of the solutions containing the notified chemical should be conducted in adequately ventilated areas and/or respiratory protection should be worn by workers. While some inhalation of the spray containing the notified chemical may occur during application in the field, the exposure is not expected to reach acutely toxic levels.

The highest potential for eye irritation is during mixing/loading of the imported product (containing the notified chemical at up to 22% concentration). The use of eye protection (goggles) will minimise ocular exposure during mixing/loading tasks. Eye irritant effects are not expected during spray application based on the low concentration of notified chemical in the diluted end-use spray.

The potential for skin sensitisation is of concern during mixing/loading tasks and during application. Coveralls and gloves are expected to be worn by workers and these are expected to minimise dermal exposure to the notified chemical.

Systemic toxicity and lifetime carcinogenicity potential are of concern for workers who will be exposed to the notified chemical on a repeated basis. The repeat dose toxicity potential for the notified chemical was estimated by calculation of a margin-of-exposure (MoE) using the estimated daily systemic exposure of 0.027 mg/kg bw/day (see Section 6.1.1.) and a NOAEL of 3 mg/kg bw/day that was established in a chronic dietary study in dogs (this NOAEL is considered to be protective of carcinogenic effects). A MoE of 100 is considered to be acceptable to account for intra-species variability and inter-species extrapolation. The MoE is estimated to be 111 (i.e., 3 mg/kg bw/day ÷ 0.027 mg/kg bw/day) and is considered acceptable.

The exposure estimate assumes that workers are wearing coveralls over normal clothing and gloves. Furthermore, it assumes that the workers that are conducting the boom spray operations are in open cabins. Therefore, provided that adequate PPE are in place, the risk of repeated exposure of workers to the notified chemical is not considered to be unreasonable. However, the need for PPE and the adequacy of the PPE selected for use during spray operations will depend on the particular use situation. For example, additional PPE may be required under certain spray application scenarios, such as respiratory protection if the spray activities are likely to result in significant inhalation exposure of workers to the notified chemical (particularly if conducting spray activities on a frequent basis). Conversely, the need for PPE may be negated through the conduct of spray activities from a closed cabin.

It should also be noted, that while the notified chemical is intended to be used in an encapsulated form, the above risk assessment is based on use of the notified chemical itself. For several acute toxicity endpoints, studies conducted on a microencapsulation formulation containing the notified chemical at <20% concentration were negative (and therefore the above risks related to acute toxicity may have been mitigated). There was no chronic toxicity data provided for the notified chemical, as a microencapsulation formulation. Therefore, the risks related to repeated exposure to the notified chemical cannot be ruled out.

Overall, provided that control measures are in place to minimise exposure of workers to the notified chemical during mixing/loading tasks (such as adequate ventilation areas and/or respiratory protection and the wearing of impervious gloves, coveralls and goggles, as appropriate) and during application (such as impervious gloves, coveralls and/or the use of a closed cabin), the risk to workers is not considered to be unreasonable.

6.3.2. Public Health

As the notified chemical is only intended to be applied in commercial settings, the direct exposure of members of the public to the chemical is expected to be limited. Therefore, the risk to public health is not considered to be unreasonable.

The available information suggests that residues of the notified chemical have not been detected in plants. However, the metabolite, 6-CPA (which is not considered to be carcinogenic based on the data provided), has been detected and plants. Although there is no information available on the potential for the notified chemical, when present as a microencapsulated formulation, and/or its metabolite, 6-CPA, to be taken up by plants, this possibility cannot be ruled out. Furthermore, the possibility that the public may experience dietary exposure to the metabolite 6-CPA as a result of food producing crops being sprayed with the notified chemical in microencapsulated form, can also not be ruled out. While a dietary risk assessment has not been conducted, this assessment report will be referred to Food Standards Australia New Zealand (FSANZ) for consideration.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will not be manufactured or reformulated in Australia. Therefore, there will be no release to the environment due to these activities. Accidental spills during transport or storage are expected to be collected and disposed of in accordance with local regulations.

RELEASE OF CHEMICAL FROM USE

The notified chemical will be used in tank mixes with fertilisers or pesticides by farmers and applied to agricultural soils. Application is expected to take place to topsoil by ground boom spraying. Empty product containers are likely to be triple-rinsed with water with residues of the notified chemical in rinsate added to spray tanks for application to agricultural soils. Containers are then expected to be disposed of via programs such as drumMUSTER or ChemClear®, or according to State and Territory legislative requirements. Notified chemical residues remaining in application equipment are expected to remain in situ, and are likely to be released to agricultural soils during subsequent use of the equipment.

RELEASE OF CHEMICAL FROM DISPOSAL

The majority of the introduction volume of the notified chemical is expected to be applied to agricultural soils. However, unused product containing the notified chemical is expected to be disposed of via an authorised waste disposal company in accordance with local regulations.

7.1.2. Environmental Fate

Details of the provided environmental fate studies are included in Appendix A and C. Supplementary environmental fate characteristics of the notified chemical were sourced from published documents (US EPA, 2004a) as required. Classification for soil mobility is according to the classifications of McCall *et al.* (1980). Classification of all other properties and environmental fate characteristics of the notified chemical are based on the scale adopted by Mensink *et al.* (1995).

When initially applied to agricultural soils, the notified chemical will be contained within capsules which will have different environmental partitioning and fate characteristics than free notified chemical. Over time, the notified chemical will become freely available in the environment following diffusion through the polymeric walls of the capsules into its surrounding environment. Thus, the discussion of the environmental fate considers both the inherent characteristics of the notified chemical and the potential influence of the delayed release (microencapsulated) form of the notified chemical.

Based on the measured vapour pressure of 4.3 x 10^{-4} kPa, the notified chemical is considered moderately volatile. The notified chemical is expected to partition to air from soil surfaces based on its reported volatility from soils: the notified chemical has a reported half-life due to volatilisation of one day when applied to the soil surface (Dow, 2007a). However, the notified chemical is expected to be imported and applied to agricultural soils in a microencapsulated form (suspension capsules). The capsules are optimised to slow volatility losses of notified chemical. Thus the overall rate of dissipation of the microencapsulated form of the notified chemical from soil surfaces is expected to be limited by the rate of release of notified chemical from the capsules to soils. This significantly increases the longevity of the notified chemical on the soil surface: the microencapsulated form of the notified chemical has a reported half-life due to volatilisation of 10 days when applied to the soil surface (ibid.).

Volatility losses of the microencapsulated form of the notified chemical from soil surfaces will be limited by incorporation into soils within 10 days of application, by light cultivation or rain/irrigation of 12.5 mm or more, according to the provided label directions. Therefore, assuming that incorporation occurs 10 days after application and based on the reported half-life of 10 days, up to 50% of the annual import volume of the microencapsulated notified chemical may volatilise to the atmospheric compartment from soil surfaces before its incorporation into soils. The amount lost through volatilisation will be reduced if incorporation occurs before the tenth day following application.

The half-life of the notified chemical in air is calculated to be 305 days based on reactions with hydroxyl radicals (AOP, v1.92; US EPA, 2009). Therefore, notified chemical that partitions to air following application to agricultural soils is considered to have the potential to persist in the atmospheric compartment.

Applied notified chemical that is not lost to the atmospheric compartment through volatilisation is expected to be associated with the terrestrial compartment following use. Free notified chemical is fairly to readily degradable when mixed with soils. The reported half-life in aerobic mineral soils is 11 days to 17.9 days (with 25.1 days as the 90th upper percentile of the two half-lives; US EPA, 2004a). The major metabolite in soils was identified as 6-CPA (ibid.). The half-life of the metabolite, 6-CPA, in an aerobic soil study ranged from 23.2 days to 47.6 days in three soils (Appendix C.1.3), indicating that it is fairly degradable in soils. These data indicate that the notified chemical and its metabolite are unlikely to persist in soils.

The effective half-life in soil of the microencapsulated form of the notified chemical can be modelled using the

above half-life of the free notified chemical and accounting for the delayed release of the microencapsulated form. The effective half-life was determined to be 41 days following a peak mass fraction of 0.31 in soils at 31 days after application, assuming incorporation at day 10 (Appendix C.1.4). Thus, the use of the microencapsulated form of the notified chemical has the effect of both delaying the peak soil concentrations and prolonging the time taken for the notified chemical to dissipate from soils.

In soils, the notified chemical is expected to have medium mobility as the adsorption coefficient (Kd) for the notified chemical ranged from 0.947 mg/L to 19.9 mg/L with Koc values ranging from 254 mg/L to 360 mg/L, respectively (Appendix A – Adsorption/Desorption). The major metabolite, 6-CPA, has very high mobility in soils based on the reported adsorption coefficient (Koc) of 1 mg/L to 9 mg/L, and Kd values ranging from 0.38 mg/L (mineral soils) to 1.02 mg/L (high organic matter soils; ibid.). Hence, the notified chemical and metabolite may move offsite due to leaching. The polymeric capsules containing the notified chemical are not expected to be soluble and are thus unlikely to be mobile in soils.

The notified chemical may enter surface waters in run-off from a treated field or from overspray or spray drift during application by ground boom sprayer. The notified chemical is moderately water soluble. The notified chemical is fairly hydrolysing with a half-life of less than or equal to 9.7 days in sterile pH 5, 7 and 9 buffer solutions and also fairly photo-degradable (ibid.) in sterile pH 7 solution with a reported half-life of 9.4 days (US EPA, 2004a). 6-CPA was identified as the major metabolite in both hydrolysis and photolysis studies (ibid.). These data suggest that notified chemical should not persist in most aquatic environments because of abiotic chemical degradation.

The half-life of the notified chemical is reported to be less than three hours in anaerobic aquatic environments (US EPA, 2004a). The total system half-life of the notified chemical in an aerobic water-sediment system was 0.9 days and the half-life in the water column was 0.8 days (Appendix C.1.2, Test 1). Therefore, the notified chemical is readily degradable in a water-sediment system and is not expected to persist in the aquatic or sediment compartments. However, it is noted that mineralisation of the notified chemical in the water sediment system was less than 1% over 30 days.

The major metabolite in the water-sediment study, and other aquatic degradation studies, was 6-CPA. While some degradation was observed of the metabolite in an aerobic water-sediment system study, the total system half-life of the metabolite could not be established within the 32-day duration of the study (Appendix C.1.2, Test 2). There was a tendency for the metabolite to partition to sediment and from two weeks onward 50% to 55% of the applied amount of 6-CPA observed to be associated with sediment compared with about 35% in water. Thus, based on the available data the metabolite is considered to have the potential to persist in the aquatic and sediment compartments.

The microencapsulated form of the notified chemical is expected to have a longer effective half-life in surface waters as notified chemical inside capsules is expected to be protected from degradation processes. Diffusion rates of the notified chemical through the capsule shell walls into water have not been provided and therefore the dissipation rate of the microencapsulated form of the notified chemical in surface waters cannot be modelled. A qualitative assessment suggests that the delayed release of the notified chemical from capsules is likely to result in low concentrations of notified chemical in surface waters for extended periods following exposure. However, it is unlikely to accumulate in surface waters due to its expected rapid degradation by biotic and abiotic mechanisms.

The notified chemical is considered moderately volatile from water based on its dimensionless Henry's Law Constant of 0.00057. The aerobic water-sediment study showed up to 14.7% loss of the notified chemical through volatilisation to air. Thus volatilisation from water may present another route of exposure of the notified chemical to the atmospheric compartment.

The notified chemical has a low partition coefficient indicating that it is unlikely to bioaccumulate. The low potential of the notified chemical for bioaccumulation is further indicated by the results of a fish accumulation study (Appendix C.1.1). The bioconcentration factor (BCF) of the notified chemical in bluegill sunfish was less than 85 after a 30 day exposure period, indicating that it is slightly concentrating. The major metabolite was 6-CPA. The total-^{14}C BCF of up to 230 in whole fish indicates that the metabolite also has low potential for bioaccumulation.

7.1.3. Predicted Environmental Concentration (PEC)

Of the potential 100 tonnes of notified chemical imported annually, it can be assumed for the purpose of the

risk assessment, that all of the notified chemical will be applied to agricultural soils. The notified chemical targets microorganisms that undertake nitrogen cycling in soils. The recommended maximum use rate, from the product label, is 500 grams per hectare (g/ha) of notified chemical per application, with no more than 1000 g/ha of the notified chemical to be applied to a site annually. For the purposes of calculating the predicted environmental concentrations (PEC) it is assumed that this represents two applications of 500 g/ha of notified chemical three months apart. A three month (90 day) interval was selected to be representative of a worst-case scenario with one pre-emergent treatment followed by one post-emergent treatment for a crop.

Exposure to birds and mammals

The notified chemical may be applied to agricultural soils by means of spraying by ground boom sprayer. Hence, use of the notified chemical may result in its deposition on potential food sources for avian wildlife including crops, vegetation and insects. Therefore, there is potential for birds to ingest food contaminated with residues of the notified chemical.

The PEC_{food} for the notified chemical resulting from a single application of 500 g/ha can be calculated according to the updated Kenaga nomogram, which relates food item residues to pesticide application rate (Pfleeger *et al.*, 1996) (see following Table).

Residues of notified chemical in food sources with an application rate of 500 g/ha

Food items	*Concentration, fresh weight (mg/kg)*
Short grass	107.1
Leaves and leafy crops	60.3
Forage crops	60.3
Small insects	60.3
Grain/long grass	49.0
Pods with seeds	6.70
Large insects	6.70
Fruit	6.70

The PEC_{food} for the notified chemical in the diet of a small bird, such as bobwhite quail, feeding exclusively on food sources contaminated with the notified chemical can be calculated on the standard assumptions that quail have a diet of 30% small insects and 70% grain (EPHC, 2009a, p. 29). The PEC for the notified chemical in the diet of the quail, $PEC_{food\ (quail)}$, when they feed exclusively on food items from areas treated with the notified chemical is 52.4 mg/kg on a fresh weight basis [(0.3 × 60.3) + (0.7 × 49.0)].

The PEC_{food} for the notified chemical in the diet of a large bird, such as mallard duck, feeding exclusively on food sources contaminated with the notified chemical can be calculated on the standard assumptions that mallard duck have a diet of 30% grain and 70% large insects (ibid., p. 29). The PEC for the notified chemical in the diet of the mallard duck, $PEC_{food\ (mallard\ duck)}$, when they feed exclusively on food items from areas treated with the notified chemical is 19.4 mg/kg on a fresh weight basis [(0.3 × 49.0) + (0.7 × 6.70)].

The application of the notified chemical to agricultural soils has the potential to expose terrestrial vertebrates other than birds to residues. This will include wild mammals which may forage for food in treated fields. The PEC_{food} for mammals is calculated using a similar approach to that used for birds. The surrogate mammalian species selected for the environmental risk assessment is the rat as an effects endpoint is available. Further, the rat has foraging behaviours for which established methodology is available for determining the PEC through dietary exposure.

The PEC_{food} for the notified chemical in the diet of rat feeding exclusively on food sources contaminated with the notified chemical can be calculated on the standard assumptions that rats have a diet of 100% grain (ibid., p. 29). The PEC for the notified chemical in the diet of the rat, $PEC_{food\ (rat)}$, when they feed exclusively on food items from areas treated with the notified chemical is 49.0 mg/kg on a fresh weight basis (1 × 49.0).

The resultant PEC_{food} for the three surrogate bird and mammalian species are summarised in the following Table.

Summary of the predicted environmental concentrations for birds and mammals

PEC_{food}	*PEC*
$PEC_{food\ (quail)}$	52.4 mg/kg diet
$PEC_{food\ (mallard\ duck)}$	19.4 mg/kg diet
$PEC_{food\ (rat)}$	49.0 mg/kg diet

Cumulative residues due to multiple applications of the notified chemical have not been calculated, based on its expected rapid dissipation from exposed surfaces due to its high volatility. This also applies to the microencapsulated form of the notified chemical as, using the only available half-life for the microencapsulated form of the notified chemical of 10 days from soil surfaces, less than 1% would be expected to remain on food surfaces 90 days after application.

A PEC_{food} for the metabolite, 6-CPA, has not been calculated as the high unqualified sub-acute dietary endpoints for birds do not allow for a quantitative risk characterisation.

Aquatic compartment

The notified chemical may enter the aquatic compartment as a result of direct overspray or off-target spray drift during application by ground boom sprayer, or in a run-off event from a treated field. The PEC_{water} for each pathway of aquatic exposure is calculated below (see following Table).

Summary of the predicted environmental concentrations for the water compartment (PEC_{water}) via exposure from direct overspray, spray drift and run-off

PEC_{water}	*PEC (µg/L)*
$PEC_{direct\ overspray}$	330
$PEC_{spray\ drift\ (0\ m)}$	60.9
$PEC_{spray\ drift\ (1\ m)}$	22.6
$PEC_{spray\ drift\ (5\ m)}$	6.9
$PEC_{run\text{-}off\ (free\ chemical)}$	2.08
$PEC_{run\text{-}off\ (capsules)}$	4.3
$PEC_{run\text{-}off\ (total)}$	6.4

The PEC_{water} has not been calculated for the metabolite, 6-CPA, for the reasons detailed below.

The available aquatic toxicity endpoints for the metabolite indicate that the notified chemical is more toxic to aquatic organisms than the metabolite. Thus, the risks from the notified chemical on an acute basis are greater than that of the metabolite. Therefore, the quantitative risk characterisation of the notified chemical is considered to be protective to the aquatic environment for acute exposures of the metabolite and a separate calculation requiring the PEC for the metabolite was not performed.

However, the metabolite may pose a greater risk than the notified chemical to the aquatic compartment on a chronic basis. This is because the metabolite is more likely to accumulate in surface waters from multiple applications due to its increased stability in water-sediment systems compared with the notified chemical. However, the half-life of the metabolite could not be established in a water-sediment system, and thus a PEC_{water} resulting from multiple applications cannot be quantitatively modelled accounting for dissipation. A worst case PEC, assuming no degradation between applications, was not calculated as chronic aquatic toxicity endpoints are not available for the metabolite for comparison.

(i) Direct overspray

Direct overspray represents a worst-case scenario for the PEC in the aquatic compartment arising from the reported use pattern of the notified chemical. It is noted that direct overspray is unlikely with ground application and is more of a risk by aerial applications, which is not expected based on the reported use pattern. The PEC for direct overspray is calculated for a shallow water body assuming a depth of 15 cm as a worst case situation (EPHC, 2009a, pp.38-40). The $PEC_{direct\ overspray}$ with an application rate of 500 g/ha of notified chemical is 0.33 mg/L.

(ii) Spray drift

Exposure to the aquatic compartment from spray drift as a result of application by ground boom sprayer can be modelled using the AgDRIFT® model (AgDRIFT Spray Drift Task Force Spray Software, Version 2.0.09). The variables in the model that affect off-target spray drift are the droplet size and height of the boom. Therefore, the model results are independent of the form of the notified chemical (free or microencapsulated).

Off-target exposure due to spray drift is reduced with coarser droplets and increases with increasing boom height.

The notified chemical is indicated for application with fertilisers or pre-emergent pesticides. It is assumed that uniform crop coverage is not critical to the intended action of the notified chemical which targets soil microorganisms. Therefore, a coarse droplet size is likely to be used for its application to agricultural soils. As the notified chemical may be used post-emergence on crops, a high boom height is assumed to represent the worst case. Therefore, the settings of the model are those described in the APVMA standard scenario High ground boom – Coarse (v1.0; APVMA, 2010).

The PEC arising from spray drift [$PEC_{spray\ drift}$] is calculated assuming a water body 15 cm deep and 3 m wide. (EPHC, 2009a, pp.38-40). The percent drift at 0 m, 1 m and 5 m is 18.3%, 6.79% and 2.1% of the nominal application rate, respectively. Therefore, with an application rate of 500 g/ha the $PEC_{spray\ drift}$ for a water body at 0 m, 1 m and 5 m are 60.9 µg/L, 22.6 µg/L and 6.9 µg/L, respectively.

The PEC of the notified chemical in surface waters was not calculated following a second application, as significant levels are not expected to remain after 90 days, due to the short half-life of the notified chemical in the water column of a water-sediment system (0.8 days, Appendix C.1.2). Encapsulation of the notified chemical may prolong its dissipation in water due to delayed transformation until after it is released from capsules. However, the notified chemical is expected to gradually diffuse through capsule walls into water where it is expected to rapidly degrade via biotic and abiotic mechanisms. Therefore, although the rate of diffusion from capsules into water is unknown, it is unlikely that a significant amount of the free notified chemical will be present in the water at the time of the second application.

(iii) Run-off

The potential for exposure of the notified chemical to the aquatic compartment as a result of run-off has been modelled, as detailed in Appendix C.1.5.

Usually, only transport in the water phase is considered for a moderately soluble chemical, such as the notified chemical, as transport in the water phase generally exceeds that of the sediment phase, due to the large volumes of run-off water compared with the volume of topsoil that is removed. However, both mechanisms for transport were determined to be of relevance to the notified chemical when applied to agricultural soils in a microencapsulated form. Therefore, the run-off model considered the consequences of the modified-release dose-form of the notified chemical and the differences in the mode of transport, in either the water or sediment phase, of the free notified chemical and its microencapsulated form, respectively.

For free notified chemical in soils, the peak mass fraction of free notified chemical in soil is greatest following incorporation. However, it is assumed that incorporation into soils will significantly reduce the potential of the free notified chemical to be solubilised in run-off water. A soil dissipation model (refer to Appendix C.1.4) of the microencapsulated form of the notified chemical indicates a peak mass fraction of 0.104 for free notified chemical released from capsules on the soil surface prior to incorporation. The PEC has been calculated assuming no interception of notified chemical by crops, an application rate of 500 g/ha and that 10.4% of the applied notified chemical is free in soils at the time of the run-off event. The $PEC_{run\text{-}off\ (free\ chemical)}$ of the free notified chemical on the soil surface that is predicted to be transported in the water phase during a run-off event, when diluted in an environmental water body, is 2.08 µg/L.

The capsules containing the notified chemical are not expected to be soluble in environmental waters and are likely to be transported as particulates with topsoil. Therefore, notified chemical in the microencapsulated form is expected to be predominantly transported in the sediment phase during a run-off event. The worst-case PEC has been calculated assuming that all of the applied notified chemical remains within capsules (i.e. the run-off event occurs immediately after application) and an application rate of 500 g/ha. The $PEC_{run\text{-}off\ (capsules)}$ of the microencapsulated form of the notified chemical on the soil surface that is transported in the sediment phase, when diluted in an environmental water body, is 4.3 µg/L.

The worst-case PEC is determined to account for the total notified chemical that may be transported in both the water and sediment phases during a run-off event. It is calculated by summing the PEC for the free and microencapsulated forms of the notified chemical. The resultant $PEC_{run\text{-}off\ (total)}$ is 6.4 µg/L. It is noted that the model used to derive the worst-case PEC for the free chemical with water phase transport assumes partial release of the notified chemical from capsules. Thus the calculated $PEC_{run\text{-}off\ (total)}$ is an overestimate of the aquatic exposure but is an adequate approximation for the purposes of undertaking the risk characterisation.

Terrestrial compartment

The notified chemical will be released into soils in the terrestrial compartment as a result of its application to agricultural soils by ground boom sprayer and drip irrigation, or off-target spray drift during application by ground boom sprayer. The PEC for non-target organisms in the terrestrial compartment is calculated below (see following Table).

Summary of the predicted environmental concentrations for the terrestrial compartment (soil-dwelling organisms and terrestrial plants)

PEC_{soil}	*PEC*
$PEC_{soil\ (total)}$	0.38 mg/kg soil
$PEC_{soil\ (free\ chemical)}$	0.23 mg/kg soil

The PEC of the notified chemical in soils following a single application was calculated to be 0.33 mg/kg soil when applied at a rate of 500 g/ha and assuming a soil bulk density of 1500 kg/m^3 and a soil-mixing zone of 10 cm. A soil dissipation model (refer to Appendix C.1.4) of the microencapsulated form of the notified chemical indicates that the mass fraction of the applied amount in soils and remaining in capsules in soils after 90 days is 0.139 assuming immediate incorporation into soils. Thus, the residual concentration in soils from the first application after 90 days will be 0.05 mg/kg soil (0.33 mg/kg soil × 0.139). Therefore, the maximum PEC for the notified chemical in soils, including both that freely available in soils and in capsules [$PEC_{soil\ (total)}$], was calculated to be 0.38 mg/kg soil, immediately following a second application with a 90 day interval between applications.

The above calculation represents a worst case PEC as the notified chemical is expected to be applied to soils in a microencapsulated form which may reduce the bioavailability of the notified chemical. The soil dissipation model predicts a peak mass fraction of free notified chemical available in soil of 0.55 of the applied microencapsulated amount, and is reached 22 days after application with immediate incorporation. This correlates to a maximum PEC of free notified chemical in soil of 0.18 mg/kg soil 22 days after a single application (0.33 mg/kg soil × 0.55). The dissipation model predicts that the majority of the remaining notified chemical from the first application (0.05 mg/kg soil) will be free in soil, and not in capsules, after 90 days. For simplicity, these two maximum values are added to derive the PEC. Therefore, the worst-case PEC of free notified chemical in soil [$PEC_{soil(free\ chemical)}$] following a second application is 0.23 mg/kg soil.

A PEC_{soil} due to off-target exposure arising from spray drift when applied by ground boom sprayer was not performed as the risk characterisation is undertaken using the above calculation for $PEC_{soil(total)}$, which represents a worst-case scenario for direct overspray.

A worst-case PEC in soils for the metabolite, 6-CPA, in soil could be calculated assuming 100% conversion of the notified chemical and a worst case half-life in soils of 47.6 days. However, this is not undertaken in this instance as there are no available endpoints for soil organisms to complete the quantitative risk characterisation.

Sediment compartment

The PEC for the sediment compartment has not been calculated as significant amounts of the notified chemical are not expected to partition to sediment from water, based on the results of a water-sediment system study (see 3rd Table in Appendix C.1.2). The study showed a maximum of 5.0% of free notified chemical added to the water surface associated with sediment. The half-life in sediment was not established in the study report due to the low concentration of the metabolite, but it is noted that the metabolite had largely dissipated from sediment within 7 days of initial application to the water surface. Therefore, significant exposure of the free notified chemical to sediment organisms is not expected.

The use of the notified chemical in a microencapsulated form may result in different partitioning behaviour in a water-sediment system as capsules are expected to have limited solubility in environmental waters. For example, capsules may settle on sediment as a result of off-target spray drift during application with ground boom sprayer. Alternately, capsules may be mixed with sediments as a result of transport in the sediment phase during a run-off event. This could potentially lead to increased exposure, and therefore increased risks, to sediment-dwelling bottom-feeders compared with use of the free notified chemical. However, the notified chemical is still expected to rapidly dissipate after it diffuses through capsule walls into the surrounding water or sediment media. Therefore, the risks to the sediment compartment were not considered to be significantly increased. Further, as effects endpoints are not available for the sediment compartment a quantitative risk characterisation could not be conducted. Therefore, a $PEC_{sediment}$ was not calculated for the notified chemical.

The metabolite of the notified chemical, 6-CPA, shows a tendency to partition to sediments in a water-sediment system where it is expected to persist between applications. However, a $PEC_{sediment}$ for the metabolite has not been calculated, as relevant effects endpoints are not available for comparison.

Atmospheric compartment
The PEC for the atmospheric compartment has not been calculated as there are no relevant ecotoxicological endpoints to complete a quantitative risk characterisation.

7.2. Environmental Effects Assessment

The ecotoxicological effects of the notified chemical and the principal environmental metabolite, 6-CPA, were investigated with representative test species from aquatic and terrestrial taxa. Details of the submitted studies can be found in Appendix C. The results are summarised in the discussion below. In some cases, additional ecotoxicological endpoints have been sourced from the published literature, as referenced, to supplement the submitted information.

Classification of effects endpoints is conducted in accordance with the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009) for aquatic species, the US EPA ecotoxicological categories for avian and mammals (US EPA, 2012b) and the scale adopted by Mensink *et al.*, (1995) for earthworms.

Effects on birds and mammals
The results of the submitted ecotoxicological studies that investigate the acute effects to birds of the notified chemical and the metabolite, 6-CPA (see following Table).

Summary of effects of the notified chemical and its metabolite, 6-CPA, on birds

Test Species	*Study Type*	*Endpoint*
Notified chemical		
Bobwhite quail	Dietary 5-day exposure, 3-day post exposure (pilot)	LC50 >1000 mg/kg diet NOEC = 100 mg/kg diet
Japanese quail	Dietary 5-day exposure, 3-day post exposure	LC50 = 820 mg/kg diet NOEC = 400 mg/kg diet
Turkey poults	Acute oral – single dose, 14 day observation	LD50 = 118 mg/kg bw
Leghorn cockerel chicks	Acute oral – single dose, 14 day observation	LD50 = 235 mg/kg bw
6-CPA		
Bobwhite quail	Dietary 5-day exposure, 3-day post exposure (pilot)	LC50 >1000 mg/kg diet NOEC = 1000 mg/kg diet
Japanese quail	Dietary 5-day exposure, 3-day post exposure	LC50 >5000 mg/kg diet NOEC = 300 mg/kg diet
Mallard duck	Dietary 5-day exposure, 3-day post exposure	LC50 >4640 mg/kg diet NOEC = 1000 mg/kg diet

Additional avian acute toxicity endpoints for the mallard duck, considered to be core studies by the US EPA, were obtained from the published literature (US EPA, 2004a) and are tabulated below.

Summary of effects of the notified chemical on birds reported by the US EPA (2004a)

Test Species	*Study Type*	*Endpoint*
Mallard duck	Acute dietary	LC50 = 1467 mg/kg diet NOAEC = 631 mg/kg bw
Mallard duck	Acute oral	LD50 >2510 mg/kg bw NOAEL = 631 mg/kg bw
Bobwhite quail	Acute dietary	LC50 = 2131 mg/kg diet NOAEC = 1000 mg/kg bw

The notified chemical is moderately toxic to turkey and leghorn cockerel on an acute oral basis with reported median lethal dose (LD50) of 118 mg/kg bw and 235 mg/kg bw, respectively. The median lethal concentration (LC50) in a sub-acute dietary study on Japanese quail was 820 mg/kg diet. Additional acute dietary LC50 endpoints for mallard duck and bobwhite quail were 1467 mg/kg diet and 2131 mg/kg diet, respectively. The results indicate that the notified chemical is slightly-to-moderately toxic to birds in sub-acute dietary exposures.

Dietary studies for the metabolite, 6-CPA, to bobwhite quail, Japanese quail and mallard duck showed no treatment related mortality at any test level. Mallard duck showed depression and reduced reaction to external stimuli at test levels in excess of 1000 mg/kg diet. Significant effects were observed on the body weight of Japanese quail at test levels in excess of 300 mg/kg diet. As the sub-acute median lethal concentration in feed for all bird species is in excess of 1000 mg/kg bw, and no treatment related mortality was observed at treatment levels of up to 5000 mg/kg bw, the metabolite is considered practically non-toxic to birds in sub-acute dietary exposures.

Information is not available on the chronic toxicity of the notified chemical, or the metabolite 6-CPA, to birds.

Effects data for wild mammals in Australia are not available. The median lethal dose for rats (LD50 = 1070 mg/kg bw) from the human health effects assessment is used as a surrogate for wild mammal species in the environmental risk characterisation. The rat is considered a suitable representative species due to its foraging behaviours. The notified chemical is slightly toxic to the rat on an acute oral basis.

Effects on aquatic organisms
The results from ecotoxicological investigations conducted on the notified chemical, notified chemical in two product formulations, and the metabolite, 6-CPA, to aquatic organisms are summarised in the Table below. Unless specified otherwise in the Study Type column, the test substance was the notified chemical. The endpoints for formulations are reported as the concentration of the notified chemical in the test solution (mg n.c/L).

Summary of effects of the notified chemical, the notified chemical in two formulations and the metabolite 6-CPA on aquatic organisms

Test Species	*Study Type*	*Endpoint*
Freshwater Fish		
Bluegill (*Lepomis macrochirus*)	Acute 96 h, flow-through	LC50 = 3.4 mg/L
	Acute 96 h, static	LC50 = 7.9 mg/L[a]
Rainbow trout *(Oncorhynchus mykiss/ Salmo gairdneri)*	Acute 96 h, static	LC50 = 4.0 mg/L
	Acute 96 h, static	LC50 = 9.1 mg/L[a]
	Acute 96 h, static	
	Notified chemical	LC50 = 7.0 mg/L
	Formulation 1	LC50 = 3.1 mg n.c./L[c]
	Formulation 2	LC50 = 1.7 mg n.c./L[c]
	6-CPA	LC50 = 40.5 mg/L
	Acute 96 h, unknown[b]	LC50 = 6.45 mg/L
Channel Catfish (*Ictalurus punctatus)*	Acute 96 h, static	
	Notified chemical	LC50 = 8.0 mg/L
	Formulation 1	LC50 = 2.9 mg n.c./L[c]
	Formulation 2	LC50 = 3.1 mg n.c./L[c]
	6-CPA	LC50 = 100 mg/L
	Acute 96 h, unknown[b]	LC50 = 5.80 mg/L
Goldfish (*Carassius auratus)*	Acute 96 h, static	
	Notified chemical	LC50 = 7.6 mg/L
	Formulation 1	LC50 = 2.5 mg n.c./L[c]
	Formulation 2	LC50 = 2.7 mg n.c./L[c]
	6-CPA	LC50 = 239 mg/L
Fathead minnow	Acute 96 h, unknown[b]	LC50 = 10.2 mg/L
Estuarine/Marine Fish		
Tidewater silverside (*Menidia beryllina)*	Acute 96 h, flow-through	LC50 = 4.28 mg/L
Freshwater Invertebrates		
Daphnia magna	Acute, 48 h, flow-through	LC50 = 5.8 mg/L[a]
	Acute, 48 h, flow-through	LC50 = 2.2 mg/L
Estuarine/Marine Invertebrates		
Eastern oyster (*Crassostrea virginica*)	Acute, 96 h, flow-through, shell deposition	EC50 = 0.56 mg/L
	Acute, 96 h, flow through, shell deposition	EC50 = 1.5 mg/L[d]
Grass shrimp (*Palaemonetes pugio*)	Acute, 96 h, flow-through	LC50 = 3.1 mg/L

Test Species	*Study Type*	*Endpoint*
Freshwater Plants, Diatoms and Algae		
Green algae (*Selenastrum capricornutum*)	Acute, 72 h, static	E_rC50 = 1.7 mg/L NOEC = 0.66 mg/L

[a] Nominal values were not verified by measured concentrations.
[b] Study reports were not submitted.
[c] The results are reported as the concentration of the notified chemical (n.c.) in the test solution.
[d] Study report not submitted, endpoint reported by the US EPA (2004a).

The acute toxicity of the notified chemical to five species of freshwater fish and one species of marine fish was investigated. The 96-hour median lethal concentrations (96 h LC50) ranged from 3.4 mg/L to 10.2 mg/L, indicating that the notified chemical is toxic to fish on an acute basis. The bluegill sunfish was the most sensitive fish species, while the marine species, tidewater silverside, was the least sensitive fish species.

The acute toxicity of the notified chemical to one species of freshwater invertebrate and two species of marine invertebrates was investigated. The most sensitive species was the eastern oyster with a 96-hour median effect concentration (shell deposition; 96 h EC50) of 0.56 mg/L. However, this result is considered reliable with restrictions due to the reduced shell deposition in the control. It is noted that the US EPA (2004a) reports a core study result for the eastern oyster 96 h EC50 (shell deposition) of 1.5 mg/L for a study that met the requirements of the test method OPPTS 850.1025. Therefore, the above endpoint is considered reliable and was used in preference to the result in the submitted study. The eastern oyster is the most sensitive aquatic invertebrate species for which acute toxicity data is available. On the basis of the eastern oyster 96 h EC50 (shell deposition) of 1.5 mg/L the notified chemical is considered toxic to aquatic invertebrates on an acute basis.

The acute toxicity of the notified chemical was only investigated for one species of algae. Effects endpoints are not available for aquatic plants or diatoms. The 72-hour median effect concentration (growth rate; 72 h E_rC50) was 1.7 mg/L, indicating the notified chemical is toxic to algae. The no-observed-effect concentration (NOEC) was 0.66 mg/L, and may be used as chronic toxicity endpoint for algae. The notified chemical is considered rapidly degradable due to its short half-life in a water sediment system and, therefore, considered to be harmful to algae with long-term effects.

The notified chemical disrupts the bacterially mediated steps in the nitrogen cycle by inhibiting conversion of ammonia to nitrate. The notified chemical may therefore serve to increase the residence time of ammonia in surface waters which may be of toxic concern in freshwater systems. However, data are not available to assess the extent to which the notified chemical inhibits ammonia conversion in aquatic systems.

The acute toxicity of the notified chemical in two formulations to three freshwater fish was also investigated. The 96-hour median lethal concentrations (96 h LC50) ranged from 1.7 mg/L to 3.1 mg/L of the notified chemical. Slightly increased toxicity of the notified chemical to fish was observed when the test system was dosed with notified chemical in a formulation. The observed increased toxicity could be due to effects of constituents in the formulation or due to increases in the effective dose of notified chemical as a result of increased availability from the presence of solubilising constituents in the formulation. It is noted that neither formulation contained the microencapsulated form of the notified chemical which is of most relevance to the reported import and use pattern in Australia. Therefore, the effects endpoints for the notified chemical are used in preference to those for the formulations in the environmental risk characterisation.

The acute toxicity of the metabolite, 6-CPA, to three species of freshwater fish was investigated. The 96-hour median lethal concentrations (96 h LC50) ranged from 40.5 mg/L to 239 mg/L, indicating that the metabolite has reduced toxicity to fish compared with the notified chemical. The metabolite is considered to be harmful to fish.

Environmental hazard classification under the GHS

The environmental hazard classification of the notified chemical is conducted in accordance with the Globally Harmonised System of Classification and Labelling of Chemicals (GHS; United Nations, 2009). Under the GHS the notified chemical is considered to be acutely toxic to fish, aquatic invertebrates and algae. Based on the acute toxicity to aquatic invertebrates the notified chemical is formally classified under the GHS as 'Acute category 2; Toxic to aquatic life'.

One adequate chronic toxicity endpoint was available. Therefore, the long-term classification for the notified

chemical was determined based on the most stringent outcome by comparing the long-term hazard classification using either the acute or chronic data. The most stringent outcome resulted from the chronic endpoint. Based on its rapid degradability and the chronic endpoint for algae, the notified chemical is formally classified under the GHS as 'Chronic category 3; Harmful to aquatic life with long lasting effects'.

Effects on soil-dwelling organisms and terrestrial plants

Ecotoxicological investigations were only provided for earthworms: the results are summarised in the following Table and the details of the study can be found in Appendix C.2.5.

Summary of effects of the notified chemical on soil dwelling organisms

Test Species	*Study Type*	*Endpoint*
Earthworm (*Eisenia foetida*)	Acute, 15 day	LC50 = 209 mg/kg soil

In addition, plant toxicity endpoints were obtained from the published literature of a comparable overseas agency (US EPA, 2004a) and are summarised in the Table below. These endpoints were not generated using standard guidelines but are sufficient to provide indicative toxicity to terrestrial plants for a screening level assessment.

Summary of effects of the notified chemical on terrestrial plants

Test Species	*Endpoint*	*Results (mg/kg soil)*
Study 1[a]		
Monocots (wheat, corn, oats)	EC50 (plant growth reduction)	43, 50, >100
	EC10 (plant growth reduction)	23, 23, 27
Dicots (cotton, sugar beets, tomatoes, cucumber)	EC50 (plant growth reduction)	41, 44, 62, >100
	EC10 (plant growth reduction)	5, 12, 21, 62
Study 2[a]		
Monocots (corn, sorghum, wheat, rice)	NOEC (fresh weight of plant tops)	10 000-50 000
Dicots (cotton, sugar beet, tomato, alfalfa, soybean)	NOEC (fresh weight of plant tops)	5000-20 000

[a] Limited further details of the study conditions are reported in the source document (US EPA, 2004a)

Effects endpoints for the inhibition of nitrification in soil by soil microorganisms were not submitted. This was considered a relevant endpoint for the risk assessment as the notified chemical is expected to inhibit the microbially-mediated nitrogen mineralisation processes in soils to which it has been applied due to its highly selective action as a bactericide to *Nitrosomonas* spp., the bacteria that oxidise ammonium ions in the soil (Tomlin, 2003). Therefore, endpoints were obtained from the published literature in order to assess the long-term risk to soil microorganisms in agricultural soils and in the wider environment due to off-target spray drift.

In soils with organic carbon content ranging from 1.2% to 2.1% incubated at 25 ºC, less than 25% inhibition of nitrification is observed at 42 days with concentrations of the notified chemical at or below 0.5 mg/kg soil (42 d $EC25_{(nitrification)}$ >0.5 mg/kg soil; see Appendix C.2.6). Inhibition of nitrification in soils is increased in soils with low organic carbon content and reduced at high temperatures. Therefore, in accordance with the criteria for agrochemicals (EPHC, 2009a, p50), concentrations of the notified chemical of 0.5 mg/kg soil or less are not considered to have a long-term influence on nitrogen transformation in soils.

Atmospheric effects

There are no standard ecotoxicological endpoints for evaluating effects in the atmospheric compartment. Generally the effects assessment for this compartment involves the evaluation of the long-range transport potential, global warming potential and ozone depleting potential. The notified chemical is considered to have long-range transport potential due to its propensity for volatilisation from soil surfaces, moderate volatility from water and as its predicted half-life in air exceeds two days. No information is available for the global warming potential of the notified chemical, but it is noted that nitrification inhibitors act to reduce the emissions of nitrous oxide from agriculture soils which is a significant contributor to global warming (Motavalli *et al.*, 2008). The ozone depleting potential of the notified chemical is unknown and the reaction rate of the notified chemical could not be calculated using established quantitative structure-activity relationships as only the reaction with olefins and acetylenes are estimated by the AOP Program (v 1.92; US EPA, 2009).

7.2.1. Predicted No-Effect Concentration

For industrial chemicals generally, release to the environment during use and disposal is incidental rather than intentional and effects are expected to be through common modes of toxic action such as narcosis. However,

according to the reported use pattern, the notified chemical is intentionally applied to agricultural soils and has a specific mode of action to microorganisms responsible for nitrogen fixation in soils. Therefore, the predicted no-effect concentration (PNEC) has not been calculated for the aquatic and terrestrial compartment using the assessment factor methodology for industrial chemicals (EPHC, 2009b, pp. 55-65). Rather, for determining the risks to the environment in the risk characterisation, the effects endpoints were directly compared to the predicted environmental concentrations, in accordance with the methodology outlined in the Environmental Risk Assessment Guidance Manual for Agricultural and Veterinary Chemicals (EPHC, 2009a, pp. 71-77).

The pivotal endpoints for the environmental effects of the notified chemical used in the quantitative risk characterisation are summarised in the Table below.

The set of pivotal endpoints for the environmental effects of the notified chemical used in the risk characterisation

Taxa	*Endpoint*	*Classification*
Birds and mammals		
Bobwhite quail	Acute dietary LC50 = 2131 mg/kg diet	Slightly toxic [a]
Mallard duck	Acute dietary LC50 = 1467 mg/kg diet	Slightly toxic [a]
Rat	Acute oral LD50 = 1070 mg/kg bw	Slightly toxic [a]
Terrestrial Compartment		
Earthworms	Acute, 15 d LC50 = 209 mg/kg soil	Slightly toxic [b]
Terrestrial plants	EC50 = 41 mg/kg soil	- [c]
Soil microorganisms	42 d EC25 >0.5 mg/kg soil	Not considered to have a long-term effect on nitrogen transformation in soils ≤0.5 mg kg soil [d]
Aquatic Compartment		
Fresh water fish	96 h LC50 = 3.4 mg/L	Toxic [e]
Marine fish	96 h LC50 = 4.28 mg/	Toxic [e]
Fresh water invertebrates	48 h LC50 = 2.2 mg/L	Toxic [e]
Marine invertebrate	96 h EC50 = 1.5 mg/L	Toxic [e]
Fresh water algae	72 h E_rC50 = 1.7 mg/L	Toxic [e]
Plants	No data	- [f]

[a] US EPA (2012b); [b] Mensink *et al.* (1995); [c] Classification criteria are not available; [d] EPHC (2009a, p50); [e] United Nations (2009); [f] Classification is not possible as no effects endpoints are available.

Endpoints for birds and mammals were selected for surrogate species representing small birds, large birds and wild mammals. The other endpoints are those of the most sensitive tested species for each trophic level in the terrestrial and aquatic compartments. Where more than one test result was available for the most sensitive species, the most relevant and reliable endpoint was selected. The endpoints for formulations were not considered relevant, as the tested formulation was not that which is proposed for use in Australia (containing a microencapsulated form of the notified chemical). Test results which were based on the nominal concentration without verification by analytical monitoring, or for which full study reports were not available, were not considered fully reliable. Endpoints reported in publications of a competent overseas authority such as the US EPA were considered reliable for regulatory decision-making.

7.3. Environmental Risk Assessment

Introductory comments to the risk characterisation

Australian environmental risk assessments for industrial chemicals generally use a deterministic approach to quantitative risk characterisation whereby point values which represent the worst-case exposure (PEC) and effects (PNEC) estimates are compared using the risk quotient, Q (EPHC, 2009b). The risk quotient is a simple ratio defined by the equation Q = PEC/PNEC. The risk to an environmental compartment is generally considered to be acceptable when the risk quotient is less than one (Q < 1). A resultant risk quotient that is greater than one (Q > 1) indicates an unacceptable risk to the aquatic environment and options to refine the risk characterisation are explored.

In this assessment, the likelihood that use of a product containing the notified chemical applied to agricultural soils in accordance with the reported use pattern will not pose an unreasonable risk to organisms in the environment, is determined based on a quantitative ecological risk assessment framework which was developed

by the US EPA (Urban and Cook, 1986). The risk characterisation step involves the integration of the environmental exposure and effects analysis through a comparison of the PEC for an exposed compartment and the level of concern (LOC) established for the most sensitive species in each trophic level. The LOCs for acute effects are products of a median lethal effect end-point and a safety factor which is taken to be 0.1. For chronic effects, the levels of concern are taken to be the chronic no observed effects concentrations (NOECs) for the most sensitive species. The notified chemical may be considered to pose an unreasonable risk to the environment if the PEC for any compartment exceeds the LOC determined for that compartment.

An alternative though equivalent approach to quantifying the ecological risks of a chemical to is to calculate the risk quotient, Q. In this case, the PNEC used in determining the risk quotient equation is the L(E)C50 for acute effects, or the NOEC for chronic effects. The threshold for unacceptable risk based on the Q metric is the safety factor. Hence, a Q value ≥ 0.1 for acute effects and ≥ 1.0 for chronic effects is considered to indicate potentially unacceptable risk to organisms in the environmental compartment under consideration (EPHC, 2009, pp. 76-77).

Only limited chronic ecotoxicological endpoints were available for the notified chemical: the algal 72 h NOEC indicated that the notified chemical was harmful to aquatic organisms with long term effects. Therefore, as the only available chronic endpoint was for an algal species, the chronic risks to organisms could not be assessed during the quantitative risk characterisation (European Commission, 2003). Repeated application of the notified chemical to agricultural soils may occur within and across growing seasons leading to the possibility of repeated exposure. However, as detailed in Section 7.1.2 and 7.1.3, it is not expected that a significant amount of the applied notified chemical will persist in the terrestrial, aquatic or sediment compartments between applications. Therefore, it is considered that organisms are unlikely to have long-term exposure to the notified chemical in environmental media.

The notified chemical is proposed for use in Australia in a microencapsulated form. Environmental effects endpoints for the microencapsulated form of the notified chemical, or a formulation containing the notified chemical in the microencapsulated form, were not available. Notified chemical within the polymeric capsules is expected to be of limited bioavailability to organisms in soils and surface waters. The notified chemical in capsules is expected to become bioavailable following diffusion through capsule walls into the surrounding environmental media. However, for the acute risk characterisation it was assumed that the microencapsulated form of the notified chemical would pose an equivalent of lesser risk than the equivalent amount of free notified chemical in the environmental compartment being considered. Thus, when determining the risks to the environment during the risk characterisation it was assumed under a worst-case scenario that all notified chemical was bioavailable.

Risks to birds and mammals

The notified chemical is moderately volatile and up to 50% of the applied amount may partition to air from soil surfaces prior to its incorporation into soils. Therefore, exposure to non-target birds and mammals by inhalation poses a potential risk for these species. However, a quantitative risk characterisation by an inhalation exposure route is not possible (EPHCa, 2009, p. 29). Further, birds and mammals are predominantly exposed to chemicals applied to crops and pastures through ingestion of contaminated foods, water or soil.

The risk characterisation quantifies the risks from dietary ingestion of residues on vegetative matter and insects from representative small bird (bobwhite quail), large bird (mallard duck) and mammalian (rat) species. For spray applications, the exposure to each surrogate species was estimated based on the likely concentration of the notified chemical in food items in a treated field, as detailed in Section 7.1.3. The PEC for each species was calculated based on their expected diet (Section 7.1.3). The median lethal concentration endpoints (LC50s) for the concentration of notified chemical in the diet of the bird species used in the risk characterisation were taken directly from the quail, mallard duck and rat studies discussed previously. The LC50 endpoint for rat of 21400 mg/kg diet was derived from the median lethal dose (LD50 = 1070 mg/kg bw) assuming a body weight of 400 grams and food consumption of 20 grams [(0.4 kg bw ÷ 0.02 kg diet) × 1070 mg/kg bw] (Urban and Cook, 1986, p. 39).

The resultant risk quotients for birds and mammals are presented in in the Table below. The level of concern for acute effects on avian wildlife and mammals is reached when the PEC_{food} reaches 10% of the level of the sub-acute dietary LC50 (i.e., Q = 0.1).

Risk quotients for birds and mammals

Risk Assessment	PEC (mg/kg diet)	LC50 (mg/kg diet)	Q	LOC
$Q_{food\ (quail)}$	52.4	2131	0.025	0.1
$Q_{food\ (mallard\ duck)}$	19.4	1467	0.013	0.1
$Q_{food\ (rat)}$	49.0	21400	0.002	0.1

Therefore, based on the calculated risk quotients, the risk from dietary exposure is acceptable to birds and mammals that feed exclusively on food items within a field treated with 500 g/ha of the notified chemical.

Risks due to dietary exposure of the metabolite, 6-CPA, to birds and mammals could not be determined as definitive effects endpoints were not available. The available effects data indicates that the metabolite is less toxic to birds than the notified chemical. Therefore, the above risk assessment for the notified chemical is taken to be protective of birds exposed to the metabolite as a result of the reported use pattern.

Risks to the aquatic compartment

The application of the notified chemical to fields by ground boom sprayer or drip irrigation has the potential to result in exposure to aquatic organisms in nearby water bodies. For the risk characterisation of the aquatic compartment, exposure estimates (PEC, Section 7.1.3) were determined based on an application rate of 500 g/ha of notified chemical and potential release of the notified chemical into an environmental water body from: direct overspray or spray drift during application by ground boom sprayer; and, run-off from a treated field. Section 7.1.3 contains further detail of the derivation of the PEC_{water} values. Although the PECs for the aquatic compartment have been calculated to account for the different exposure pathways for the free notified chemical and the microencapsulated form, it is assumed for the worst-case exposure that the entire amount of notified chemical is freely available to organisms on entry into the environmental water body, regardless of whether it enters in a free or microencapsulated form.

The effects estimate, L(E)C50, selected for characterising the acute risks to the aquatic compartment is the acute toxicity endpoint for most sensitive aquatic species, the eastern oyster 96-hour median effect concentration (EC50, shell deposition) of 1.5 mg/L. Although this is a marine species, it is considered to be a suitable representative species for use in the risk characterisation for the aquatic compartment as the endpoint is similar to those reported for freshwater and other marine species. Further, as the notified chemical may be used in a wide variety of crops and pastures including sugarcane, it could potentially be used in locations that result in estuarine or marine exposure.

The resultant risk quotients for the aquatic compartment are presented in the Table below. The level of concern for acute effects of the notified chemical on aquatic life is reached when PEC_{water} is 10% of the L(E)C50 for the most sensitive species (i.e., Q = 0.1).

Risk quotients for the aquatic compartment

Risk Assessment	*PEC (µg/L)*	*L(E)C50 (µg/L)*	*Q*	*LOC*
$Q_{direct\ overspray}$	330	1500	0.22	0.1
$Q_{spray\ drift\ (0\ m)}$	60.9	1500	0.041	0.1
$Q_{run\text{-}off\ (total)}$	6.4	1500	0.0043	0.1

Based on the calculated risk quotient for the aquatic compartment with exposure as a result of direct overspray ($Q_{direct\ overspray}$ = 0.22) the risk is unacceptable to aquatic organisms. However, assuming good agricultural practice is employed during application it is considered unlikely that direct overspray of an environmental water body will occur when the notified chemical is applied to agricultural soils using ground equipment. Therefore, the risk characterisation was refined by assessing the potential for aquatic exposure arising from spray drift during application by ground boom sprayer.

The risk quotient for the aquatic compartment with exposure as a result of spray drift during application by ground boom sprayer indicates acceptable risk for organisms in an environmental water body immediately adjacent to the edge of the treated field ($Q_{spray\ drift\ (0m)}$ = 0.041). Therefore, on the basis of the $Q_{spraydrift}$ and the reported use pattern, the notified chemical is not considered to pose an unreasonable risk to the aquatic environment due to off-target spray drift when applied at a rate of 500 g/ha by ground boom spray equipment in accordance with good agricultural practice.

The risk quotient for the aquatic compartment with exposure as a result of a run-off event from a treated field

($Q_{run\text{-}off\ (total)}$ = 0.0043) indicates an acceptable risk to aquatic organisms in an environmental water body. Therefore, on the basis of the $Q_{run\text{-}off}$ and the reported use pattern, the notified chemical is not considered to pose an unreasonable risk to the aquatic environment due to run-off from a field treated with the notified chemical at a rate of 500 g/ha.

It is noted that the notified chemical is a nitrification inhibitor. Therefore, use of the notified chemical may result in indirect toxicity to aquatic organisms by modifying the water concentrations of ammonia. However, data is not available to quantitatively assess these risks.

Acute risks of the metabolite, 6-CPA, to the aquatic compartment are considered to be less than that of the notified chemical due to its reduced toxicity (based on the limited available endpoints). Therefore the above risk characterisation is considered to be protective to the aquatic compartment for acute exposures of the metabolite. However, the metabolite may pose a chronic risk to the aquatic organisms based on its expected persistence in this compartment. However, insufficient effects data is available on the chronic toxicity of the metabolite to assess the chronic risks.

Risks to the terrestrial compartment

The notified chemical is applied to agricultural soils to inhibit the nitrification in soils. The mode of action of the notified chemical is as a selective bactericide to soil microorganisms that are responsible for nitrogen cycling. The risk to soil-dwelling organisms, such as earthworms and microorganisms, and terrestrial plants is assessed using the estimated concentrations in soils in a field treated twice annually at a rate of 500 g/ha with three months between applications and assuming immediate incorporation into soils ($PEC_{soil\ (total)}$, Section 7.1.3).

The acute effects endpoints used in the quantitative risk characterisation for the terrestrial compartment were taken from Section 7.2.1. The level of concern (LOC) for earthworms and terrestrial plants is reached when the PEC is 10% of the L(E)C50 (i.e., Q = 0.1). The effects endpoint for soil microorganisms, and its interpretation for long-term effects, is considered to be an indicator of chronic risks. Therefore, the level of concern (LOC) for soil microorganisms is reached when the PEC is 100% of the L(E)C50 (i.e., Q = 1.0). The resultant risk quotients for the terrestrial compartment are presented in the following Table.

Risk quotients for the terrestrial compartment

Risk Assessment	*PEC_{soil} (mg/kg soil)*	*L(E)C50 (mg/kg soil)*	*Q*	*LOC*
$Q_{earthworms}$	0.38	209	0.002	0.1
$Q_{terrestrial\ plants}$	0.38	41	0.009	0.1
$Q_{soil\ microorganisms}$	0.38	> 0.5	< 0.76	1.0

The resultant risk quotients for terrestrial organisms of each trophic level – producers (plants), consumers (earthworms), and decomposers (microorganisms) – indicates an acceptable risk to the terrestrial compartment based on the reported use pattern of the notified chemical.

Effects data for the metabolite, 6-CPA, are not available and, therefore, a quantitative risk characterisation for the terrestrial compartment cannot be completed.

Risks to the sediment compartment

A quantitative risk characterisation was not performed for the sediment compartment as significant exposure is not expected based on the fate characteristics of the notified chemical, and as ecotoxicological endpoints were not available for sediment species.

There is potential for exposure to the sediment compartment for the metabolite, 6-CPA, based on its tendency to partition to sediment from water and its expected persistence in the sediment compartment. However, a quantitative risk characterisation for the sediment compartment cannot be completed as effects endpoints are not available.

Persistence, bioaccumlation and toxicity (PBT) Assessment

The reported use pattern indicates that the majority of the notified chemical will be applied to agricultural soils as a nitrogen stabiliser in a microencapsulated form.

The notified chemical is not expected to persist in agricultural soils to which it has been applied, or in surface waters which it may enter due to spray drift or run-off, based on its measured half-life in soils and water-

sediment systems. The use of the microencapsulated form of the notified chemical is expected to extend the effective half-life of the notified chemical in soils and water due to delayed release of the notified chemical into the environment. That is, as the notified chemical inside capsules is protected from degradation mechanisms, it has a reduced dissipation due to volatilisation. However, the notified chemical is expected to readily undergo degradation or dissipation following diffusion through the capsule walls into the surrounding environment.

The notified chemical predominantly degrades to the metabolite, 6-CPA, in water, sediments, soils and fish. The metabolite is not expected to persist in soils based on its measured half-life. The half-life of the metabolite in a water-sediment system could not be established in a study of 32-days duration. Therefore, the metabolite is considered to have the potential to persist in water and sediment.

The notified chemical is moderately volatile and readily partitions to air from soil surfaces. The volatilisation of the notified chemical from soil surfaces following application is partially restricted by use of the microencapsulated form of the notified chemical, which is designed to limit volatility losses, and by its expected incorporation into soils within 10 days following application. The notified chemical is moderately volatile from water and may also be released into the atmospheric compartment by volatilisation from contaminated surface waters. The notified chemical has the potential to persist in the atmospheric compartment based on its calculated atmospheric half-life in air of more than 2 days. The notified chemical is therefore considered to have the potential to undergo long-range transport.

The notified chemical is not expected to bioaccumulate in the environment on the basis of its low octanol-water partition coefficient and is considered only slightly concentrating based on its measured bioconcentration factor in fish. The metabolite, 6-CPA is also considered to have low potential for bioaccumulation.

The notified chemical is considered to be slightly-to-moderately toxic to birds, slightly toxic to mammals, toxic to aquatic organisms on an acute basis and slightly toxic to earthworms. The notified chemical has a highly selective mode of action against soil microorganisms that undertake nitrogen mineralisation in soils. However, the effects on microorganisms are not expected to have a long-term influence on nitrogen transformation in soils at the reported application rate. Only limited chronic ecotoxicological endpoints were available for the notified chemical: the algal 72 h NOEC indicated that the notified chemical was harmful to aquatic organisms with long term effects. Therefore, the notified chemical is not considered toxic under the PBT criteria.

The use of the encapsulated form of the notified chemical has the potential to increase the chronic risks as the delayed release may result in low concentrations in the various environmental compartments over a longer period of time. In the soil compartment, the modelled effective half-life for the notified chemical in the encapsulated form was extended from 25 to 41 days. In the aquatic compartment, the notified chemical is expected to rapidly dissipate once it diffuses through the capsule walls based on its half-life of less than one day. Therefore, although exposure may be extended in the environment due to delayed release of the notified chemical, the chronic risk is considered to be low.

The limited effects data for the metabolite, 6-CPA, indicated lower toxicity associated with the metabolite compared with the notified chemical. The metabolite was harmful to fish and practically non-toxic to birds on a sub-acute dietary basis. No chronic effects data was available.

Risk assessment conclusion

The notified chemical may volatilise from agricultural soils to which it has been applied into the atmospheric compartment where it is considered to have long-range transport potential. However, the notified chemical is unlikely to persist in other environmental compartments. The notified chemical is not expected to bioaccumulate.

The major metabolite of the notified chemical by biotic and abiotic degradation is 6-CPA. The limited available toxicity data indicates lower toxicity for the metabolite compared to the notified chemical on an acute basis. Therefore, the quantitative risk characterisation on an acute basis for the notified chemical was considered protective of the environment exposed to the metabolite based on the reported use pattern. However, the metabolite was considered to have greater potential for chronic risks than the notified chemical based on its expected persistence in the aquatic and sediment compartments. These risks could not be quantified due to a lack of half-life and chronic effects endpoints for the metabolite in these compartments. The metabolite is not expected to bioaccumulate.

On the basis of the risk quotients to organisms in the aquatic and terrestrial compartments and the assessed use

pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point 63.6 °C

Method	OPPTS 830.7200 Melting Point/Melting Range Similar to OECD TG 102 Melting Point/Melting Range
Remarks	The melting point was determined using differential scanning calorimetry (DSC), with scanning from 55 to 70 °C at 0.5 °C/minute.
Test Facility	Dow (2007b)

Density 1550.4 kg/m^3 at 20 °C

Method	Pesticide Assessment Guidelines, Subdivision D, Section 63-7
Remarks	The notified chemical was heated to its melting point and then transferred into a 10 mL volumetric flask, that was then stoppered. The test substance was allowed to solidify at 20 °C and was then weighed.
Test Facility	Dow (1990a)

Vapour Pressure 4.3 x 10^{-4} kPa at 25 °C

Method	OECD TG 104 Vapour Pressure
Remarks	Determined using a vapour pressure balance.
Test Facility	Huntingdon (2006)

Water Solubility 0.072 g/L at 25 °C

Method	Pesticide Assessment Guidelines, Subdivision D: Product Chemistry, Section 63-8
Remarks	Generator column method with recirculating system and HPLC/UV determination.
Test Facility	Dow (1987a)

0.020 g/L at 18.5 °C

Method	EC Directive 84/449/EEC A.6 Water Solubility
Remarks	The study report stated that the shake flask method was used. However, the study details indicate the test was performed by column elution method with recirculating system and determination by GC/FID. The test was performed at one flow rate (30 mL/h) without confirmatory results of a second run at half the initial flow rate to verify that solubility was not affected by the flow rate. A quadratic regression equation was produced from the calibration data (peak height) used for quantitation indicating poor linearity of the method. Three of the five test samples showed a response in excess of the highest calibration solution indicating calibration was not performed over an appropriate range. The extraction recovery of the test substance was 78% at ~30% of the nominal concentration: accuracy of the test method was not demonstrated over an appropriate range. Thus, the results of the study should be used with caution due to variations to the study protocol and lack of appropriate analytical method validation.
Test Facility	Huntingdon (1985)

Partition Coefficient (n-octanol/water) log Pow = 3.32 at 35 °C

Method	EC Directive 84/449/EEC A.8 Partition Coefficient
Remarks	Flask Method with GC/ECD determination.
Test Facility	Huntingdon (1985)

Surface Tension 71.7 mN/m at 21 °C

Method	EC Directive 84/449/EEC A.5 Surface Tension Similar to OECD TG 115 Surface Tension of Aqueous Solutions
Remarks	Concentration: 90% saturation solution (1.96 x 10^{-2} g/L)
Test Facility	Huntingdon (1985)

Adsorption/Desorption log K_{oc} = 2.40-2.56 mL/g

Method Pesticide Assessment Guidelines, Subdivision D: Product Chemistry, Section 163-1
Similar to OECD TG 106 Adsorption – Desorption Using a Batch Equilibrium Method

Remarks A batch laboratory adsorption/desorption with radiolabelled notified chemical (2,6-^{14}C-nitrapyrin; radiopurity > 99.9%) was conducted on five different soils. The five soils covered a range of pH from 5.4 to 7.1, a range of organic carbon content from 0.3% to 5.6% and four different USDA textural classes: two sandy loam, one loam, one clay loam and one silty clay loam. The aquatic medium was 0.01N calcium chloride. The tests were performed with a soil: solution ratio of 4:1 and an equilibrium time of 3 hours.

The adsorption isotherm test was performed with a solution blank and four concentrations of test substance (nominal concentrations of 0.1, 0.5, 1.0 and 10 µg/L; duplicate). Desorption studies were carried out on the soil samples with a concentration of 1.02 ppm of the notified chemical that had been aged 30 days. Desorption studies were also conducted for two intermediate products of aerobic soil metabolism, 2-chloro-6-dichloromethyl-pyridine and 6-CPA (unspecified nominal concentrations).

The preliminary desorption test indicated that equilibrium between soils and solution was reached within 3 hours. While a 3 hour equilibrium was found to be suitable for the desorption study, the adsorption study appeared to require longer to reach equilibrium. However, adsorption samples in excess of 6 hours showed evidence of increasing hydrolysis and a shaking time of 3 hours was chosen for the adsorption study as a compromise between equilibrium and avoiding complications due to hydrolysis.

The amount of test substance adsorbed to soil was calculated as the difference between the amount of test substance present in solution after 3 hours contact with soil samples and that initially added to each tube. The concentration of test substance in test solutions was determined by reverse-phase HPLC and radioactivity assays of test solutions by liquid scintillation counting (LSC). To verify the mass balance of the test substance, the concentration of the test substance for selected soil samples were determined by LSC analysis of the combusted samples. Similarly, the desorption study determined the test substances in solution by LSC and HPLC and in combusted soils by LSC.

Freundlich adsorption isotherms were calculated by linear regression analysis of $\ln C_{equilibrium}$ (i.e., concentration in solution at equilibrium) versus ln(x/m) (i.e., concentration in soil).

RESULTS

Table 1 Adsorption of the notified chemical

Soil Type (%sand/silt/clay)	*Organic Carbon Content (%)*	*pH*	*Kd (mL/g)*	*Koc (mL/g)*	*Classification for mobility*
Sandy loam (64/27/9)	0.33	6.0	0.947	287	Medium
Loam (38/50/12)	0.63	7.1	2.22	359	Medium
Clay loam (30/38/32)	5.65	6.3	19.9	352	Medium
Sandy loam (69/18/13)	0.81	6.4	2.92	360	Medium
Silty clay loam (58/28/14)	2.05	5.4	5.2	254	Medium

Table 2 Desorption for the notified chemical and the two metabolites of the notified chemical in aerobic soils, 6-CPA and 2-chloro-6-(dichloromethyl)-pyridine

Soil Type (%sand/silt/clay)	*Organic Carbon Content (%)*	*pH*	*Kd (mL/g)*			*Koc (mL/g)*		
			n.c. [a]	6-CPA [b]	DCMPyr [c]	n.c. [a]	6-CPA [b]	DCMPyr [c]
Sandy loam (64/27/9)	0.33	6.0	4.9	0.03	3.2	1500	6.8	97
Loam (38/50/12)	0.63	7.1	57	0.86	9.2	9100[d]	140	1500
Clay loam (30/38/32)	5.65	6.3	87	10	15	1500	180	270
Sandy loam (69/18/13)	0.81	6.4	18	3.6	9.3	2200	450	1200
Silty clay loam (58/28/14)	2.05	5.4	30	3.6	8.9	1500	170	430

[a] notified chemical, nitrapyrin; [b] 6-chloropicolinic acid; [c] 2-chloro-6-(dichloromethyl)pyridine; [d] This result was considered an outlier based on a Q test and was excluded from the calculation of the average.

Remarks Recovery from the adsorption study of notified chemical ranged from 48% to 94% (average of 75%). The low recovery was attributed to volatilisation of the test substance from the soil during air drying prior to combustion. Recovery from desorption soils ranges 93.7-100%.

The average value obtained for the adsorption coefficient Koc constant of the notified chemical (Table 1) was 321 mL/g, the results ranged from 254 mL/g to 360 mL/g, corresponding to log Koc 2.40 to 2.56. Based on these values for Koc, the notified chemical is classified as having medium mobility in soil (McCall *et al.*, 1980). The average value measured for the desorption Koc constant of the notified chemical (Table 2) was 1700 mL/g. The higher result for the desorption study indicates gradual incorporation of the notified chemical into the matrix over time, resulting in a decreased amount of notified chemical available for desorption.

Desorption Koc values of 860 mL/g and 190 mL/g were determined for 2-chloro-6-(dichloromethyl)pyridine and 6-CPA, respectively (Table 2). 2-Chloro-6-(dichloromethyl)pyridine is therefore considered to have low soil mobility while 6-CPA is considered to have high soil mobility.

The study report cited previous adsorption results for both the notified chemical and 6-CPA. Average values of 267 mL/g and 560 mL/g were reported for the Koc (normalized for soil organic carbon content) of the notified chemical. The study results are consistent with the previous results. An adsorption Koc va1ue of 1 mL/g to 5 mL/g was calculated for 6-CPA via the Freundlich equation (for three soils and a one-day equilibration time). An adsorption Koc value of 9 mL/g was reported independently for this acid. The adsorption Koc for 6-CPA was not determined in this study to allow for comparison.

Test Facility Dow (1987b)

Flammability Not flammable

Method EC Directive 84/449/EEC A.10 Flammability (Solids)
Remarks The notified chemical was not flammable in contact with an ignition source (performed 6 times; hot wire ignition source with a minimum temperature of 1000 °C). Melting of the test substance in the region of the ignition source and the evolution of some light grey smoke was noted when initially in contact with the ignition source.
Test Facility Huntingdon (1985)

Explosive Properties No indication of explosive properties

Method OPPTS 830.6316 Explodability
Remarks DSC was used to determine the thermal explodability of the notified chemical. Approximately 1 g of notified chemical was placed in a sealed glass capillary tube and placed in the DSC then heated from 30 to 420 °C. There was an exothermic event at 371 °C (157 J/g), but overall the data did not indicate explosive potential.
To determine impact explodability, 20 mg of notified chemical was placed in a solid

sample holder for each test. The drop weight was raised (to 20 cm) and allowed to fall onto the sample holder. This was then repeated (at 38 cm, then 10 times at 51 cm). The samples were observed for flame, smoke or explosive report. There were no indications of impact sensitivity.

Test Facility Dow (2006a)

Explosive Properties No indication of explosive properties

Method EC Directive 84/449/EEC A.14 Explosive Properties

Remarks The notified chemical was found not to explode under the effect of flame, was not sensitive to shock (fall height 40 cm with a drop weight of 10 kg) and was not sensitive to friction.

Test Facility Huntingdon (1985)

Oxidising Properties No indication of oxidising properties

Method EC Directive 84/449/EEC A.17 Oxidizing Properties (Solids)

Remarks When the notified chemical was tested at 5-90% concentration (in cellulose), the burning rate could only be determined at 5 and 10% concentration. The burning rates were lower than that of the reference mixture (barium nitrate/cellulose).

Test Facility Huntingdon (1985)

Stability Testing Stable at normal and elevated temperatures and in the presence of metals and metal ions

Method OPPTS 830.6313 Stability to Normal and Elevated Temperature, Metals, and Metal Ions

Remarks The stability of the notified chemical to metals and metal ions under normal and elevated conditions was tested. Mixtures of the notified chemical (~2 g) and the metal or metal ions in glass bottles were stored at either 20 or 54 °C for 14 days. Aliquots were removed at 1, 2, 7 or 14 days and analysed by GC-FID to determine the concentration of notified chemical. The metals and metal ions tested were: copper, brass, aluminium, 304 stainless steel, 316 stainless steel, copper (I) chloride, nickel (II) chloride and iron (III) chloride. A control sample without metal or metal ions was also analysed.

Some variability in results obtained for samples in the presence of metal salts was noted, with the study authors commenting that this may have been due to sample inhomogeneity. As the magnitude of any concentration decreases did not increase over time (or at elevated temperatures), the decreases were not considered to be a result of degradation of the notified chemical.

Test Facility Dow (2006b)

Oxidation/Reduction: Chemical Incompatibility No indication of chemical incompatibility

Method OPPTS 830.6314 Oxidation/Reduction: Chemical Incompatibility

Remarks The notified chemical [solid form or as a solution: ~25 g in 45 mL of deionised water (dH_20)] was added to four flasks that were each equipped with a magnetic stirrer. The temperature of the solution was recorded and then the chemical was treated with potassium permanganate (0.3224 g in 25.0528 g of dH_20), monoammonium phosphate (1.2543 g), zinc powder (0.2525 g) or dH_20. The temperatures of the solutions were measured following mixing of the test substance and reagents and after 1, 2, 4 and 24 hours.

There were no significant temperature changes noted. A dark purple colour change was noted in the potassium permanganate flask. The zinc reagent settled to the bottom of the flask and made the notified chemical appear grey. No colour change was observed in the ammonium phosphate solution.

Test Facility Dow (2006a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Notified chemical

METHOD: Similar to OECD TG 401 Acute Oral Toxicity

Species/Strain:
Rat/Wistar
Rat/Sprague-Dawley
Mouse/Swiss Webster
Rabbit/New Zealand albino
Guinea Pig/Hartley

Vehicle:
a) Corn oil
b) Corn oil:acetone (9:1)

Remarks - Method: The acute oral toxicity of the notified chemical was investigated in a number of species. In general, the animals (excluding mice) were administered the notified chemical by gavage, after 15 hours fasting. The animals were observed for 14 days. From the brief record of the studies provided in the study report, it is not clear that the animals were necropsied.

The studies were conducted on the different species at different times and are summarised in a single report [mouse (1961), rat (Wistar; 1961), rabbit (1961), guinea pig (1962) and rat (Sprague-Dawley; 1971)].

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
Rat – male (Sprague-Dawley; 5% in 9:1 corn oil:acetone)			
1	5 M	126	0/5
2	5 M	252	0/5
3	5 M	500	0/5
4	5 M	1000	0/5
Rat – male (Sprague-Dawley; 20% in 9:1 corn oil:acetone)			
5	5 M	1000	2/5
6	5 M	2000	5/5
7	5 M	3980	5/5
Rat – female (Sprague-Dawley; 5% in corn oil)			
8	5 F	252	0/5
9	5 F	500	0/5
Rat – female (Wistar; 10% in corn oil)			
10	5 F	252	2/5
11	5 F	500	0/5
12	5 F	1000	1/5
13	5 F	2000	5/5
14	5 F	3980	5/5
Mouse – female (10% in corn oil)			
15	5 F	252	0/5
16	5 F	500	0/5
17	5 F	1000	5/5
18	5 F	2000	5/5
19	5 F	3980	5/5
Rabbit – mixed sexes (39.8% in corn oil)			
20	2	252	0/2
21	2	500	0/2
22	2	1000	2/2
23	2	2000	2/2
24	2	3980	2/2
Guinea pig – males (39.8% in corn oil)			

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
25	5 M	252	3/5
26	5 M	500	3/5
27	5 M	1000	5/5
28	5 M	2000	5/5
29	5 M	3980	5/5

LD50 1072 mg/kg bw (Sprague-Dawley rat – male)
1231 mg/kg bw (Wistar rat – female)
713 mg/kg bw (Swiss Webster mouse – male)
713 mg/kg bw (New Zealand albino rabbit – mixed sexes)
<252 mg/kg bw (Hartley guinea pig – male)

Signs of Toxicity In general, the deaths occurred within 5 days of dosing.

Clinical signs in high dose rats include diuresis (males and females) incoordination of the hind quarters (females) and bloody nares (males). Diarrhea and hyperexcitability was noted in rabbits prior to death.

Remarks - Results The study authors did not consider the two female Wistar rat mortalities at 252 mg/kg bw to be test-substance related. This is supported by the absence of mortalities that occurred at i) the higher dosage level and ii) in a supplementary study that was conducted in female Sprague-Dawley rats (in 1972) at 252 and 500 mg/kg bw/day. Therefore, these deaths were not used to calculate the LD50.

Mortalities were observed in all species. The guinea pig was the most sensitive species.

CONCLUSION The notified chemical is harmful via the oral route.

TEST FACILITY Dow (1971a)

B.2. Acute toxicity – oral

TEST SUBSTANCE Notified chemical (<20%; microencapsulation formulation)

METHOD OECD TG 425 Acute Oral Toxicity: Up-and-Down Procedure
Species/Strain Rat/Fisher 344
Vehicle None
Remarks - Method No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	1 F	880	0/1
2	1 F	1400	0/1
3	2 F	2220	0/2
4	1 F	3500	0/1
5	3 F	5000	0/3

LD50 >5000 mg/kg bw
Signs of Toxicity A single animal treated at 5000 mg/kg bw was hypoactive with a hunched posture from 1-5 hours post dosing. All animals gained body weight over the study period.
Effects in Organs No abnormalities noted at necropsy.

CONCLUSION The microencapsulated formulation containing the notified chemical at <20% concentration is of low toxicity via the oral route.

TEST FACILITY Eurofins (2007a)

B.3. Acute toxicity – dermal

TEST SUBSTANCE Notified chemical

METHOD Similar to OECD TG 402 Acute Dermal Toxicity – Limit Test
Species/Strain Rabbit/New Zealand albino
Vehicle None or Dowanol DPM glycol ether
Type of dressing Semi-occlusive
Remarks - Method The test substance was administered as a solid or in a vehicle to the skin of rabbits, held in place by a porous gauze patch and tape. A plastic cuff was fitted using rubber bands. The animals were exposed for 24 hours, followed by cleansing of the application site with soap and water, followed by a two week observation period.

The sex of the rabbits was not specified. From the brief record of the study provided in the study report, it is not clear that the animals were necropsied.

RESULTS

Group	*Number of Animals*	*Dose (mg/kg bw)*	*Mortality*
Solid test substance			
1	4	2000	0/4
2	4	3980	4/4
25% in Dowanol DPM glycol ether			
3	2	252	0/2
4	2	500	0/2
5	2	1000	1/2
50% in Dowanol DPM glycol ether			
6	2	500	0/2
7	2	1000	2/2
8	2	2000	2/2

LD50 2830 mg/kg bw (solid)
848 mg/kg bw (applied with vehicle)
Signs of Toxicity – Systemic None in surviving animals. It was noted that some of the rabbits that died during the study were hyperexcitable and vocal prior to death.
Remarks - Results All animals gained weight over the study period. Mortalities were observed at lower doses when the notified chemical was administered in a vehicle.

CONCLUSION The notified chemical is harmful via the dermal route.

TEST FACILITY Dow (1971a)

B.4. Acute toxicity – dermal

TEST SUBSTANCE Notified chemical

METHOD Similar to OECD TG 402 Acute Dermal Toxicity – Limit Test
Species/Strain Rabbit/New Zealand white
Vehicle Water
Type of dressing Semi-occlusive
Remarks - Method The test substance was administered as a solid to the skin of rabbits, held in place by a porous gauze patch and non-irritating tape. A plastic cuff was fitted using rubber bands and 5 mL of water was injected under the cuff to enhance skin contact. The animals were exposed for 24 hours,

followed by cleansing of the application site with soap and water.

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	Slight-moderate erythema was observed in all animals at 24 hours post-exposure.
Signs of Toxicity – Systemic	Clinical signs following dosing included lethargy and decreased appetite in all animals and rapid shallow respiration in 3/5 males and 2/5 females. There were no clinical signs after the first day.
Remarks - Results	All animals gained weight over the study period. A slight body weight decrease was observed in most animals 1-day post-treatment (coinciding with the decreased appetite), but all animals, with the exception of one male, gained weight over the 14 day observation period.

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

TEST FACILITY Dow (1986a)

B.5. Acute toxicity – dermal

TEST SUBSTANCE Notified chemical (<20%; microencapsulation formulation)

METHOD OECD TG 402 Acute Dermal Toxicity – Limit Test

Species/Strain	Rat/Fischer 344
Vehicle	None
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	5000	0/5
2	5 F	5000	0/5

LD50	>5000 mg/kg bw
Signs of Toxicity - Local	None
Signs of Toxicity - Systemic	None
Effects in Organs	No gross abnormalities
Remarks - Results	All animals gained weight over the study period

CONCLUSION The microencapsulated formulation containing the notified chemical at <20% concentration is of low toxicity via the dermal route.

TEST FACILITY Eurofins (2007b)

B.6. Acute toxicity – inhalation

TEST SUBSTANCE Notified chemical

METHOD Similar OECD TG 403 Acute Inhalation Toxicity – Limit Test

Species/Strain	Rat/Fischer F344
Vehicle	None
Method of Exposure	Whole-body exposure

Exposure Period	4 hours
Physical Form	Gas
Remarks – Method	Solid test substance (in a J-tube) was volatilised using compressed air that was heated using a flameless heat torch. It is noted that the air through the J-tube was diluted with the main chamber air before entering the chamber, which resulted in the substance recrystallising in the main chamber airstream before entering the chamber. It is further noted that no crystals were observed in the main chamber..

RESULTS

Group	*Number and Sex of Animals*	*Concentration (ppm)*		*Mortality*
		Nominal	*Actual*	
1	6 M	17.6	2.75	0/6
2	6 F	17.6	2.75	0/6

LC50	>2.75 ppm (~0.03 mg/L; 4 hours)
Signs of Toxicity	None.
Effects in Organs	There were single observations of distended ovary and herniated liver in females. However, these effects were not considered treatment related.
Remarks - Results	Body weight losses were observed in animals of both sexes between days 2 and 5, but all animals gained weight for the remainder of the study. Very slight eye irritation was noted in all animals at the end of the exposure period but was reported to have reversed within one hour.

CONCLUSION Based on the low concentration of notified chemical in this study, a conclusion on the toxicity via inhalation cannot be made.

TEST FACILITY Dow (1986b)

B.7. Acute toxicity – inhalation

TEST SUBSTANCE Notified chemical (<20%; microencapsulation formulation)

METHOD OECD TG 403 Acute Inhalation Toxicity – Limit Test

Species/Strain	Rat/F344 DuCrl
Vehicle	None
Method of Exposure	Oro-nasal exposure
Exposure Period	4 hours
Physical Form	Liquid aerosol
Particle Size	MMAD = 2.84 μm (GSD = 2.65)
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Concentration (mg/L)*		*Mortality*
		Nominal	*Actual*	
1	5 M	91	3.51	0/5
2	5 F	91	3.51	0/5

LC50	>3.51 mg/L (4 hours)
Signs of Toxicity	None
Effects in Organs	None
Remarks - Results	Body weight losses were observed in animals of both sexes on day 2, but all animals exceeded their initial body weights on day 4 and continued to gain weight throughout the study.

	Maximum attainable concentration with a respirable particle size was 3.51 mg/L. Approximately 76% of the aerosolised particles had a diameter less than 6.1 µm.
CONCLUSION	Under the conditions of the study, the microencapsulated formulation containing the notified chemical at <20% concentration was of low toxicity via inhalation.
TEST FACILITY	Dow (2007c)

B.8. Irritation – skin

TEST SUBSTANCE	Notified chemical
METHOD	In-house method
Species/Strain	Rabbit/New Zealand albino
Number of Animals	3
Vehicle	None
Observation Period	3 week study period
Type of Dressing	Semi-occlusive
Remarks - Method	The abdomens of three rabbits were shaved. Approximately one gram of test substance was applied to intact and freshly abraded skin. For intact skin, the test substance was applied for five days per week for two weeks. For abraded skin, the exposure period was of three days duration. The animals were examined for irritation daily. Body weights and behaviour were recorded.
RESULTS	
Remarks - Results	The test substance was noted as causing very slight to slight erythema, and slight exfoliation at the intact and abraded sites. Additionally, slight scabbing was observed on abraded sites. There were no signs of toxicity. Week 2 body weights were greater than the pre-exposure weights in 2/3 animals, but was less in the other animal. The terminal body weights were greater than pre-exposure weights in all animals.
CONCLUSION	Under the conditions of the study, the notified chemical was slightly irritating to the skin.
TEST FACILITY	Dow (1971a)

B.9. Irritation – skin

Formulation

TEST SUBSTANCE	Notified chemical (<20%; microencapsulation formulation)
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion
Species/Strain	Rabbit/New Zealand albino
Number of Animals	3 males
Vehicle	None
Observation Period	10 days
Type of Dressing	Semi-occlusive
Remarks - Method	Skin examined at 1, 24, 48, 67 hours, and at 7 and 10 days, post dosing.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	1.7	1.7	1.7	3	<10 days	0

Oedema	1.0	0.7	0.7	2	<7 days	0

*Calculated on the basis of the scores at 24, 48, and 67 hours for each animal.

Remarks - Results: The standard 72 hour examination was made 5 hours early but this deviation is not expected to have affected the outcome of the study.

CONCLUSION: The microencapsulated formulation containing the notified chemical at <20% concentration is slightly irritating to the skin.

TEST FACILITY: Eurofins (2007c)

B.10. Irritation – eye

TEST SUBSTANCE: Notified chemical

METHOD: Similar to OECD TG 405 Acute Eye Irritation/Corrosion
Species/Strain: Rabbit/New Zealand albino
Number of Animals: 4
Observation Period: 7 days
Remarks - Method: The eyes of two rabbits were examined for signs of defects or irritation prior to commencing.

One rabbit was administered 0.1 mg of solid test substance into the conjunctival sac of the right eye. After 30 seconds after, the eye was washed with water. The left eye was then administered the same dose but was left unwashed.

The second rabbit was administered 0.1 mL of 10% test substance in propylene glycol (into both eyes) using the above procedure. The animals were observed for irritation.

RESULTS

Remarks - Results: It is noted that administration of the solid test substance resulted in moderate conjunctival inflammation, slight transient iritis and transient corneal haziness in the unwashed eye, whereas the washed eye exhibited slight transient conjunctival inflammation. Both eyes appeared normal after 48 hours.

It is noted that administration of the test substance in propylene glycol resulted in moderate conjunctival inflammation in the unwashed eye, whereas the washed eye displayed slight conjunctival inflammation. Both eyes appeared normal after one week.

CONCLUSION: Under the conditions of the study, the notified chemical was irritating to the eye.

TEST FACILITY: Dow (1971a)

B.11. Irritation – eye

TEST SUBSTANCE: Notified chemical

METHOD: Similar to OECD TG 405 Acute Eye Irritation/Corrosion
Species/Strain: Rabbit/New Zealand White
Number of Animals: 6 (4F + 2M)
Observation Period: 21 days
Remarks - Method: 0.1 g of the notified chemical was administered as a solid into the conjunctival sac, the eyes remained unwashed.

RESULTS

Lesion	*Mean Score**	*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
Conjunctiva: redness	2.6	3	<21 days	0
Conjunctiva: chemosis	1.3	3	<7 days	0
Conjunctiva: discharge	0.6	3	<72 hours	0
Corneal opacity	0.3	2	<14 days	0
Iridial inflammation	1.0	1	<7 days	0

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

CONCLUSION The notified chemical is irritating to the eye.

TEST FACILITY Dow (1986c)

B.12. Irritation – eye

TEST SUBSTANCE Notified chemical (<20%; microencapsulation formulation)

METHOD OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain Rabbit/New Zealand White
Number of Animals 3 M
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	0.7	0.7	0.7	2	<72 hours	0
Conjunctiva: chemosis	0	0	0	0	no effects	0
Conjunctiva: discharge	0.3	0.3	0	1	<48 hours	0
Corneal opacity	0	0	0	0	no effects	0
Iridial inflammation	0	0	0	0	no effects	0

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

CONCLUSION The microencapsulated formulation containing the notified chemical at 17.9 % concentration is slightly irritating to the eye.

TEST FACILITY Eurofins (2007d)

B.13. Skin sensitisation

TEST SUBSTANCE Notified chemical

METHOD Similar to OECD TG 406 Skin Sensitisation (1981) – Split Adjuvant
Species/Strain Guinea pig/Hartley albino
PRELIMINARY STUDY Not conducted
MAIN STUDY
Number of Animals Test Group: 10 Positive Control Group: 10
INDUCTION PHASE Induction Concentration: 10% in Dowanol DPM/Tween 80 (9:1).
Topical: 4 × 0.1 mL topical applications made on days 2, 5, 7 and 9.
Intradermal: 2 × 0.1 mL of Freund's Complete Adjuvant, adjacent to the topical site, at the third induction.
Signs of Irritation No irritation was observed during induction in animals of either the test or positive control groups.
CHALLENGE PHASE
1st challenge Topical: 10% in Dowanol DPM/Tween 80 (9:1), following a two week rest period.

Remarks - Method

The induction and challenge concentrations were the same. The challenge applications were not occluded.

A test substance/negative control component of the study was not conducted. The animals of the positive control group were treated with DER 331 epoxy resin in a similar fashion as the animals of the test group.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions*	
		24 h	*48 h*
Test Group	10% notified chemical	3/10	3/10
Positive Control Group	10% DER 331 epoxy resin	10/10	10/10

Remarks - Results

A positive response was observed in 30% of test animals. The study authors concluded that the test substance should be considered as possessing some potential to induce human skin sensitisation.

In the absence of a negative control component to this study (and considering the concentration of test substance that was tested at the induction and challenge phases), it is considered that the study provides evidence of reactions of skin sensitisation.

CONCLUSION

There was evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.

TEST FACILITY

Dow (1986d)

B.14. Skin sensitisation – mouse local lymph node assay

TEST SUBSTANCE

Notified chemical (<20% microencapsulation formulation)

METHOD

OECD TG 429 Skin Sensitisation: Local Lymph Node Assay

Species/Strain

Mouse/ CBA/J (female)

Vehicle

Pluronic L92 surfactant (1% w/v) in water

Remarks - Method

In an irritation screening study, mice (1 animal/concentration) were administered three consecutive daily 25 µL doses of the test substance at 1, 5, 25, 50, 75 or 100% on the dorsal surface of each ear. While no irritation was noted at any of the tested concentrations, a notable weight loss in the animal treated with 100% test substance was recorded.

The main study was conducted using 5 mice/group at 0, 5, 25 and 75% concentration. A concurrent positive control study using 30% α-hexylcinammaldehyde (HCA) was also conducted.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM)*	*Stimulation Index (Test/Control Ratio)*
Test substance		
0 (vehicle control)	607	-
5	680	1.2
25	1174	1.9
75	964	1.6
Positive Control (HCA)		
30	7371	12.2

Remarks - Results

The stimulation index values for the test substance groups were <3, indicating the absence of a skin sensitisation response.

CONCLUSION	There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	Dow (2007d)

B.15. Short-term toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 410 Repeated Dose Dermal Toxicity: 21/28-day Study.
Species/Strain	Rabbit/New Zealand white
Route of Administration	Dermal – semi-occluded
Exposure Information	Total exposure days: 21 days Dose regimen: 5 days per week (at least two days prior to necropsy)
Vehicle	None (gauze patch moistened with water)
Remarks – Method	The test material was applied solid to a 10×15 cm area on the back of the rabbits and was held in place by a moistened gauze patch, backed by nonabsorbent cotton held in place by an elastic jacket. Patches were removed after six hours and the treatment site was cleansed to remove residual test material. In a range-finding study, rabbits (2/sex/dose) were administered 1000 mg/kg bw/day for four days. There were no signs of toxicity observed and therefore 1000 mg/kg bw/day was selected as the high dose.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
control	5M + 5F	0	0/10
low dose	5M + 5F	100	0/10
mid dose	5M + 5F	500	0/10
high dose	5M + 5F	1000	0/10

Clinical Observations

There were no clinical signs of toxicity in any group. Very slight to well-defined erythema was observed in all treatment groups and was dose dependent. Very slight oedema was observed in 500 and 1000 mg/kg bw/day groups only. These results are indicative of dermal irritation. These findings were confirmed during pathological examination. There were no treatment related effects on body weights.

Laboratory Findings – Clinical Chemistry and Haematology

There were no treatment related findings in clinical chemistry or haematology in any treatment group.

Effects in Organs

Increases in absolute and relative liver weights were statistically significantly increased in males (↑16%/↑20% absolute/relative) and females (↑18%/↑22% absolute/relative) treated at 1000 mg/kg bw/day and there was evidence of a dose response in both sexes, with non-statistically significant increases noted in 500 mg/kg bw/day females (↑18%/↑20% absolute/relative). The findings were not accompanied by associated clinical pathology or histopathological findings but the statistically significant changes at 1000 mg/kg bw/day are considered to be treatment related toxicologically relevant effects given that liver toxicity has been noted in other repeat dose studies.

Histopathological analysis revealed local effects in treatment groups including very slight to moderate epidermal hyperplasia, focal to multifocal epidermal pustules, multifocal to diffuse hyperkeratosis, multifocal parakeratosis, very slight to moderate dermal inflammation, and multifocal dermal oedema. These effects were generally dose related. There were no other macroscopic or microscopic findings in the examined tissues (liver, kidney and testes).

CONCLUSION
The NOAEL was established as 500 mg/kg bw/day in this study, based on increased liver weights at 1000 mg/kg bw/day.

TEST FACILITY Dow (1992)

B.16. Subchronic toxicity

TEST SUBSTANCE Notified chemical

METHOD Similar to OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents.

Species/Strain	Rat/Fischer-344
Route of Administration	Oral – diet
Exposure Information	Total exposure days: 91 days Dose regimen: 7 days per week
Remarks - Method	The brain, liver, kidneys and gonads were the only organs weighed.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
control	10M + 10F	0	0/20
low dose	9M* + 10F	10	0/19
mid dose	10M + 10F	40	1/20
high dose	10M + 10F	120	0/20

*One animal removed from this group early in the study because it was incorrectly classified as male.

Mortality and Time to Death
There were no treatment related mortalities during the study. A female treated at 40 mg/kg bw/day was found moribund on day 7 and removed from the study. This mortality was attributed to a urinary tract infection.

Clinical Observations
There were no treatment related clinical observations. One control female had transient weight loss and decreased faeces on day 57 but recovery was observed. One female treated at 120 mg/kg bw/day had a single observation of wet perineum.

There were statistically significant decreases in absolute body weights in males and females treated at 120 mg/kg bw/day, which corresponded to decreased body weight gains in males (↓13%) and females (↓11%) treated at 120 mg/kg bw/day. Body weight decreases at other doses were not considered treatment related. There was no effect on feed consumption.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
There were statistically significant decreases in packed cell volume, haemoglobin and red blood cell count in males (↓2%, ↓4% and ↓6%, respectively) and females (↓4%, ↓6% and ↓5%, respectively) treated at 10, 40 and 120 mg/kg bw/day. Red blood cells were reported as being of normal size.

Total bilirubin was statistically increased in (↑95%) and females (↑70%) treated at 120 mg/kg bw/day, and males treated at 40 mg/kg bw/day had a non-statistically significant increase (↑60%). The study authors stated that this was likely a result of an interference with hepatocyte processing of bilirubin. Serum glucose was statistically decreased in males (↓15%) and females (↓12%) treated at 120 mg/kg bw/day. There were increases in albumin in males (↑6%) and females (↑6%) treated at 120 mg/kg bw/day, that were statistically significant in males only. The changes in glucose and albumin are considered to be small and within the expect limits of biological variability for this species of rat.

The only change observed in urinalysis was a statistically significant decrease in specific gravity in males treated at 120 mg/kg bw/day (↓2%).

Effects in Organs
Effects in organs were generally limited to the liver and kidneys. Absolute and relative liver weights were

statistically increased in males treated at 40 mg/kg bw/day (↑1%/13; absolute/relative) and 120 mg/kg bw/day (↑69%/80%; absolute/relative). Increases were smaller in females but were statistically significant in the 120 mg/kg bw/day (40%/49%; absolute/relative) group. Macroscopic examination revealed general paleness to the hepatic parenchyma in males (8/10) and females (10/10) treated 120 mg/kg bw/day. These liver effects were associated with treatment related histopathological changes (see following Table) such as diffuse centrilobular hypertrophy, and diffuse centrilobular and midzonal vacuolation of hepatocytes (consistent with fatty changes) in males and females treated at 120 mg/kg bw/day. The low incidence and severity of hypertrophy in 40 mg/kg bw/day groups is not considered to be toxicological significant and likely indicates the threshold for toxicity. Hyperplasia was observed in all treatment groups other than controls but as there was no dose response, the relevance of this effect remains unclear and is possibly incidental.

Absolute and relative kidney weights were statistically increased in 120 mg/kg bw/day males (9%/16% absolute/relative). A non-statistical increase in absolute kidney weights was observed in 120 mg/kg bw/day females, accompanied by a statistical increase in relative kidney weights (5%/13% absolute/relative), consistent with the observation of more severe histopathological changes in males. Very slight nephrosis (1-2 ectatic tubules lined by necrotic epithelium per kidney section) was observed in males treated at 40 and 120 mg/kg bw/day males, with some rats in the 120 mg/kg bw/day group exhibiting slight nephrosis (3-6 ectatic tubules lined by necrotic epithelium per kidney section). The study authors attributed the aggregated mononuclear cells (mostly lymphoid) and increased incidence and severity of tubule degeneration/regeneration in 40 and 120 mg/kg bw/day males as indicators of tubulointestitial disease, which is common in this type of rat. The high incidence in controls suggests that the tubule degeneration/regeneration may be of limited toxicological significance but the increased severity of the lesion does suggest an association to treatment. The only clear treatment related kidney effect in females was a dose related increase of brown pigment in the convoluted tubule in 40 and 120 mg/kg bw/day groups.

	Males (mg/kg bw/day)				*Females (mg/kg bw/day)*			
	0	*10*	*40*	*120*	*0*	*10*	*40*	*120*
Liver (10 sex/dose)								
hyperplasia, epithelial bile duct, multifocal	0	2(1.0)	1(1.0)	1(1.0)	0	1(1.0)	3(1.0)	1(1.0)
hypertrophy, centrilobular	0	0	1(1.0)	10(2.0)	0	0	1(1.0)	10(1.0)
hypertrophy, diffuse vacuolation, centrilobular and midzonal, diffuse	0	0	0	10(3.9)	0	0	0	10(3.6)
Kidney (10 sex/dose)								
aggregate of mononuclear cells	0	0	2(1.0)	9(1.1)	0	1(1.0)	0	0
nephrosis, multifocal	0	0	2(1.0)	10(1.4)	0	0	0	0
brown pigment, convoluted tubule, multifocal	0	0	0	0	0	0	3(1.0)	10(1.0)
tubule, degen./regen.	8(1.0)	6(1.0)	10(1.8)	10(2.5)	1(1.0)	2(1.0)	1(1.0)	3(1.0)

( ), Average severity of affected animals: 1=very slight, 2=slight, 3=moderate, 4=severe.

CONCLUSION

The NOAEL was established as 10 mg/kg bw/day in this study, based on increased liver weights, and kidney nephrosis and tubule degeneration/regeneration in males treated at 40 mg/kg bw/day and above, and brown pigment in the convoluted tubule in females treated at 40 mg/kg bw/day and above.

TEST FACILITY Dow (1986e)

B.17. Subchronic toxicity

TEST SUBSTANCE Notified chemical

METHOD OPP No 82-1 90 Day Oral Toxicity in Rodents
Similar to OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents

Species/Strain	Mouse/B6C3F1
Route of Administration	Oral – diet
Exposure Information	Total exposure days: 95-96 days Dose regimen: 7 days per week
Vehicle	Acetone
Remarks - Method	Urinalysis was not conducted in this study.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*			*Mortality*
		Nominal	*Achieved**		
			Males	*Females*	
control	10M + 10F	0	0	0	2/20
low dose	10M + 10F	200	195.6	195.9	0/20
mid dose	10M	300	293.7	-	0/10
high dose	10M + 10F	400	393.5	388.8	0/20
high dose	10M + 10F	600	541.8	515.8	20/20
high dose	10F	800	-	615.8	10/10

*Mean intake values over the duration of the study.

Mortality and Time to Death

Nine of the females treated at 800 mg/kg bw/day were found dead or moribund between days 4 and 6 and the remaining animal was sacrificed on day 8. All males and females treated at 600 mg/kg bw/day, except one male, were found dead or moribund (days 38-41 for females and 46-53 for males) and the remaining animal was sacrificed on day 54. One control male was found dead on day 82 and one control female was found moribund and sacrificed on day 89. The cause of death for the control animals could not be determined.

Clinical Observations

There were no treatment related clinical observations in 200, 300 or 400 mg/kg bw/day groups. Decreased respiratory rate, decreased faecal volume, lethargy, hypothermia and general poor condition were observed prior to death in the 600 and 800 mg/kg bw/day groups.

Ophthalmological examination in surviving animals did not reveal any treatment related findings.

Body weights were markedly reduced in the 600 and 800 mg/kg bw/day groups prior to death or sacrifice (The body weights of 200 and 300 mg/kg bw/day groups were similar to controls over the study period). The absolute body weights of males and females treated at 400 mg/kg bw/day were slightly reduced compared to the controls throughout the treatment period. Overall, the data suggests a treatment related effect on body weights in males and females in animals treated at 400 mg/kg bw/day and above.

Feed consumption was markedly decreased in the 600 and 800 mg/kg bw/day groups prior to death. Feed consumption was generally lower in the 400 mg/kg bw/day groups compared to the control group, but the decreases were considered minor.

Laboratory Findings – Clinical Chemistry and Haematology

There were statistically significant decreases in males and females treated at 400 mg/kg bw/day of haemoglobin concentration (↓10%/11%; males/females), haematocrit (↓7%/6%; males/females) and platelet counts (↓16%/20%; males/females), with a statistically significant decrease of platelet counts in 300 mg/kg bw/day males (↓11%) suggesting a dose response. Additionally, females treated at 400 mg/kg bw/day exhibited a statistically significant increase in mean reticulocyte count (↑21%) and mean total leukocyte count (↑99%). The increase in leukocytes was attributed to a significant increase in absolute lymphocyte counts. These haematology effects are considered treatment related.

There were non-significant increases in aspartate aminotransferase in males treated at 400 mg/kg bw/day (↑128%). Alanine aminotransferase was statistically increased in males (↑335%) and females (↑135%) treated at 400 mg/kg bw/day, and in males (↑81%) treated at 300 mg/kg bw/day. These enzymes are indicative of liver function and are suggestive of hepatocellular injury. There were statistically significant decreases in females treated at 400 mg/kg bw/day females of glucose levels (↓30%), total protein (↓5%), albumin (↓11%) and albumin/globulin ratio (↓17%). Alkaline phosphatase was significantly increased in males but decreased in females and is therefore considered incidental.

Effects in Organs

There were statistically significant increases in absolute and relative liver weights in all treated males and females, with a clear dose response (see following Table). Additionally, there were statistically significant increases in absolute and relative kidney and epididymis/testis weights in males treated at 400 mg/kg bw/day. The only treatment related macroscopic findings was enlarged liver in males (7/10) and females (6/10) treated at 600 mg/kg bw/day, which is consistent with these groups having survived long enough for the effect to develop.

	Males (mg/kg bw/day)				*Females (mg/kg bw/day)*		
	0	*200*	*300*	*400*	*0*	*200*	*400*
Liver (10/sex/dose)							
absolute weight (g)	1.04	1.34** (↑29%)	1.53** (↑48%)	1.64** (↑58%)	1.00	1.26** (↑26%)	1.68** (↑69%)
relative to bw	5.06	6.30* (↑25%	7.48** (↑48%)	8.61** (↑70%)	5.03	6.39* (↑27%)	8.60** (↑71%)
Kidney (10/sex/dose)							
absolute weight (g)	0.46	0.50	0.44	0.40** (↓14%)	0.36	0.37	0.35
relative to bw	2.26	2.37	2.13	2.09* (↓8%)	1.84	1.86	1.79
Epididymis/testis (10/sex/dose)							
absolute weight (g)	0.35	0.33	0.32	0.28** (↓18%)	-	-	-
relative to bw	1.70	1.60	1.56	1.49* (↓12%)	-	-	-

Statistically significant compared to control (**P<0.01, *P<0.05)

There were incidences of extramedullary haematopoiesis in the spleen in all treatment groups in both sexes with increased severity at 400 mg/kg bw/day, but there were also high incidences in control groups and therefore this effect is not considered to be toxicologically significant.

The uterus and ovaries were slightly to moderately hypoplastic/atrophic in females treated at 400 mg/kg bw/day with no observations reported in the control or 200 mg/kg bw/day groups. The hypoplastic/atrophic uterus and ovaries were smaller, and ovaries had fewer corpora lutea than controls. There were also 3 females treated at 400 mg/kg bw/day with minimal to slight atretic corpora lutea with no observations in controls. The study authors were unsure whether these effects were due to atrophy or because the animals had not fully matured.

Effects in the liver occurred at all dose levels in males and females (see following Table). Hypertrophy was the only effect observed in males and females treated at 200 mg/kg bw/day, with severity increasing in both sexes. Observations in males and females treated at 400 mg/kg bw/day include centrilobular intracytoplasmic vacuoles, clear vacuoles, single cell necrosis, inflammatory cell infiltrate and intracytoplasmic green/brown pigment in the Kupffer cells. Centrilobular intracytoplasmic vacuoles, clear vacuoles, single cell necrosis were also observed in males treated at 300 mg/kg bw/day and were dose related. These effects are clear indicators of liver toxicity at 200 mg/kg bw/day and above.

	Males (mg/kg bw/day)				*Females (mg/kg bw/day)*		
	0	*200*	*300*	*400*	*0*	*200*	*400*
Liver (10/sex/dose)							
centrilobular to panlobular hypertrophy	0	10(2.7)	10(3.2)	10(4.0)	0	10(2.5)	10(4.0)
centrilobular intracytoplasmic vacuoles	0	0	10(1.9)	10(2.4)	0	0	10(1.6)
clear vacuoles	0	0	5(1.2)	6(1.7)	0	0	9(1.4)
single cell necrosis	0	0	8(1.2)	10(2.2)	0	0	10(1.2)
inflammatory cell infiltrate	0	0	0	10(1.6)	0	0	7(1.0)
intracytoplasmic pigment in Kupffer cells	0	0	0	10(1.8)	0	0	10(1.3)

( ), Average severity of affected animals: 1=minimal, 2=slight, 3=moderate, 4=moderately severe.

CONCLUSION
The LOAEL was established as 200 mg/k gbw/day, based on liver hypertrophy and increased liver weights at the lowest dose tested.

TEST FACILITY Pharmaco (1995)

B.18. Chronic toxicity

TEST SUBSTANCE Notified chemical

METHOD OPP 83-1 Chronic toxicity
Similar to OECD TG 452 Chronic Toxicity Studies
Species/Strain Dog/beagle
Route of Administration Oral – diet
Exposure Information Total exposure: 12 months
Dose regimen: 7 days per week
Remarks - Method Blood was collected for haematological and clinical chemistry determinations just prior to commencement, and at 3, 6 and 12 months. Urinalysis was conducted at 12 months.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
control	4M + 4F	0	0/8
low dose	4M + 4F	0.5	0/8
mid dose	4M + 4F	3	0/8
high dose	4M + 4F	15	0/8

Mortality and Time to Death
No mortalities were observed.

Clinical Observations
The dogs were reported as being healthy during the study period, with the exception of demodectic mange, which was initially noted in 1 female treated at 0.5 mg/kg bw/day and one male treated at 15 mg/kg bw/day. The symptoms appeared from day 155 to day 199. All dogs were treated (including prophylactic treatment) with Mitaban four to five times during this time period. There were no treatment related effects on body weights or body weight gains, or on feed consumption during the study.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
There were no treatment related findings in haematology or urinalysis.

There were increases in alkaline phosphatase (ALP) levels at 6 and 12 months in males (↑95% and ↑187%, respectively) and females (↑67% and ↑137%, respectively) treated at 15 mg/kg bw/day. Increases in cholesterol levels were also noted in males (↑35-62%) and females (↑38-64%) treated at 15 mg/kg bw/day, from 3 months treatment, which were considered by the study authors to be treatment related.

Effects in Organs
Absolute and relative liver weights were statistically significantly increased in males (↑49%/↑49%; absolute/relative) and females (↑29%/↑21%; absolute/relative) treated at 15 mg/kg bw/day and the increases were considered to be treatment related. The relative heart weight was statistically significantly decreased in males and females treated at 15 mg/kg bw/day, but was not considered to be treatment related due to the absence of related findings (including clinical chemistry and histopathology).

The only macroscopic observation at necropsy was a single incidence of decreased size of the right testes in 1 male treated at 3 mg/kg mg/day, but this was considered a sporadic finding, due to the lack of a dose-response relationship.

Slight hepatocellular hypertrophy was observed in males and females treated at 15 mg/kg bw/day. In all 4 males and 2 females, the hypertrophy was diffuse and panlobular, but was centrilobular with extension into

midzonal region of the hepatic lobule in the remaining 2 females.

CONCLUSION
The NOAEL was established as 3 mg/kg bw/day, based on increased levels of ALP and cholesterol, increased liver weights and hepatocellular hypertrophy in both males and females at 15 mg/kg bw/day.

TEST FACILITY Dow (1989a)

B.19. Chronic toxicity/carcinogenicity

TEST SUBSTANCE Notified chemical

METHOD OPP 83-1 Chronic toxicity and OPP 83-2 Carcinogenicity
Similar to OECD TG 453 Combined Chronic Toxicity/Carcinogenicity Studies, respectively

Species/Strain Rat/Fischer 344

Route of Administration Oral – diet

Exposure Information Total exposure: 12 months (chronic), 24 months (carcinogenicity)
Dose regimen: 7 days per week

Remarks - Method In a combined chronic/carcinogenicity study, rats (60/sex/dose) were administered the test substance in the diet for 24 months. The chronic study animals (10/sex/dose) were randomly assigned at the beginning of the study and were sacrificed at 12 months and subject to necropsy and histopathology (for the low and mid-dose groups, histopathologic examination was limited to the liver, kidneys and tissues with gross lesions).

Haematology, clinical chemistry determinations and urinalysis were conducted at 6, 12, 18 and 24.

Surviving animals were sacrificed at 24 months. Necropsy and histopathology was conducted on the animals of the control and high dose groups (and any mortalities). Histopathology was only conducted on animals of the low and middle dose groups for selected endpoints, based on detected lesions at the high dose. The brain, kidney, adrenal glands, liver and gonads were weighed.

RESULTS

Group	*Number and Sex of Animals*	*Nominal dose (mg/kg bw/day)*	*Mortality[a]*	
			Males	*Females*
control	60M + 60F	0	12/50	18/50
low dose	60M + 60F	5	10/50	23/50
mid dose	60M + 60F	20	12/50	16/50
high dose	60M + 60F	60	23/50*	19/50

*Statistically significant compared to control (P<0.05).
[a]Data presented for the carcinogenicity phase of the study only.

Mortality and Time to Death
There was a statistically significant increase in the incidence of mortalities in males treated at 60 mg/kg bw/day. The most common cause of death of the animals in this group was severe chronic progressive glomerulonephropathy (10/23 mortalities), although this was also observed in the control group (1/12). Mortalities in other treatment groups were similar to the controls in terms of incidence and time of death, with the exception of single females from the 20 and 60 mg/kg bw/day groups that died at day 85 (meningeal haemorrhage) and 175 (undetermined cause), respectively. Common causes of mortality in the treated and control groups were leukaemia and pituitary neoplasms.

Clinical Observations, Body weights and Feed Consumption
There were no clinical signs that were attributed to treatment with the test substance by the study authors, as all observations were similar to controls.

Body weight gains of the treated animals were similar to the control animals during the first year of the study, although there was a statistically significant body weight gain decrease in females treated at 60 mg/kg bw/day (↓5%).

There were statistically significant decreases in absolute body weights in males treated at 20 and 60 mg/kg bw/day during the second year of the study and the overall body weight gains (day 0 to 735) were decreased in males treated at 20 and 60 mg/kg bw/day (↓9% and ↓20%, respectively). In females, there were statistically significant decreases in absolute body weights in animals of the 60 mg/kg bw/day groups throughout the second year and the overall body weight gain was decreased (↓15%). The decreases in body weight gain were considered treatment related in males and females treated at 60 mg/kg bw/day, and in males treated at 20 mg/kg bw/day.

The final fasted absolute body weights were statistically decreased at 24 months in males (↓15%) and females (↓12%) of the 60 mg/kg bw/day group, and in males treated at 20 mg/kg bw/day (↓8%).

Feed consumption was similar in treated and control groups.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Changes in haematology parameters in males treated at 60 mg/kg bw/day included statistically significant decreases in erythrocyte count, haemoglobin and/or haematocrit at the 6, 12 and 18 month evaluations, but no changes were observed at 24 months. Additionally, in 20 mg/kg bw/day males there were statistically significant decreases in erythrocyte count and haemoglobin at 12 months. Statistically significant changes in platelet count in males at 6 and 18 months were considered incidental due to the fluctuating nature of the changes. In females, there was a statistically significant decrease in platelet count in females treated at 60 mg/kg bw/day. The study authors did not consider the haematology changes to be a primary effect of the test substance.

Treatment related findings included decreased creatine phosphokinase in males at most observation points, and in females during the second year (see following Table). Treatment related clinical chemistry parameters in males during the first year only include decreases in alanine aminotransferase (ALT), aspartate aminotransferase (AST), and increased total bilirubin. Blood urea nitrogen concentration was increased in males treated at 60 mg/kg bw/day at all observations points. Other changes in males include decreases in, albumin and total protein, and increased total bilirubin and phosphorus. There were also increases in blood urea nitrogen in males treated at 60 mg/kg bw/day. Changes in females were mostly limited to the 60 mg/kg bw/day groups (increased ALT, AST, blood urea nitrogen, cholesterol and total bilirubin).

The study authors state that the clinical chemistry parameters at the 12 month sacrifice (ALT, AST and creatine phosphokinase) were consistent with a histomorphologic lesion in the liver, but that the clinical chemistry changes at 24 months did not reflect a response in the liver because of potential loss of serum enzymes due to the severity of renal lesions.

	Males (mg/kg bw/day)				*Females (mg/kg bw/day)*			
	0	*5*	*20*	*60*	*0*	*5*	*20*	*60*
Creatine phosphokinase[a] (IU/L)								
6 month	348	276 (↓□□ %)	210* (↓40%)	187* (↓46%)	157	153	152	148
12 month	364	188* (↓49%)	245 (↓33%)	218* (↓41%)	157	144	141	139
18 month	364	333 (↓9%)	254* (↓31%)	180* (↓51%)	311	246	229	170* (↓45%)
24 month	424	154* (↓64%)	245* (↓42%)	267* (↓37%)	23	157* (↓33%)	133* (↓43%)	162* (↓31%)

*Statistically significant compared to control (P<0.05).
[a] values rounded to whole numbers.

The only treatment related finding from urinalysis was a statistically significant decrease in specific gravity in 60 mg/kg bw/day males.

Effects in Organs – General

In 60 mg/kg bw/day males there were statistically significant increases in absolute and relative liver weights at 12 months (↑27%/↑31%; absolute/relative) and at 24 months (↑41%/↑67%; absolute/relative). Statistically significant increases in liver weights were also observed in females treated at 60 mg/kg bw/day at 12 months (↑13%/↑20%; absolute/relative) and 24 months (↑46%/↑64%; absolute/relative).

Other organ weight changes were mostly observed at 24 months, including statistically significant increases in kidney weights in 60 mg/kg bw/day males (↑17%/↑38%; absolute/relative) and females (↑10%/↑24%; absolute/relative) with a statistically significant increase in relative kidney weight in 20 mg/kg bw/day males (↑12%). Additionally, there were increases in adrenal weights in males treated at 60 mg/kg bw/day (↑69%/↑90%; absolute/relative), which were considered by the study authors to be a secondary due to stress from advanced renal disease. Relative brain weights were statistically significantly increased at 24 months in males (↑16%) and females (↑12%), although the study authors associated this with the decrease in final body weights.

Macroscopic observations possibly related to treatment were increased liver size in 60 mg/kg bw/day males and females at 12 months and roughened surface of the kidneys in males treated at 60 mg/kg bw/day at 24 months.

Treatment related histopathological changes were found in the liver and kidney. There were increases in protein droplet nephropathy in males treated at 20 and 60 mg/kg bw/day at 12 months, but this was not observed at 24 months or in females. In females there were increased observations of renal tubules dilated with proteinaceous casts at 60 mg/kg bw/day, however, there were also high incidences in control males. Chronic progressive glumerulonephropathy was observed in the majority of animals in the treated and controls groups (slight to moderate severity), and there was an increase in severity in 60 mg/kg bw/day males (severe). Regarding the changes in the liver, hepatocellular centrilobular hypertrophy and vacuolation consistent with fatty changes were observed in 60 mg/kg bw/day males and females at 12 and 24 months. Hypertrophy was also observed at a lower incidence and severity in 20 mg/kg bw/day males.

There was a statistically significant increase in cystic thyroid follicles in the 60 mg/kg bw/day males (14/50) compared to controls (3/50) at 24 months that was not observed at 12 months or in females. The toxicological significance of this effect is unknown. The study authors hypothesised that this may be a physiological response to the loss of thyroid hormone in the urine of animals with severe renal disease.

Effects in Organs – Tumours

In the kidneys, there were increases of tubular adenomas (3/50) and adenocarcinomas (3/50) in males treated at 60 mg/kg bw/day, compared to controls (0/50). The study authors conclude that this is weak evidence of carcinogenic potential in male rats, possibly due to a sex-specific accumulation of protein in the proximal tubules and exacerbation of naturally occurring progressive glomerulonephropathy, accompanied by increased cellular turnover and proliferative response. The US EPA (2005, 2012a) attributed these tumours to chemically-induced $\alpha_{2\mu}$-globulin accumulation, a mechanism which was determined to be irrelevant for human cancer hazard assessment.

CONCLUSION

The NOAEL was established as 5 mg/kg bw/day, based on decreased body weight gains in males treated at 20 mg/kg bw/day and above. There were treatment related increases of tubular adenoma and adenocarcinoma in 60 mg/kg bw/day males, however, these tumours are not relevant to human carcinogenic safety assessment. Therefore, this study is not considered to demonstrate carcinogenic potential in humans.

TEST FACILITY Dow (1989b)

B.20. Carcinogenicity

TEST SUBSTANCE Notified chemical

METHOD OPP 83-2 Carcinogenicity
Similar to OECD TG 451 Carcinogenicity Studies

Species/Strain	Mouse/B6C3F1
Route of Administration	Oral – diet
Exposure Information	Total exposure: 24 months Dose regimen: 7 days per week
Remarks - Method	In a carcinogenicity study, mice (60/sex/dose) were administered the test substance in the diet for 24 months. Satellite groups (10/sex/dose) were randomly assigned at the beginning of the study and were sacrificed at 12 months and subject to necropsy and histopathology. Haematology was conducted at 12 (10/sex/dose) and 24 months (20/sex/dose). Differential leukocytes counts were determined at 12, 18 (10/sex/dose) and 24 months (20/sex/dose). Clinical chemistry was conducted at 12 months (10/sex/dose). All animals were subject to gross and microscopic pathology. Urinalysis was not conducted. Animals were not faster prior to sacrifice.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*			
			12 months		*24 months*	
			M	*F*	*M*	*F*
control	60M + 60F	0	0/10	0/10	6/50	14/50
low dose	60M + 60F	5	2/10	0/10	7/50	14/50
mid dose	60M + 60F	25	0/10	0/10	10/50	9/50
high dose	60M + 60F	75	0/10	1/10	8/50	13/50

Mortality and Time to Death

The deaths in the chronic phase of the study were considered incidental and not related to treatment. The two male mortalities at 5 mg/kg bw/day were attributed to trauma and the single high dose female mortality was attributed to a bone tumour that was causing compression to the spinal cord. Mortalities at 24 months were similar in the control and treated groups.

Clinical Observations

Clinical observations were similar in treated and control groups. In general, absolute body weights and body weight gains were similar to controls. Feed consumption was similar in treated and control groups.

Laboratory Findings – Haematology, Differential Leukocyte Count and Clinical Chemistry

There were no treatment related effects on haematology (including differential leukocyte parameters) at any of the measured observation points in males or females.

There were no treatment related effects in clinical chemistry parameters in females at 12 months. In males at 12 months, alanine aminotransferase (ALT) levels were increased at all treatment levels and the increase was statistically significant in the 75 mg/kg bw/day group (↑37%). Glucose levels were also increased in all treated male groups at 12 months with a statistically significant increase at 20 (↑21%) and 75 mg/kg bw/day (↑17%). Cholesterol levels were statistically significantly decreased in 25 (↓19%) and 75 mg/kg bw/day (↓29%) males.

Effects in Organs – General

The terminal body weights of the animals of the control groups at the 12 month sacrifice taken on day 371 were dissimilar to the control body weights for all animals taken on day 370. The day 370 and day 371 body weights were similar for the treated groups. At day 371, the 12 month control groups generally weighed less than the treated groups, for males and females. For this reason, several statistically significant organ weight changes (brain, heart and kidney) were not considered by the study authors to be treatment related. However, the study authors concluded that there was a treatment related increase in liver weights in males and females treated at 75 mg/kg bw/day at 12 months was treatment related.

At 24 months, there were statistically significant increases in absolute and relative kidney weights in males treated at 75 mg/kg bw/day (↑6%/↑7% absolute/relative). There were also increases in absolute and relative liver weights in 75 mg/kg bw/day males (↑11%/↑14% absolute/relative) but these were not statistically significant. Additionally, absolute and relative liver weights were statistically significantly increased in females treated at 75 mg/kg bw/day (↑8%/↑9%).

The only treatment related macroscopic effect at 12 months was an increase incidence of pale mucosa of the duodenum in 25 and 75 mg/kg bw/day males and in 75 mg/kg bw/day females. Increases were also observed at 24 months in 25 and 75 mg/kg bw/day males and females. These changes were accompanied by histopathological finding of altered tinctorial properties of epithelial cells in the duodenum at 12 and 24 months in males and females, at these doses. These changes were observed lining the tips of the villi and characterised by a plump appearance with a pale staining cytoplasm. The study pathologists attributed this change to be associated with the presence of the test substance within the lumen of the intestinal tract, since the mice were not fasted prior to necropsy. As there were no indications of degenerative changes in these cells, it was not considered to be of toxicological concern.

The only other treatment related toxicologically significant histopathological finding at 12 months was an increase of altered cytoplasmic homogeneity in the liver in 25 and 75 mg/kg bw/day males, and in 75 mg/kg bw/day females. At 24 months, increases compared to concurrent controls were only observed in 75 mg/kg bw/day males at 24 months.

Effects in Organs – Tumours
There were no treatment related increases in neoplasms in treated groups.

Remarks – Results
The toxicological effects observed in this study (increased kidney and liver weights at the high dose) are considered to be treatment related, toxicologically significant effects.

CONCLUSION
The NOAEL was established as 25 mg/kg bw/day, based on increased kidney and liver weights at 75 mg/kg bw/day. While there was no evidence of carcinogenic effect of the notified chemical, the dosing may not be sufficient to adequately determine the carcinogenic potential of the notified chemical.

TEST FACILITY Dow (1990b)

B.21. Chronic toxicity/carcinogenicity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 451 Carcinogenicity Studies
Species/Strain Mouse/B6C3F1
Route of Administration Oral – diet
Exposure Information Total exposure: 24 months
Dose regimen: 7 days per week;
Remarks - Method Mice (60/sex/dose) were administered the notified chemical in the diet for 24 months at 0, 125 or 250 mg/kg bw/day. A satellite group (10/sex/dose) were randomly assigned at the beginning of the study and sacrificed at 12 months then subject to necropsy and histopathology.

Clinical chemistry and urinalysis were not conducted. All animals were subject to ophthalmological analysis before commencing treatment, and at 12 and 24 months. Haematology analysis was conducted at 12 (10/sex/dose) and 24 months (20/sex/dose).

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*			*Mortality**	
		Nominal	*Achieved*		*Males*	*Females*
			Males	*Females*		
control	60M + 60F	0	0	0	10/50	13/50
low dose	60M + 60F	125	130	127	2/50	13/50
high dose	60M + 60F	250	260	254	17/50	10/50

*Data presented for 24 month study only

Mortality and Time to Death

Mortalities in females were similar in treated and control groups. The decrease in mortalities in males treated at 125 mg/kg bw/day was statistically significant. The increase in mortalities in 250 mg/kg bw/day males (one of which was due to a squamous cell carcinoma of the stomach and seven others due to hepatocellular adenoma or carcinoma) was not statistically significant.

Clinical Observations

There were no treatment related clinical observations recorded throughout the study.

Body weight gains were statistically significantly decreased in males treated at 250 mg/kg bw/day over much of the exposure period, and were decreased at 12 months (↓30%) and at 24 months (↓27%). Body weight gain decreases in females treated at 250 mg/kg bw/day were only slightly decreased at 24 months (↓9%).

Final body weights were decreased in males treated at 125 (↓10%) and 250 mg/kg bw/day (↓14%) at 12 months, and in males treated at 250 mg/kg bw/day (↓9%) at 24 months.

Feed consumption was similar in treated and control groups.

Laboratory Findings – Haematology

The only noticeable change in haematology was a non-statistically significant increase in total white blood cell count in males treated at 250 mg/kg bw/day males (↑20%) and females treated at 250 mg/kg bw/day (↑144%). The study authors noted slight non-statistically significant increases in neutrophil levels (↑27%/↑19%; males/females) and decreases in lymphocyte levels (↓13%/↓6%; males/females) at 24 months in 250 mg/kg bw/day groups.

Effects in Organs – General

Treatment related statistically significant increases in absolute and relative liver weights in 125 and 250 mg/kg bw/day males and females at 12 and 24 months (see following Table) and there was a dose response in both sexes.

	Males (mg/kg bw/day)			*Females (mg/kg bw/day)*		
	0	*125*	*250*	*0*	*125*	*250*
Liver weights (12-month) 10/sex/dose						
Absolute (g)	2.033	2.262* (↑11%)	2.668* (↑31%)	1.544	1.818* (↑18%)	2.394* (↑55%)
Relative to bw	5.160	6.405* (↑24%)	7.890* (↑53%)	5.144	5.964* (↑16%)	7.872* (↑53%)
Liver weights (24-month) 50/sex/dose						
Absolute (g)	1.975	2.545* (↑29%)	3.599* (↑82%)	1.724	2.132* (↑24%)	2.743* (↑59%)
Relative to bw	5.757	7.148* (↑24%)	11.557* (↑101%)	5.494	6.867* (↑25%)	9.001* (↑64%)

*Statistically significant compared to control (P<0.05).

There were no other organ weight changes in females.

In males at 12 months there were increases in the relative brain weights in animals of the 125 and 250 mg/kg bw/day groups, but absolute brain weights were similar to controls. At 24 months, there were increases in both the absolute and relative brain weights in males treated at 250 mg/kg bw/day. Absolute kidney weights in males treated at 250 mg/kg bw/day at 12 month were statistically significantly decreased with no associated decrease in relative weight. At 24 months, absolute kidney weights were statistically increased in males treated at 125 mg/kg bw/day but statistically decreased at 250 mg/kg bw/day. The relative testes weights were statistically significantly increased at 12 months in males treated at 250 mg/kg bw/day but there was a decrease in absolute testes weights at 24 months. In general, the study authors attributed these weight alterations to the treatment related decreases in body weights of the animals.

Macroscopic observations were noted in the liver, stomach and duodenum in males and females at 24 months. In the liver, observations of pale/dark foci were marginally increased in treated groups, with a clear increase in females treated at 250 mg/kg bw/day. There were also increased observations of mass/nodules in males and females treated at 250 mg/kg bw/day, and in females treated at 125 mg/kg bw/day. Increased incidences of mass/nodules were also observed in the stomach in males and females treated at 125 and 250 mg/kg bw/day. Increased incidences of pale appearance of the duodenum was observed in 125 and 250 mg/kg bw/day males

and females (see following Table).

	Males (mg□kg bw/day)			*Females (mg/kg bw/day)*		
	0	*125*	*250*	*0*	*125*	*250*
Liver			*24 months (50/sex/dose)*			
pale or dark foci, any distribution	3	12	12	5	11	29
mass/nodule, single	12	14	17	9	14	20
mass/nodule, multiple	4	4	27	0	4	7
mass/nodule, total	16	18	44	9	18	27
Stomach			*24 months (50/sex/dose)*			
mass/nodule, single	1	8	11	1	6	17
mass/nodule, multiple	0	0	4	0	0	5
mass/nodule, total	1	8	15	1	6	22
Duodenum			*24 months (50/sex/dose)*			
Pale	0	49	34	0	40	40

The macroscopic findings and liver weight alterations were accompanied by histopathological changes in the liver and gastrointestinal tract (see following Table).

	Males (mg/kg bw/day)			*Females (mg/kg bw/day)*		
	0	*125*	*250*	*0*	*125*	*250*
Stomach			*12 months (10/sex/dose)*			
hyperplasia, nonglandular mucosa, any distribution	0	2(2.0)	3(2.0)	1(2.0)	5(2.0)	4(2.0)
			24 months (50/sex/dose)			
hyperkeratosis, nonglandular mucosa, any distribution	1(2.0)	13*(2.0)	17*(2.0)	1(2.0)	16*(2.1)	15*(2.0)
hyperplasia, nonglandular mucosa, any distribution	0	16*(2.0)	14*(2.0)	4(2.0)	18*(2.0)	20*(2.0)
Duodenum			*12 months (10/sex/dose)*			
vacuolation, epithelial	0	10(2.0)	10(2.0)	0	9(2.0)	9(2.0)
hyperplasia and hypertrophy	0	10(2.0)	10(2.0)	0	10(2.0)	9(2.0)
			24 months (50/sex/dose)			
vacuolation, epithelial	0	48*(2.0)	37*(2.0)	0	39*(1.9)	39*(1.9)
hyperplasia and hypertrophy	0	48*(2.0)	37*(2.0)	0	39*(1.9)	39*(1.9)
Jejunum			*12 months (10/sex/dose)*			
vacuolation, epithelial	0	2(1.0)	5(1.0)	0	2(1.0)	7(1.4)
hyperplasia and hypertrophy	0	2(1.0)	5(1.0)	0	2(1.0)	7(1.4)
			24 months (50/sex/dose)			
vacuolation, epithelial	0	40*(1.3)	27*(1.7)	0	11*(1.5)	28*(1.6)
hyperplasia and hypertrophy	0	39*(1.3)	27*(1.7)	0	11*(1.5)	28*(1.6)
Liver			*12 months (10/sex/dose)*			
necrosis, individual cell	1(1.0)	3(1.0)	8(1.0)	1(1.0)	1(1.0)	2(1.0)
pigment, centrilobular	0	0	5(1.2)	0	0	0
increased hepatocyte size[a]	0	10(2.0)	8(3.0)	0	10(1.0)	10(2.0)
inflammation	1(1.0)	8(1.0)	6(1.0)	7(1.0)	9(1.0)	8(1.0)
			24 months (50/sex/dose)			
necrosis, individual cell	5(1.6)	48*(1.6)	48*(1.7)	3(2.0)	11*(1.5)	19*(1.6)
basophilic foci	0	1(1.0)	8*(1.3)	1(1.0)	1(1.0)	12*(1.0)
eosinophilic foci	5(1.0)	5(1.0)	12(1.6)	2(1.0)	2(1.0)	23*(1.0)
hyperplasia, bile duct	0	0	5*(2.4)	0	0	0
cytoplasmic inclusions	0	0	10(1.3)	0	0	3(1.3)
pigment, multifocal	0	1(1.0)	41*(1.6)	0	1(1.0)	3(1.0)
vacuolation	0	4(2.3)	39*(1.8)	1(1.0)	3(2.0)	15*(1.9)
increased hepatocyte size, panlobular[a]	0	49*(2.9)	26*(3.0)	0	46*(2.2)	44(2.8)
increased hepatocyte size, centrilobular[a]	0	1(3.0)	23*(3.0)	0	0	4(3.0)
inflammation	5(2.0)	44*(1.6)	45*(1.6)	4(2.3)	5(1.4)	12(1.8)
pelliosis	1	12*	5	1	5	10

Spleen			*24 months (50/sex/dose)*			
increased extramedullary haematopoiesis	6	6	18*	6	7	7

( ), Average severity of affected animals: 1=very slight, 2=slight, 3=moderate, 4=moderately severe. *Statistically significant compared to control (P<0.05). [a]often accompanied by altered tinctorial properties.

There were treatment related occurrences of hyperplasia in the nonglandular mucosa of the stomach in males and females treated at 125 and 250 mg/kg bw/day, at 12 and 24 months. This effect was often associated with hyperkeratosis. Vacuolation, hypertrophy and hyperplasia were also observed in the jejunum and duodenal mucosal cells in males and females treated at 125 and 250 mg/kg bw/day. The hyperplasia was characterised by increased numbers of epithelial cells from the base to the tips of villi, with hyperchromatic nuclei and an increase of mitotic figures.

Liver histopathology included numerous effects at both treatment levels in males and females. Hepatocytes were increased in size (often accompanied by altered tinctorial properties) at 12 and 24 months at both treatment levels in males and females. The affected cells were reported to have enlarged nuclei and were hyperchromatic, with prominent nucleoli and frequent invaginations of the cytoplasm into the nuclear space.

Increased incidences of hepatocellular single cell necrosis were noted in males treated at 125 and 250 mg/kg bw/day at 12 months and were randomly located in the centrilobular or midzonal portions of the liver lobules, and were sometimes associated with inflammation (subacute to chronic). Single celled necrosis was observed at 24 months in males treated at 125 and 250 mg/kg bw/day with an increased severity compared to the 12 months observation. This effect was also observed at a lower incidence in 24 month females and was considered treatment related at both doses. Other notable liver effects were more numerous at 24 months, and mostly occurred at 250 mg/kg bw/day, included hyperplasia of the bile duct (low incidence in 250 mg/kg bw/day males only) and vacuolation (both sexes) and an increase in the number of mice eosinophilic and/or basophilic foci (both sexes).

Electron microscopy was conducted on the livers of 2 male controls and 2 males treated at 250 mg/kg bw/day at 12 months. Treatment related findings included decreased cytoplasmic glycogen, increased cytoplasmic lipid deposits, increased amount of rough endoplasmic reticulum and the presence of enlarged lysosomes (filled with homogenous granular material).

Observations of extramedullary haematopoiesis were statistically significantly increased in males treated at 250 mg/kg bw/day males. The study authors attributed this effect as secondary to the liver tumours at this dose (see discussion following this section), noting that in some animals, the tumours were partially necrotic and haemorrhagic, and the consequent blood loss was the likely stimulus for increased haematopoiesis.

Effects in Organs – Tumours

Notable neoplasms in the study were observed in the liver, stomach, epididymis and lacrimal gland (see following Table).

	Males (mg/kg bw/day)			*Females (mg/kg bw/day)*		
	0	*125*	*250*	*0*	*125*	*250*
Stomach			*24 months (50/sex/dose)*			
papilloma, nonglandular mucosa, benign	1	9*	12*	1	8*	21*
squamous cell carcinoma, nonglandular mucosa	0	0	3	0	0	2
Liver			*24 months (50/sex/dose)*			
hepatocellular adenoma	12	19	45*	6	27*	32*
hepatocellular carcinoma	7	3	12	0	1	2
hemangiosarcoma, combined metastasis and no metastasis	1	2	4	1	0	2
Epididymis			*24 months (50/sex/dose)*			
sarcoma, malignant	0	2	4	-	-	-
Lacrimal/Harderian gland			*24 months (50/sex/dose)*			
Adenoma	6	4	2	1	8*	9*

*Statistically significant compared to control (P<0.05).

There were statistically significant increases in the incidences of hepatocellular adenomas in males treated at 250 mg/kg bw/day, and in females treated at 125 and 250 mg/kg bw/day, with a clear dose response relationship in both sexes. The incidence of carcinomas were increased compared to controls in males and females treated at 250 mg/kg bw/day males and females, but the increase was not statistically significant.

There was also indication of a dose response relationship in incidence of malignant hemangiosarcoma observed in males at 125 and 250 mg/kg bw/day. However, the increases were not statistically significant.

There were clear dose-related statistically significant increases of benign papilloma in the nonglandular mucosa of the stomach in both sexes at 125 and 250 mg/kg bw/day. Squamous cell carcinomas were also increased in high dose males and females, relative to controls.

There were non-statistically significant increases in the incidence of epididymal sarcomas in both treated groups of males and there was some indication of a dose-response relationship. The study authors reported that the tumours had similar appearance to tumours that were diagnosed in previous B6C3F1 mouse studies conducted by the laboratory and that the incidences were similar to historical control values at the laboratory. Additionally, the study authors note a lack of preneoplastic effects in the epididymis.

Increases of lacrimal gland adenomas were statistically significant in females treated at 125 and 250 mg/kg bw/day, with no increases in treated male groups. The study authors considered the increased incidence to be treatment related as the values were similar to historical control values at the laboratory.

Remarks – Results

There were clear indications of toxicity at both dosage levels and in both sexes including, decreased body weight gains and histopathological effects in the liver, stomach, duodenum and jejunum. The study also provided evidence of carcinogenic effects at both dosage levels

CONCLUSION

The LOAEL was established as 125 mg/kg bw/day in this study (the lowest dose tested)was not established in this study based on effects at 125 and 250 mg/kg bw/day in both sexes. The study provided evidence of carcinogenic effect based on increases in the number tumours in males and females, at both treatment levels.

TEST FACILITY	Dow (1997a)

B.22. Chronic toxicity

TEST SUBSTANCE	6-chloropicolinic acid (6-CPA)
METHOD	Similar to OECD TG 451 Chronic Toxicity Studies
Species/Strain	Dog/beagle
Route of Administration	Oral – diet
Exposure Information	Total exposure: 12 months Dose regimen: 7 days per week
Vehicle	None
Remarks - Method	In a chronic study, beagle dogs (7 months old) were administered 6-CPA in the diet for 12 (1/sex/dose) or 24 months (3/sex/dose) at concentrations of 0, 200, 600 or 2000 ppm (equivalent to 0, 5, 16 or 56 mg/kg bw/day). Animals were weighed throughout the study and were observed frequently for any changes in appearance and behaviour. Haematology (haematocrit, red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin concentration and differential leukocyte counts) and clinical chemistry (urea nitrogen, alkaline phosphatase, serum glutamic transaminase and bromosulfophthalein dye retention) analyses were conducted just prior to dosing and at 3, 6, 9, 12, 18 and 24 months. Organs were weighed at necropsy and macroscopic and microscopic histopathology analyses were conducted.

RESULTS

Group	Number and Sex of Animals	Dose		Mortality
		ppm	(mg/kg bw/day)	
control	4M + 4F	0	0	0/8
low dose	4M + 4F	200	5	0/8
mid dose	4M + 4F	600	16	0/8
high dose	4M + 4F	2000	56	0/8

Mortality and Time to Death
No mortalities were reported.

Clinical Observations
No treatment related clinical observations were reported. While there was no apparent effect of treatment on body weight gains in males, in females, there were decreases in body weight gains in all treated groups. However, the average initial body weight for the female control group was lower than the corresponding treatment groups. Feed consumption was similar between the groups.

Laboratory Findings – Clinical Chemistry and Haematology
There was no apparent treatment related effect on the measured haematology or clinical chemistry parameters.

Effects in Organs – General
There was no apparent dose related changes in organ weights.

The study authors reported slight reversible liver and kidney changes in the dogs treated at 600 and 2000 ppm that were sacrificed at 12 months (1/sex/dose) but that there were no treatment related microscopic observations after 24 months (relative to control animals).

CONCLUSION
The NOAEL was established as 56 mg/kg bw/day by the study authors.

TEST FACILITY Dow (1967a)

B.23. Chronic toxicity/carcinogenicity

TEST SUBSTANCE 6-chloropicolinic acid (6-CPA)

METHOD Similar to OECD TG 453 Combined Chronic Toxicity\Carcinogenicity Studies
Species/Strain Mouse/B6C3F1
Route of Administration Oral – diet
Exposure Information Total exposure: 24 months
Dose regimen: 7 days per week
Vehicle None
Remarks - Method Mice (50/sex/dose) were administered 6-CPA in the diet for 24 months. Satellite groups were randomly assigned at the beginning of the study and sacrificed at 6 (10/sex/dose) and 12 months (10/sex/dose). Haematology and clinical chemistry analyses were conducted for both satellite groups prior to necropsy. Haematology and clinical chemistry were also conducted at 24 months (10/sex/dose). Urinalysis was not conducted. Brain, heart, kidneys, liver and testes were weighed at 6, 12 and 24 months (10/sex/dose/observation).

RESULTS

Group	Number and Sex of Animals	Dose (mg/kg bw/day)	Mortality*	
			Males	Females
control	70M + 70F	0	8/50	15/50
low dose	70M + 70F	100	6/50	14/50
mid dose	70M + 70F	300	5/50	11/50
high dose	70M + 70F	900	13/50	7/50

*Data presented for 24 month study only.

Mortality and Time to Death

There was a slight increase in mortalities in males treated at 900 mg/kg bw/day. The majority of mortalities occurred after 12 months.

Clinical Observations

There were no treatment related clinical signs noted. There were some statistically significant decreases in absolute body weights noted in males treated at 900 mg/kg bw/day throughout the treatment period but the overall body weight gain was not significantly affected. There were no dose-related statistically significant decreases in the absolute body weights in females and the overall body weight gain were not significantly affected. Feed consumption was similar between control and treated groups.

Laboratory Findings – Clinical Chemistry and Haematology

There were no treatment related findings in clinical chemistry or haematological analyses.

Effects in Organs – General

There was a statistically significant decrease in absolute brain weight in males treated at 900 mg/kg bw/day at 6 months but the change was small (↓3%). There was also a statistically significant decrease in relative kidney weights in this group (↓7%). There were no other treatment related organ weight changes reported.

All macroscopic observations in treated groups were similar to controls at 6, 12 and 24 months. The only non-neoplastic histopathological change was an increased incidence of decreased vacuolation of the proximal convoluted tubule of the kidney in males treated at 900 mg/kg bw/day at 6 and 24 months. There were no other treatment related histopathological findings reported.

Effects in Organs – Tumours

There was a non-statistically significant increase in the incidence of hepatocellular carcinomas in treated female groups compared to the concurrent control group (see following Table). While there was an indication of a dose-response of these tumours, the trend analysis was not statistically significant. There was no increase in hepatocellular carcinomas in males. Hepatocellular adenomas were similar in treated and control groups for males and females.

	Males (mg/kg bw/day)				*Females (mg/kg bw/day)*			
	0	*100*	*300*	*900*	*0*	*100*	*300*	*900*
Liver	*24 months (50 animals/group)*							
hepatocellular carcinoma, no metastasis	6	8	6	3	1	2	3	4
hepatocellular carcinoma, metastasis	1	1	0	2	0	1	0	2
hepatocellular carcinoma, total	7	9	6	5	1	3 (6%)	4 (8%)	6 (12%)

Laboratory historical control (1979-85) data for hepatocellular carcinoma in female B6C3F1 mice: 13/372 (3.5%), range 0-8% (dietary and inhalation studies); 9/322 (2.8%), range 0-8% (dietary).
Published historical control data (Haseman *et al.*, 1984) for hepatocellular carcinoma in female B6C3F1 mice: 101/2469 (4.1%), range 0-15% (dietary).

The non-statistically significant increased incidence of hepatocellular carcinomas in females was considered equivocal by the study authors. The total incidence of this tumour in females treated at 100 and 300 mg/kg bw/day (6% and 8%, respectively) was within the historical control range for the laboratory (0-8%). However, the incidence in females treated at 900 mg/kg bw/day females (12%) was outside the laboratory historical control range. The historical control range from the National Toxicology Program with B6C3F1 female mice was 0-15% for studies conducted around the same time period, although it is noted that there may be inter-study and/or inter-laboratory variability (Haseman *et al.*, 1984).

The study authors also note the questionable biological significance of the increased incidence of liver carcinomas in females in the absence of identified liver toxicity.

CONCLUSION

The NOAEL was established as 300 mg/kg bw/day, based on decreased body weights and decreased vacuolation in the proximal convoluted tubule in males treated at 900 mg/kg bw/day. A marginal increase in liver carcinomas in 900 mg/kg bw/day females was not considered to be treatment related, thus the test material was not considered to be carcinogenic under the conditions of the study.

TEST FACILITY Dow (1986f)

B.24. 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: WP2*uvr*A

Metabolic Activation System S9 fraction from Aroclor 1254 induced rat liver

Concentration Range in Main Test a) With metabolic activation: 10-1000 µg/plate (all strains)
b) Without metabolic activation: 5-500 µg/plate (WP2*uvr*A only)
c) Without metabolic activation: 1-200 µg/plate (all other strains)

Vehicle Dimethyl sulfoxide

Remarks - Method A range-finding study was conducted in strains TA100 and WP2*uvr*A in the presence and absence of metabolic activation between 10-5000 µg/plate.

In the main mutagenicity assay, a 2-fold increase in the number of revertant colonies for strain TA100 was considered a positive result, while a 3-fold increase for all other strains was considered positive. Vehicle and positive controls were used in parallel with the test substance.

RESULTS

Metabolic Activation	*Test substance Concentration (µg/plate) Resulting in:*				
	Cytotoxicity in Preliminary Test		*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
	TA100	*WP2*uvr*A*			
Absent					
Test 1	≥100	≥333	≥100	-	negative
Test 2	-	-	≥100	-	negative
Present					
Test 1	≥333	≥1000	≥200	-	negative
Test 2	-	-	≥200	-	negative

Remarks - Results In Test 1 (strain TA98; without metabolic activation), the mean vehicle control value for the number of revertants per plate was below the acceptable levels (as outlined in the acceptability criteria of the study report). This resulted in relative increases in the number of revertants at the 25 and 50 µg/plate concentrations (3.0 and 3.9 fold increases, respectively). Therefore, this component of Test 1 was repeated and subsequently gave a negative result.

In Test 1, there were also increases in the number of revertant colonies for strains TA1537 (without metabolic activation) and TA100 (with metabolic activation), but these were below the 3-fold and 2-fold criteria, respectively.

In the second test, there were also increases in the number of revertant colonies for strains TA98 (with metabolic activation) and TA100 (with metabolic activation), but these were below the 3-fold and 2-fold criteria, respectively.

The positive controls gave satisfactory responses, confirming the validity of the test system.

CONCLUSION The notified chemical was not mutagenic to bacteria under the conditions of the test.

TEST FACILITY Covance (2007)

B.25. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD Similar to OECD TG 471 Bacterial Reverse Mutation Test – Plate incorporation procedure

Species/Strain	*S. typhimurium*: TA97, TA1535, TA98, TA100
Metabolic Activation System	S9 fraction from Aroclor 1254 induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 0.8-500 µg/plate b) Without metabolic activation: 0.8-500 µg/plate
Vehicle	Dimethyl sulfoxide
Remarks - Method	A range-finding study was conducted in strain TA100 in the presence and absence of metabolic activation up to a concentration of 5000 µg/plate. As cytotoxicity was observed at 1000 and 5000 µg/plate, 500 µg/plate was selected as the highest dose for the main study. Two mutagenicity assays were conducted (Tests 1 and 2). Due to statistically significant increases in the numbers of revertant colonies (see remarks below), a third assay was conducted in TA97 (with S9) and TA100 (without S9). The study authors considered a positive result to be when there was a statistically significant increase compared to a control, together with a dose response. Strains capable of detecting cross-linking mutagens (*E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102) were not tested in this study.

RESULTS

Metabolic Activation	*Test substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥1000	≥500	none reported	negative
Test 2	-	≥500	none reported	negative
Present				
Test 1	≥1000	≥500	none reported	negative
Test 2	-	≥500	none reported	negative

Remarks - Results There were statistically significant increases in the number of revertants per plate in strain TA97, with and without metabolic activation (up to a 1.5 fold increase). Similarly, there were statistically significant increases in the number of revertants per plate in strain TA100 without metabolic activation (up to a 1.3 fold increase). The third mutagenicity assay conducted in these strains produced similar results. Overall, given the small magnitude of the increases and the lack of dose-response relationships, the increases were not considered to be indicative of a mutagenic response.

There were no other statistically significant increases in the other strains. All positive controls gave satisfactory responses, confirming the validity of the test system.

CONCLUSION The notified chemical was not mutagenic to bacteria under the conditions of the test.

TEST FACILITY Microtest (1985a)

B.26. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD Similar to OECD TG 471 Bacterial Reverse Mutation Test – Preincubation procedure

Species/Strain	*S. typhimurium*: TA97, TA98, TA100, TA1535
Metabolic Activation System	S9 fraction from Aroclor 1254 induced rat and hamster liver
Concentration Range in Main Test	a) With metabolic activation: 0-333 µg/plate b) Without metabolic activation: 0-666 µg/plate
Vehicle	Dimethyl sulfoxide
Remarks - Method	The laboratory conducted cytotoxicity range-finding assays, but the results were not reported. All strains were tested without metabolic activation and with 10% rat and hamster metabolic activation. In addition, strains TA98 and TA100 were conducted with 5% and 30% rat and hamster metabolic activation. At least five doses were conducted in triplicate. The assay was repeated to confirm findings, but only the results of the second study were reported. Individual trials were considered mutagenic (+), weakly mutagenic (w+), questionable (q; if results were not reproducible) or non-mutagenic based on the relative increase in revertant colonies and any dose-response relationship.

RESULTS

TA1535: non mutagenic – no increase in the frequency of revertant colonies was reported, with or without S9 metabolic activation.
TA97: mutagenic – increase was noted in the presence of metabolic activation (10% rat w+, up to 1.41 fold increase; 10% hamster +, up to 1.85 fold increase).
TA98: mutagenic – increase was noted in the presence of metabolic activation (10% rat q, up to 2.34 fold increase; 30% rat +, up to 2.75 fold increase; 10% hamster +, up to 2.04 fold increase; 30% hamster +, up to 3.48 fold increase).
TA100: mutagenic – increase was noted in the presence of metabolic activation (10% rat +, up to 1.81 fold; 30% rat +, up to 1.80 fold increase; 10% hamster +, up to 1.99 fold increase; 30% hamster +, up to 2.45 fold increase).

Remarks - Results There were numerous mutagenic, weak mutagenic and questionable responses with and without metabolic activation. However, it is noted that the relative increases in this study may not be considered positive responses by contemporary standards.

CONCLUSION The notified chemical was considered to be weakly mutagenic to bacteria under the conditions of the test.

TEST FACILITY Original study data presented in Zeiger *et al.* (1988). Study interpretations and discussion also presented in Zeiger (2010).

B.27. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

METHOD	Similar to OECD TG 476 In vitro Mammalian Cell Gene Mutation Test
Cell Type/Cell Line	Chinese hamster ovary (CHO)/CHO-K1-BH4
Metabolic Activation System	S9 fraction from Aroclor 1254 induced rat liver
Vehicle	Dimethyl sulfoxide
Remarks - Method	A range-finding study was conducted in the presence and absence of metabolic activation at concentrations of up to 1000 µg/mL. The main assay was conducted three times without metabolic activation (and once with activation).

Metabolic Activation	*Test substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	0, 20, 40, 60, 80, 100	4 hours	8 days	7-9 days
Test 2	0, 20, 40, 60, 80	4 hours	8 days	7-9 days
Test 3	0, 20, 40, 60, 80	4 hours	8 days	7-9 days
Present				
Test 1	0, 120, 140, 160, 180, 200	4 hours	8 days	7-9 days

Positive controls: ethyl methanesulfonate (without metabolic activation), 20-methyl-cholanthrene (with metabolic activation).

RESULTS

Metabolic Activation	*Test substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test*	*Cytotoxicity* in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	≥125	≥80	none reported	negative
Test 2	-	≥80	none reported	negative
Test 3	-	>80	none reported	negative
Present				
Test 1	≥250	≥160	none reported	negative

*less than 10% relative cell survival.

Remarks - Results	Sporadic non-statistically significant increases in the frequencies of mutations at non cytotoxic doses were noted, but these were within historical control ranges. There was a statistically significant increase in the mutation frequency at 80 µg/mL without metabolic activation (Test 1), but this was at cytotoxic levels (only 1.9% relative cell survival was observed). Additionally, the increase was within historical control ranges for the laboratory and was not reproduced in repeat tests.
CONCLUSION	The notified chemical was not mutagenic to CHO cells treated *in vitro* under the conditions of the test.
TEST FACILITY	Dow (1986g)

B.28. Genotoxicity – in vivo

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 486 Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo
Species/Strain	Mouse/B6C3F1(Male)
Route of Administration	Oral – gavage
Vehicle	Corn oil
Remarks - Method	A range-finding study was conducted to determine the dose selection for the main study. The study was initially conducted at 1000 mg/kg bw (2 mice) and then additional groups were dosed at 125, 250, 500 or 750

mg/kg bw (3 mice/dose).

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	6M	0	2-4 (3M) and 12-16 (3M)
II (low dose)	6M	125	2-4 (3M) and 12-16 (3M)
IV (high dose)	6M	250	2-4 (3M) and 12-16 (3M)
IV (positive control; DMN)	6M	10	2-4 (3M) and 12-16 (3M)

DMN=dimethylnitrosamine

RESULTS

Doses Producing Toxicity: In the range-finding study, mortalities were observed at 500, 750 and 1000 mg/kg bw, and clinical signs (piloerection and lethargy) were observed at 250 mg/kg bw. Therefore, the main study was conducted at 125 and 250 mg/kg bw.

In the main study, piloerection was observed in all animals treated at 250 mg/kg bw up to four hours post-dosing.

Genotoxic Effects: There were no statistically significant increases in the mean net nuclear grain counts in hepatocytes from treated mice when compared to controls.

The positive control exhibited statistically significant increases in the mean net nuclear grain counts.

CONCLUSION: The test substance did not induce DNA damage *in vivo* under the conditions of the study.

TEST FACILITY: BioReliance (2009)

B.29. Genotoxicity – in vivo

TEST SUBSTANCE: Notified chemical

METHOD: Similar to OECD TG 474 Mammalian Erythrocyte Micronucleus Test

Species/Strain: Mouse/CD-1

Route of Administration: Oral

Vehicle: Corn oil

Remarks - Method: A range-finding study (3 stages) was conducted to determine the dose selection for the main study. Groups of mice were administered a single dose of the test substance at the following concentrations:
a) 0, 500, 750 or 1000 mg/kg bw (2 mice/concentration) – one death at 500 mg/kg bw and one death at 1000 mg/kg bw.
b) 400 or 600 mg/kg bw (2 mice/concentration) – no deaths.
c) 800 or 1000 mg/kg bw (2 and 3 mice/concentration, respectively) – one death at 1000 mg/kg bw.

In the main study, animals were administered a single dose of the test (or control) substance. Based on the range-finding study, the maximum tolerable dose for the main study was determined to be 800 mg/kg bw. The animals were sacrificed after 24-72 hours.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	5M + 5F	0	24 hours
II (vehicle control)	5M + 5F	0	48 hours
III (vehicle control)	5M + 5F	0	72 hours
IV (high dose)	5M + 5F	800	24 hours
V (high dose)	5M + 5F	800	48 hours
VI (high dose)	5M + 5F	800	72 hours
VII (positive control, CP)	5M + 5F	80	48 hours

CP, cyclophosphamide.

RESULTS

Doses Producing Toxicity	In the main study, there was a decrease in the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) at 72 hours (and 48 hours to a lesser extent).
Genotoxic Effects	There was no statistically significant increase in the mean frequency of micronucleated PCE in treated groups compared to concurrent controls
Remarks – Results	A statistically significant increase in micronucleated PCE was observed in positive control-treated mice.

CONCLUSION The notified chemical was not clastogenic under the conditions of this *in vivo* micronucleus test.

TEST FACILITY Microtest (1985b)

B.30. Developmental toxicity

TEST SUBSTANCE Notified chemical

METHOD OPP 83-3(a)
Similar to OECD TG 414 Prenatal Developmental Toxicity Study

Species/Strain	Rat/Fischer 344
Route of Administration	Oral – gavage
Exposure Information	Exposure days: gestation days 6-15 Post-exposure observation period: none (sacrificed on gestation day 16)
Vehicle	Corn oil
Remarks - Method	To determine the appropriate doses for the main developmental toxicity study, mated female rats were administered the notified chemical by gavage at 0, 15, 50 or 100 mg/kg bw/day (9-10/dose) during gestation days (GD) 6-15. The rats were sacrificed on day 16. The uterus was examined for implantations and resorptions. Liver and kidneys were weighed, and the number of corpora lutea was determined. The livers were subject to histopathological analysis.

RESULTS

Group	*Number of Animals*	*Number pregnant**	*Dose mg/kg bw/day*
control	10	10	0
low dose	9	9	15
mid dose	10	4	50
high dose	10	8	100

*Only pregnant animals subject to post-mortem analyses.

Effects on Dams

There were no mortalities or clinical signs of toxicity. There was an apparent dose related decrease in body weight gains with a statistically significant decrease in the 100 mg/kg bw/day group. There were also associated decreases in feed and water consumption at 100 mg/kg bw/day.

Absolute liver weights were slightly increased at 50 mg/kg bw/day (↑10%) and were statistically increased at 100 mg/kg bw/day (↑23%). Additionally, relative liver weights were statistically increased at 50 (↑8%) and 100 mg/kg bw/day (↑26%). There were no gross pathological findings at necropsy. Histopathological findings (see following Table) in the liver included altered tinctorial properties (decreased basophilia) mostly at 100 mg/kg bw/day, and vacuolation consistent with fatty change at 50 and 100 mg/kg bw/day. Both microscopic findings in the liver increased with severity between 50 and 100 mg/kg bw/day.

	Females (mg/kg bw/day)			
	0	*15*	*50*	*100*
Group size	10	9	4	8

Liver				
altered tinctorial properties-decreased basophilia	0	0	1(1.0)	8(3.0)
vacuolation consistent with fatty change	0	0	4(1.0)	8(3.0)

( ), Average severity of affected animals: 1=very slight, 2=slight, 3=moderate.

There was a statistically significant decrease in pregnancies at 50 mg/kg bw/day, but because the 100 mg/kg bw/day group was similar to controls and because there was no dose response, this is unlikely to be treatment related. A statistically significant increase in resorptions was observed at 50 mg/kg bw/day but is also not associated with treatment due to lack of a dose response.

CONCLUSION

The NOAEL was established as 15 mg/kg bw/day in this study, based on increased liver weights and liver vacuolation consistent with fatty change at 50 mg/kg bw/day and above.

TEST FACILITY Dow (1986h)

B.31. Developmental toxicity

TEST SUBSTANCE Notified chemical

METHOD

	OPP 83-3(a) Similar to OECD TG 414 Prenatal Developmental Toxicity Study
Species/Strain	Rat/Fischer 344
Route of Administration	Oral – gavage
Exposure Information	Exposure days: gestation days 6-15 Post-exposure observation period: gestation days 16-21
Vehicle	Corn oil
Remarks - Method	In a developmental toxicity study, mated female rats were administered the notified chemical by gavage at 0, 5, 15 or 50 mg/kg bw/day (29-30/dose) on GD 6 to 15. The animals were sacrificed on GD 21. Liver weights were recorded for all animals and liver histopathology was conducted on the control and high dose (10/dose). Foetal examinations/observations included gravid uterine weight, number and position of foetuses *in utero*, number of live and dead foetuses, number and position of resorptions, number of corpora lutea, foetal sex and body weight, gross external alterations, and skeletal alterations.

RESULTS

Group	*Number of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	30	0	0/30
low dose	30	5	0/30
mid dose	30	15	0/30
high dose	29	50	0/29

Effects on Dams

There were no clinical signs of toxicity in maternal animals. Body weights and body weight gains were similar to controls. There was no treatment related effect on liver weights. Very slight vacuolation of centrilobular hepatocytes was observed in 5/10 rats at 50 mg/kg bw/day.

There were no statistically significant changes in the measured reproductive parameters. However, there was a slight dose related increase in the number of implantations resorbed (5/220, 7/248, 10/263 and 12/228 for 0, 5, 15 and 50 mg/kg bw/day, respectively). But due to the small magnitude, this change is likely to be within expected biological variability and thus is not treatment related.

Effects on Foetus

There were no treatment related visceral or skeletal malformations. A single 50 mg/kg bw/day foetus exhibited anophthalmia, internal hydrocephaly, fused vertebrae, and delayed ossification of vertebrae, centra and

sternum, but is unlikely to be treatment related based on the low incidence. There were other single incidences of malformations in treated groups but are not considered treatment related. More common malformations (cervical spur, delayed ossification of centra and sternum) were observed at similar levels in controls.

CONCLUSION
The maternal NOAEL was established as 15 mg/kg bw/day based on hepatocellular vacuolation at 50 mg/kg bw/day. There was no evidence of foetal toxicity under the conditions of the study.

TEST FACILITY Dow (1986i)

B.32. Developmental toxicity

TEST SUBSTANCE Notified chemical

METHOD Similar to OECD TG 414 Prenatal Developmental Toxicity Study
Species/Strain Rat/CD (Sprague-Dawley)
Route of Administration Oral – gavage
Exposure Information Exposure days: gestation days 6-15
Post-exposure observation period: none (sacrificed on gestation day 16)
Vehicle Corn oil
Remarks - Method In a developmental toxicity range-finding study, mated rats (10/dose) were administered the notified chemical at 0, 50, 100 or 200 mg/kg bw/day by gavage on GD 6-15. Due to excessive toxicity, the high dose was terminated early. Animals were sacrificed on GD 16 and reproductive performance was measured. Histopathology was not conducted.

RESULTS

Group	*Number of Animals*	*Dose (mg/kg bw/day)*	*Mortality*	*Number pregnant*
control	10	0	0/10	8
low dose	10	50	0/10	10
mid dose	10	100	0/10	10
high dose	10	200	1/10*	ND

*One animal found dead on GD 13, the remaining animals were sacrificed due to excessive body weight losses. ND, not determined due to excessive toxicity.

Mortality and Time to Death
There was one mortality on GD 13 at 200 mg/kg bw/day and the remaining animals at this dose were sacrificed early (GD 12-14) due to excessive toxicity, based on excessive body weight losses in the remaining animals.

Effects on Dams
There were no clinical observations in 50 or 100 mg/kg bw/day groups. Numerous observations were observed at 200 mg/kg bw/day including lethargy/decreased activity, chromodacryorrhea, staining of the skin/fur in the ano-genital region, and red vaginal discharge.

There were slight but non-statistically significant decreases in body weight gain at 100 mg/kg bw/day over GD 6-9 (↓31%) and GD 6-16 (↓10%). The study authors considered these changes to be treatment related, however, given the lack of statistical significance these changes are indicators of minimal toxicity. The corrected absolute body weight and body weight gain (adjusted for gravid uterine weight) on GD 16 were slightly lower than controls at 100 mg/kg bw/day (↓6%/↓11% absolute/relative) but were not statistically significant.

The study authors considered a non-statistically significant decrease in feed consumption between GD 6-9 (↓10%) at 100 mg/kg bw/day to be treatment related. There were no differences in the other measurement points. Based on the small magnitude of the decrease and the lack of statistical significance, this effect is not considered to be toxicologically significant.

There were slight increases in absolute liver and kidney weights at 50 and 100 mg/kg bw/day and the increases in relative (to corrected body weight) liver and kidney weights were statistically significant.

There were no changes in reproductive parameters in the 50 mg/kg bw/day group. In the 100 mg/kg bw/day group there were slight non-statistically significant increases in the mean number of resorptions, mean resorption/implant ratio and the incidence of females with resorptions (se e following Table). The increases similar to historical control ranges for the resorptions/litter and resorption/implant ratio, and overall are not considered treatment related.

	Females (mg/kg bw/day)			
	0	*50*	*100*	*Historical control**
Reproductive performance				
viable litters	8	10	10	
viable foetuses	121	149	146	
mean litter size	15.1	14.9	14.6	
number of resorptions	6	7	14	
Resorptions/litter	0.8	0.7	1.4	mean: 0.9, range: 0.5-1.2
resorption/implant ratio	0.047	0.051	0.089	mean: 0.058, range: 0.033-0.088

*Laboratory historical control from 18 developmental toxicity studies.

Remarks - Results

Maternal toxicity was indicated by decreases in body weight gain at 100 mg/kg bw/day. Additionally, there were increases in relative liver and kidney weights at 50 and 100 mg/kg bw/day.

CONCLUSION

The maternal NOAEL was not established based on increased relative liver and kidney weights at 50 mg/kg bw/day and above. There were marginal increases in some reproductive parameters at 100 mg/kg bw/day (number of resorptions, resorptions per litter and resorption/implant ratio), but these changes were not considered to be treatment related and thus there was no effect on reproductive performance under the conditions of the study.

TEST FACILITY Pharmaco (1994a)

B.33. Developmental toxicity

TEST SUBSTANCE Notified chemical

METHOD
- Species/Strain: Rat/CD (Sprague-Dawley)
- Route of Administration: Oral – gavage
- Exposure Information: Exposure days: gestation days 6-15; Post-exposure observation period: gestation days 16-20
- Vehicle: Corn oil
- Remarks - Method: In a developmental toxicity study, mated female rats were administered the notified chemical by gavage at 0, 15, 50 or 120 mg/kg bw/day (28/dose) on GD 6 to 15. The animals were sacrificed on GD 20. Histopathological analyses were not conducted.

RESULTS

Group	*Number of Animals*	*Dose (mg/kg bw/day)*	*Mortality*	*Number pregnant*
control	28	0	0/28	25
low dose	28	15	0/28	26
mid dose	28	50	0/28	26
high dose	28	120	0/28	23

Effects on Dams

Excessive salivation was observed in high dose animals but was attributed to administration of the test substance.

There were statistically significant decreases in absolute body weights for the 120 mg/kg bw/day group on GD 9, 12 and 16, (↓6%-9%) but there was only a slight non-statistically significant decrease on GD 20 (↓6%)), indicating some recovery from treatment. There were statistically significant decreases in body weight gains over GD 6-16 for the 50 (↓13%) and 120 mg/kg bw/day (↓35%) groups. Thirteen of the 120 mg/kg bw/day groups lost weight over GD 6-9 and four lost weight over GD 9-12. Body weight gain effects are considered treatment related at 50 and 120 mg/kg bw/day. Corrected body weight gains for the 120 mg/kg bw/day group were statistically decreased (↓39%). Gravid uterine weights were similar in control and treated groups. Feed consumption was statistically decreased over GD 6-9 (↓19%) in the 120 mg/kg bw/day group, but there were statistically significant increases in feed consumption over GD 12-16 and 16-20.

Increases in relative (corrected by GD 20 gravid uterine weight) liver (↑9%) and kidney (↑8%) weights were statistically increased at 120 mg/kg bw/day. These relative organ weight changes may be secondary to the decrease in final body weights in these groups.

Small changes in reproductive parameters (increased resorptions per litter, resorption/implant ratio and females with resorptions) occurred in all treated groups but were within historical control ranges and were not statistically significant, thus these increases are not considered treatment related (see following Table).

	Females (mg/kg bw/day)			
	0	*15*	*50*	*120*
Reproductive performance				
viable litters	25	26	26	23
viable foetuses	393	398	379	356
mean litter size	15.7	15.3	14.6	15.5
number of resorptions	13	30	26	33
resorptions/litter[a]	0.5	1.2	1.0	1.4
resorption/implant ratio[b]	0.031	0.072	0.073	0.085
females with resorptions[c]	36%	69%	54%	65%

[a]historical control data for resorptions/litter; mean: 0.9, range: 0.5-1.6
[b]historical control data for resorption/implant ratio; mean: 0.059, range: 0.033-0.107
[c]historical control data for % females with resorption; mean: 51.6%, range: 37.5-81.0%

Effects on Foetus

Foetal body weights were statistically decreased for females at 120 mg/kg bw/day (↓6%) but is considered to be minor due to the small magnitude of the decrease. The 120 mg/kg bw/day males and combined sexes were also decreased (↓4-5%) but were not statistically significant.

There were no treatment related increases of external or visceral malformations in treated groups, although there were sporadic observations of folded retina, distended lateral ventricle, cleft palate and unilateral microphthalmia.

There were no treatment related increases of skeletal malformations but ossification variations (unossified 5th and 6th sternebra, rudimentary 1st lumbar rib and total ossification variations) occurred at higher levels in the 50 and 100 mg/kg bw/day groups (see following Table). The increases at 120 mg/kg bw/day (unossified 5th and 6th sternebra, and rudimentary 1st lumbar rib) at the high dose were considered treatment related based on the increase over concurrent controls. The foetal and litter incidence of unossified 5th and 6th sternebra in the 50 mg/kg bw/day group were close historical control means and are therefore not considered to be treatment related. These variations are likely to be secondary effects of maternal toxicity.

		Females (mg/kg bw/day)				*Historical*
		0	*15*	*50*	*120*	*control**
Ossification variations						
unossified 5th sternebra	– foetal	38/193 (20%)	46/194 (24%)	62/183 (34%)	74/174 (43%)	mean: 44% range: 13-64%
	– litter	14/25 (56%)	18/26 (69%)	19/26 (73%)	21/23 (91%)	mean: 79% range: 50-96%
unossified 6th sternebra	– foetal	10/193 (5%)	13/194 (7%)	25/183 (14%)	30/174 (17%)	mean: 12% range: 2-37%
	– litter	7/25	10/26	11/26	13/23	mean: 37%

		(28%)	(39%)	(42%)	(57%)	range: 10-74%
Rudimentary 1st lumbar rib	– foetal	6/193	11/194	10/183	41/174	mean: 5%
		(3%)	(6%)	(6%)	(24%)	range: 0-19%
	– litter	4/25	8/26	6/26	16/23	mean: 18%
		(16%)	(31%)	(23%)	(70%)	range: 0-64%
Total variations	– foetal	108/193	116/194	142/183	138/174	mean: 79%
		(56%)	(60%)	(78%)	(79%)	range: 61-94%
	– litter	25/25	25/26	26/26	23/23	
		(100%)	(96%)	(100%)	(100%)	

*Obtained from 21 studies conducted by Pharmaco LSR between 1986-1991 (3519 foetuses from 513 litters).

There was a statistically significant increased incidence of total foetal malformations at 50 mg/kg bw/day (presence of five lumbar vertebrae, wavy ribs, thoracic/lumbar vetetbral defects) but due to the absence of a dose response, were not considered treatment related.

CONCLUSION
The maternal NOAEL was established as 15 mg/kg bw/day based on decreased body weight gains. The test substance did not exhibit reproductive toxicity. The NOAEL for foetal toxicity is 50 mg/kg bw/day based on ossification variations observed at the high dose. The test substance did elicit foetal toxicity in the absence of maternal toxicity under the conditions of the study.

TEST FACILITY Pharmaco (1994b)

B.34. Developmental toxicity

TEST SUBSTANCE Notified chemical

METHOD Not stated
Similar to OECD TG 414 Prenatal Developmental Toxicity Study

Species/Strain	Rabbit/New Zealand albino
Route of Administration	Oral – gavage
Exposure Information	Exposure days: Gestation days 6-18 Post-exposure observation period: Gestation days 19-28
Vehicle	Corn oil
Remarks - Method	In a developmental toxicity study, mated rabbits (25-27/dose) were administered the notified chemical by gavage on GD 6-18 at 0, 3, 10 or 30 mg/kg bw/day. Animals were sacrificed on GD 28 and subject to post mortem examination. Histopathology was not conducted on the examined maternal organs (liver and kidney). Dose selection was based on a range-finding study which determined a maximum tolerable dose of 30 mg/kg bw/day.

RESULTS

Group	*Number of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
control	27	0	0/27
low dose	27	3	0/27
mid dose	27	10	1/27
high dose	25	30	1/25

Mortality and Time to Death
There was one mortality at 10 and one at 30 mg/kg bw/day, and both were attributed to starvation as a result of a hairball. The 30 mg/kg bw/day animal also exhibited a ventricular discoloration, and an enlarged and pale liver. These deaths were not attributed to treatment.

Effects on Dams
No clinical signs of toxicity were observed in maternal animals. There were no statistically significant differences in absolute body weights, but there were statistically significant decreases in body weight gain at 30 mg/kg bw/day between GD 12-15 and 6-19 and is likely to indicate maternal toxicity at this dose, although

it is considered to be minimal.

Absolute and relative liver weights were statistically increased at 30 mg/kg bw/day (↑22%/↑24% absolute/relative). There were small non-statistically significant increases at 10 mg/kg bw/day (↑10%/↑6% absolute/relative), suggesting a dose response. Kidney weights were similar to controls.

Reproductive parameters were not affected by treatment, although there was a statistically significant increase in resorption rate at 3 mg/kg bw/day but this was within historical control values, and is not considered to be treatment related in the absence of a dose response.

Effects on Foetus

There were sporadic observations of various malformations in control and treated groups (severe forelimb flexor with inward rotation of the forelimb, hemivertebra, fused rib, forked rib, missing half of a vertebra with other half fused to adjacent vertebra, scoliosis, ectropic hydronephrotic kidney, kyphosis) but were not considered to be treatment related.

There was a statistically significant increase in crooked hyoid bones (see following Table) at 30 mg/kg bw/day that appears to be treatment related, thus indicating the threshold for foetal toxicity in this study. There were slight increases of this malformation at 3 and 10 mg/kg bw/day compared to the concurrent control, but these increases were within the historical control ranges of the laboratory and there was no dose response, thus they are not considered to be treatment related.

	Females (mg/kg bw/day)			
	0	*15*	*50*	*100*
Hyoid, crooked				
number of foetuses (percent)	9/169 (5%)	14/185 (8%)	11/129 (9%)	25/131* (19%)
number of litters (percent)	8/25 (32%)	11/25 (44%)	9/22 (41%)	12/21* (57%)

Laboratory historical control: foetuses, 0/220-12/128 (0-9%); litters, 0/28-9/17 (0-53%).
Statistically significant compared to control (*P<0.05).

Remarks - Results

There were signs of maternal (decreased body weight gains and increased liver weights) and foetal toxicity (increased incidence of crooked hyoid) at 30 mg/kg bw/day. There was no developmental toxicity at non-maternotoxic doses, thus the test substance is not considered to be teratogenic.

CONCLUSION

The maternal NOAEL was established as 10 mg/kg bw/day based on decreased body weight gains and increased liver weights. The foetal NOAEL was established as 10 mg/kg bw/day based on an increased incidence of crooked hyoid. The test substance was not teratogenic under the conditions of the study.

TEST FACILITY Dow (1985)

B.35. Reproductive toxicity – two generation study

TEST SUBSTANCE Notified chemical

METHOD OPP 83-4
Similar to OECD TG 416 Two-Generation Reproductive Toxicity Study

Species/Strain	Rat/Fischer 344
Route of Administration	Oral – diet
Exposure Information	Exposure period: 22-24 weeks
Remarks - Method	In a two-generation reproductive toxicity study, P generation rats approximately 6 weeks old were administered dietary concentrations of the test substance at 0, 5, 20 or 75 mg/kg bw/day (30/sex/dose) for approximately 10 weeks. The animals were then mated over three weeks to produce the F1 generation (pregnancy detected by vaginal smear). Females were fed their premating dietary concentration during the mating period, lactation, weaning and post-weaning. The P generation were sacrificed after approximately 22 weeks of dosing.

The F1 generation was culled on day 4 postpartum (4/sex/litter) then weaned on postpartum day 28, and F1 adults (30/sex/dose) and F1 weanlings (10/sex/dose) were selected. F1 adults were administered the test substance for approximately 12 weeks before mating to produce the F2 generation. The F1 generation were sacrificed approximately 24 weeks postpartum. The F2 generation were culled on day 4 postpartum and maintained until weaning then sacrificed and subject to necropsy (10/sex/dose).

Histopathology was conducted on the liver, kidney and reproductive organs of P and F1 generations at the control and high dose, and the low and intermediate dose where necessary to examine possible treatment related effects. Sacrificed weanling pups were examined for gross abnormalities and the livers were examined histologically.

Weeks on study	*P*	*F1*	*F2*
0-10	- premating exposure		
11-13	- mating period		
14-17	- exposure continues	- litter born and culled on day 4 postpartum to 4/sex/litter	
18-20	- exposure continues	- litters weaned 28 days postpartum - adult population selected (30/sex/dose) - weanlings necropsied (10/sex/dose)	
22	- sacrifice and necropsy	- premating exposure begins	
23-33		- exposure continues	
34-37		- mating period	
37-40		- exposure continues	- litter born and culled on day 4 postpartum to 4/sex/litter
40-42		- exposure continues	- litters weaned 28 days postpartum - weanlings necropsied (10/sex/dose)
43		- sacrifice and necropsy	

Generation	*Group*	*Number and Sex of Animals*	*Dose mg/kg bw/day*
P, F1	control	30M + 30F/generation	0
P, F1	low dose	30M + 30F/generation	5
P, F1	mid dose	30M + 30F/generation	20
P, F1	high dose	30M + 30F/generation	75

RESULTS

Mortality and Time to Death

There were no treatment related mortalities in P or F1 generation animals. One P generation 5 mg/kg bw/day female was found moribund on day 96 and was submitted for pathological examination. Previously, an enlarged area on the right side of the head with a slight haemorrhage was observed on this animal. Histopathology revealed that morbidity in this animal was cause by a large infiltrative squamous cell carcinoma of the head. An F1 generation 75 mg/kg bw/day female was found dead on day 92 and was attributed to urolith formation resulting from a urethral blockage.

Effects on Parental (P) animals

There were no treatment related clinical signs of toxicity observed in P generation adults.

In males, there were no treatment related effects on absolute body weights or body weight gains in P generation males over the duration of the study, in fact, there were statistically significant increases in absolute body weight and body weight gain in some treated groups compared to controls after 3 months dosing until the

end of the study. Feed consumption was similar to controls during premating exposure but was generally higher in treated groups postmating, although the increases were not statistically significant. Gross pathological examination revealed treatment related incidences of increased size of the liver (27/30) at 75 mg/kg bw/day. There were associated increases in absolute and relative liver weights at all treatment levels and there was a dose response (see following Table). Additionally there was hepatocellular hypertrophy at 20 and 75 mg/kg bw/day, and vacuolation (consistent with fatty change) at 75 mg/kg bw/day. Liver weight increases at 5 mg/kg bw/day are considered to be of low toxicological concern based on the small magnitude and this dose is considered to be a no adverse effect level in males. Absolute and relative kidney weights were statistically increased at 20 and 75 mg/kg bw/day and there were associated tubular necrotic effects at 75 mg/kg bw/day.

	P males (mg/kg bw/day)			
	0	*5*	*20*	*75*
Group size	30	30	30	30
Liver				
absolute weight (g)	7.36	8.03*(↑9%)	8.57*(↑16%)	11.74*(↑60%)
relative to body weight	2.36	2.46*(↑4%)	2.56*(↑8%)	3.57*(↑51%)
hypertrophy, centrilobular	0	0	28(2.0)	30(3.0)
vacuolation, centrilobular	0	0	0	30(3.0)
Kidney				
absolute weight (g)	2.06	2.16	2.31*(↑12%)	2.44*(↑18%)
relative to body weight	0.66	0.66	0.69*(↑4%)	0.74*(↑11%)
mineralisation, intratubular	0	2	0	20
necrosis, intratubular epithelial	0	0	0	30(2.0)

Statistically significant compared to control (*P<0.05).
( ), Average severity of affected animals for histopathological parameters: 1=very slight, 2=slight, 3=moderate.

Body weight gain in P generation females during the premating period was statistically decreased (↓10%) but was not affected by treatment during gestation at 75 mg/kg bw/day. During lactation days 1-21 there was a statistically significant decrease in body weight gain in the 75 mg/kg bw/day (↓29%) but the body weight gain from days 1-28 were not different from controls. Body weights for P generation females were not reported after the lactation period. There were no statistically significant changes in feed consumption in treated groups. Absolute and relative liver weights were statistically increased at 75 mg/kg bw/day and there was associated hepatocellular hypertrophy and vacuolation (consistent with fatty change) at this dose (see following Table). Absolute and relative kidney weights were statistically increased at 75 mg/kg bw/day (increase in relative kidney weight at 20 mg/kg bw/day is considered minimal). The no adverse effect level for P generation females is 20 mg/kg bw/day.

	P females (mg/kg bw/day)			
	0	*5*	*20*	*75*
Group size	30	29	30	30
Liver				
absolute weight (g)	4.96	4.83	5.08	6.01*(↑21%)
relative to body weight	2.53	2.47	2.6	3.21*(↑27%)
hypertrophy, centrilobular	0	0	2(2.0)	29(2.0)
vacuolation, centrilobular	0	0	0	16(2.0)
Kidney				
Absolute weight (g)	1.45	1.46	1.48	1.58*(↑9%)
relative to body weight	0.74	0.74	0.77*(↑4%)	0.85*(↑15%)

Statistically significant compared to control (*P<0.05).
( ), Average severity of affected animals for histopathological parameters: 1=very slight, 2=slight, 3=moderate.

There was no effect on the various tested reproductive parameters (male and female mating index; male and female conception index; gestation index; gestation survival index; survival index; sex ratio on day 1; or gestation length) or the mean litter size.

Effects on 1st Filial Generation (F1)

Body weights of 75 mg/kg bw/day F1 pups were statistically lower than controls from postpartum day 4 to 28

(↓20% on day 28), which is considered to be a treatment related effect, likely due to the test substance administered to the pups through lactation, given the initial pup weights were similar to controls. The 75 mg/kg bw/day pups culled on day 4 exhibited a slight increase in runts (5/290 compared to 1/231 in the control group) but was attributed to the lower body weights in this group. In the weanlings sacrificed on postpartum day 28, there were incidences of vacuolation consistent with fatty change in 75 mg/kg bw/day males (8/10) and females (7/10).

There were no treatment related clinical signs of toxicity observed in F1 generation adults.

In males, absolute body weights were statistically decreased at 75 mg/kg bw/day group at all weekly intervals but there was no effect on the body weight gain in this group, suggesting that the decreases in absolute body weights were the result of the lower pup body weight, i.e., the test substance did not affect growth. Feed consumption was slightly higher in treated groups after mating but the changes were not statistically significant and are therefore of limited toxicological significance. There were treatment related increases in absolute and relative liver weights at 20 and 75 mg/kg bw/day. All 75 mg/kg bw/day males (30/30) had increased size of the liver, with associated dose related hypertrophy at 20 and 75 mg/kg bw/day and similar to the P generation there was centrilobular vacuolation (consistent with fatty change) at 75 mg/kg bw/day (see following Table). In the kidney there were increases in intratubular mineralisation, tubular dilation and tubular degeneration/regeneration at 75 mg/kg bw/day, additionally there were minimal statistically significant increases in absolute and relative kidney weights at 20 mg/kg bw/day with more marked increases at 75 mg/kg bw/day. The no adverse effect level in F1 generation males is 5 mg/kg bw/day.

	F1 males (mg/kg bw/day)			
	0	*5*	*20*	*75*
Group size	30	30	30	30
Liver				
absolute weight (g)	8.29	8.65	9.35*(↑13%)	12.39*(↑49%)
relative to body weight	2.30	2.38*(↑3%)	2.58*(↑12%)	3.66*(↑59%)
hypertrophy, centrilobular	0	0	20(2.0)	30(2.9)
vacuolation, centrilobular	0	0	1(2.0)	30(2.9)
Kidney				
absolute weight (g)	2.38	2.4	2.50*(↑5%)	2.62*(↑10%)
relative to body weight	0.66	0.67	0.69*(↑5%)	0.77*(↑17%)
mineralisation, intratubuar	19	26	16	28
tubular dilation with proteinaceous casts	10(2.0)	10(2.0)	12(2.0)	30(2.0)
degeneration/regeneration, tubular	11(2.0)	15(2.0)	10□2.0)	30(2.0

Statistically significant compared to control (*P<0.05).
( ), Average severity of affected animals for histopathological parameters: 1=very slight, 2=slight, 3=moderate.

The 75 mg/kg bw/day females also had statistically significant decreases in absolute body weights at most observation points during the study but the body weight gains were only affected during the first week of lactation. Like the males, the body weight effects are likely the result of the initial lower pup body weight. Feed consumption was decreased at 75 mg/kg bw/day during lactation (↓16-26%), although the decreases were not statistically significant. There were minimal statistically significant increases in absolute and relative liver and kidney weights at 20 mg/kg bw/day with more marked increases at 75 mg/kg bw/day (see following Table). Enlarged liver was observed in all (30/30) 75 mg/kg bw/day animals and there was associated hypertrophy and vacuolation detected histologically. The increased liver and kidney weights at 20 mg/kg bw/day are unlikely to be treatment related given their small magnitude and the lack of associated histopathological findings, thus the no adverse effect level in F1 generation females is 20 mg/kg bw/day.

	F1 females (mg/kg bw/day)			
	0	*5*	*20*	*75*
Group size	30	30	30	29
Liver				
absolute weight (g)	5.21	5.32	5.57*(↑7%)	6.53*(↑25%)
relative to body weight	2.53	2.52	2.67*(↑6%)	3.43*(↑36%)
hypertrophy, centrilobular	0	0	0	29(2.5)

vacuolation, centrilobular	0	0	0	28(2.5)
hyperplasia, bile duct	1	0	3	8
Kidney				
absolute weight (g)	1.47	1.49	1.56*(↑6%)	1.58*(↑7%)
relative to body weight	0.71	0.71	0.75*(↑6%)	0.83*(↑17%)

Statistically significant compared to control (*P<0.05).
( ), Average severity of affected animals for histopathological parameters: 1=very slight, 2=slight, 3=moderate.

There was no effect on the various tested reproductive parameters. The mean litter size was slightly decreased in all treatment groups and was statistically significant at 5 and 75 mg/kg bw/day. The study authors considered this effect to be due to random variation, noting the lack of a dose response.

Effects on 2nd Filial Generation (F2)
Body weights of 75 mg/kg bw/day F2 pups were statistically lower than controls at all observation points and is likely to be treatment related. There were no treatment related external or internal findings in the F2 pups culled on postpartum day 4. Weanlings sacrificed on postpartum day 28 exhibited increased liver size in 75 mg/kg bw/day males (7/10) and females (10/10). Histopathological findings in the weanlings were limited to slight hepatocellular vacuolation in 75 mg/kg bw/day males (9/10) and females (10/10).

Remarks – Results
Concentrations were verified for the P and F1 generation (three times each during the study) and were generally found to be higher than the target concentrations (up to ↑20%).

Similar toxicity was observed across the P and F1 adult generations. In males there were effects in the liver and kidney at 20 mg/kg bw/day and above, but maternal toxicity was only observed at 75 mg/kg bw/day as effects in females at 20 mg/kg bw/day were considered minor. Reproductive performance was not affected at any dose level in both generations, thus the test substance is not considered to be a reproductive toxicant. The only sign of foetal toxicity was decreased pup birth weights in F1 and F2 generations. Under the conditions of the study, there was no evidence of reproductive or foetal toxicity at doses that do no induce maternal toxicity.

CONCLUSION
The parental NOAEL was established as 5 mg/kg bw/day based on increased kidney and liver weights, and hepatocellular hypertrophy in 20 mg/kg bw/day males and above.

The maternal NOAEL was established as 20 mg/kg bw/day based on increased kidney and liver weights, and hepatocellular hypertrophy and vacuolation, and decreased body weight gain at 75 mg/kg bw/day.

A reproductive NOAEL was established as 75 mg/kg bw/day as there were no treatment related effects in reproductive performance.

The foetal NOAEL was established as 20 mg/kg bw/day based on decreased pup birth weights and liver effects at 75 mg/kg bw/day.

TEST FACILITY Dow (1988)

B.36. Toxicokinetic

TEST SUBSTANCE Notified chemical

METHOD OPPTS 870.7485 (85-1) Metabolism and Pharmacokinetics
Similar to OECD TG 417 Toxicokinetics

STUDY DESIGN AND OBJECTIVE

The aim of this study was to determine the distribution, excretion and metabolism of the notified chemical in rats. Three groups of Fischer 344 rats (5/sex/group) were administered a single gavage dose of ^{14}C radiolabelled test substance in a corn oil vehicle (radiolabelled at the 2 and 6 positions of the pyridine ring). Group 1 was administered a single 1 mg/kg bw radiolabelled dose, group 2 was administered a single 60 mg/kg bw dose, and group 3 was administered 14 daily 1 mg/kg bw/day doses of unlabelled test substance, followed by a single 1 mg/kg bw radiolabelled dose on day 15. All rats were placed in glass Roth-type metabolism cages for collection of excreta over 72 hours. Urine was collected every 12 hours and faeces every 24 hours. Expired air was collected for the 60 mg/kg bw group after 12 and 24 hours, however, because no radioactivity was detected, expired air was no longer collected. Urine and faecal samples were analysed by liquid scintillation counting (LSC). The urine samples were further analysed by HPLC and GC/MS to identify metabolites. All rats were sacrificed by CO_2 asphyxiation and exsanguinated at 72 hours. The following tissues were analysed by LSC to determine the content of residual test substance: bone, brain, liver, kidneys, fat, gonads, lung, heart, blood (RBCs and plasma), skeletal muscle, spleen, skin and the remaining carcass.

Two additional groups of male rats (3/group) were administered radiolabelled test substance at 1 or 60 mg/kg bw to examine plasma concentration over time. The rats were fitted with jugular vein cannulae and blood was sampled at 15, 30, 45 minutes, 1, 2, 4, 6, 8, 12, 18, 24, 30 and 48 hours. The blood was analysed by LSC. Three additional groups of males (3/group) were administered 60 mg/kg bw radiolabelled test substance and sacrificed at 2, 10, 24 or 72 hours to determine plasma, liver, kidney and fat concentrations.

RESULTS

No signs of toxicity were observed in any group. The total recovery was within acceptable limits (94.81-99.24%) and therefore the results of this study are considered adequate to characterise the toxicokinetics of the notified chemical. Almost all the notified chemical had been excreted by 72 hours as less than 1% of the administered radioactivity remained in the carcass (see following Table). The majority of the administered radioactivity was excreted in urine (79.56-85.48% at 72 hours) with a smaller amount in faeces (11.04-14.16% at 72 hours). The urinary excretion indicates high oral bioavailability of the test substance and that it is almost completely absorbed from the gastrointestinal tract. There were no notable differences in excretion at 72 hours between the 1 and 60 mg/kg bw test groups or between sexes. There were also no notable differences between the 1 mg/kg bw single and repeated dose groups, and therefore the test substance is not bioaccumulating following repeated administration. Almost all the administered dose (~90%) was excreted by 24 hours and only small amounts were detected in the carcass after 72 hours (<1%). The highest levels of radioactivity in the carcass were found in the liver (up to 0.84%) with small amounts found in the kidneys, lung and blood (plasma and red blood cells).

Two metabolites (6-CPA and the 6-CPA glycine conjugate) were detected in urine at the 12 and 24 hour points. Unchanged parent compound was not detected in the urine, which is consistent with its low water solubility.

	1 mg/kg bw		*60 mg/kg bw*		*1 mg/kg bw/day*	
	Males	*Females*	*Males*	*Females*	*Males*	*Females*
			(% of administered radioactivity at 12 hours)			
*Urine**	69.59	63.93	41.60	38.57	73.61	71.70
(6-CPA)	(40.3)	(27.7)	(45.2)	(40.9)	(29.5)	(18.3)
(6-CPA-Gly)	(59.7)	(72.3)	(54.8)	(59.1)	(70.5)	(81.7)
			(% of administered radioactivity at 24 hours)			
*Urine**	80.47	76.67	77.96	77.31	83.29	82.15
(6-CPA)	(52.5)	(46.5)	(69.0)	(54.0)	(41.6)	(20.5)
(6-CPA-Gly)	(47.5)	(53.5)	(31.0)	(46.0)	(58.4)	(79.5)
Faeces	10.13	11.12	10.04	10.98	9.37	8.44
Total	90.60	87.79	88.00	88.29	92.66	90.59
			(% of administered radioactivity at 48 hours)			
*Urine**	82.27	78.95	82.17	83.27	85.01	84.33
Faeces	11.52	13.25	12.62	13.58	10.88	11.19
Total	93.79	92.20	94.79	96.85	95.89	95.52
			(% of administered radioactivity at 72 hours)			
Urine	82.73	79.56	82.64	83.91	85.48	84.91
Faeces	11.84	13.63	13.06	14.16	11.04	11.51
Tissue	0.93	0.95	0.55	0.51	0.85	0.79
Cage wash	0.36	0.67	0.48	0.66	0.10	0.36
Total	95.86	94.81	96.73	99.24	97.47	97.57

*includes cage wash.
( ), indicates the proportion of each metabolite present in the urinary portion of administered dose.
6-CPA, 6-chloropicolinic acid. 6-CPA-Gly, 6-6-chloropicolinic acid glycine conjugate.

The plasma concentration over time (see following Table) showed that maximum plasma concentration was reached after 2 hours (Cmax 0.599 and 23.849 µg/g plasma for 1 and 60 mg/kg bw groups, respectively), demonstrating rapid oral absorption. The study authors propose a 2 compartment biphasic model for absorption and elimination of the notified chemical. The primary difference between the 1 and 60 mg/kg bw/day groups was a slower rate of absorption, which was 2.6 fold higher in the 60 mg/kg bw/day group.

	µg equivalents the notified chemical /g plasma	
Collection time (hours)	*1 mg/kg bw*	*60 mg/kg bw*
0.25	0.212	8.108
0.50	0.320	13.548
0.75	0.425	16.252
1	0.453	18.799
2	0.599	23.849
4	0.525	20.308
6	0.409	15.532
8	0.264	14.142
12	0.075	14.570
18	0.024	5.542
24	0.010	2.126
30	0.008	0.798
48	0.003	0.225

The concentration in tissue generally decreased from the first observation point (see following Table) with the exception of the fat and kidneys, which increased from 2 to 10 hours then decreased from 10 to 72 hours. The high fat content at 2 and 10 hours could be due to the lipophilic nature of the notified chemical.

	µg equivalents the notified chemical /g plasma			
Collection time (hours)*	*Plasma*	*Liver*	*Kidney*	*Fat*
2	38.55	11.60	26.49	72.46
10	31.42	8.84	35.47	183.10
24	3.76	6.83	4.13	4.22
72	NQ	2.02	1.00	NQ

*3 animals/collection time, except at 72 hours was 5 animals/collection time. NQ, not quantifiable (less than 3 times background signal).

The area under the curve (4.96 and 307.51 µg/hr/kg for 1 and 60 mg/kg bw groups, respectively) was proportional to dose and the plasma clearance rates were identical for the two groups (162 g/hr/kg).

CONCLUSION
The notified chemical is rapidly absorbed in rats following oral administration with maximum plasma concentration reached after 2 hours, regardless of dose. Excretion was also rapid with approximately 90% excreted after 24 hours and 99% excreted after 72 hours. The urine was the primary route of excretion accounting for around 80-85% of the total excretion after 72 hours, as either 6-CPA or the glycine conjugate of 6-CPA. The faeces accounted for approximately 11-13% of excretion after 72 hours.

TEST FACILITY Dow (1987c)

B.37. Toxicokinetic

TEST SUBSTANCE Notified chemical

METHOD OPPTS 870.7485 Metabolism and Pharmacokinetics
Similar to OECD TG 417 Toxicokinetics

STUDY DESIGN AND OBJECTIVE

The aim of this study was to determine the distribution, excretion and metabolism of the notified chemical in mice. The doses were selected to determine whether non-linearity in the pharmacokinetics or metabolism of the notified chemical is observed at a dose level which caused tumours in mice, in comparison with a lower dose where no tumours were observed.

Groups of B6C3F1 male mice (10/group) were administered single gavages doses of ^{14}C radiolabelled notified chemical at 25 or 250 mg/kg bw, formulated in corn oil. Males only were used because of the lack of sex specific differences in a chronic mouse study. The animals were placed in Rothe-type metabolism cages and urine (and cage wash) was collected at 12 hour intervals and faeces was collected at 24 hours intervals. The mice were sacrificed at 72 hours and tissues (kidney, liver, glandular stomach, nonglandular stomach, duodenum, blood/plasma and remaining carcass) were collected and analysed to determine residual test substance. All analyses were conducted using LSC. The urine was subject to HPLC and LC/MS to identify metabolites.

Two groups of male F344 rats (2/group) were administered a single gavage dose of radiolabelled test substance at 1 or 60 mg/kg bw and urine was collected at 12 and 24 hours, to compare the metabolite excretion profile between species.

Additional groups of B6C3F1 male mice (3/group) were administered single gavages doses of unlabelled test substance at 0, 25 or 250 mg/kg bw to determine toxic effects. These groups were sacrificed at 72 hours and the tissues analysed.

RESULTS

The total recovery was within acceptable limits (99-101%) and therefore the results of this study are considered adequate to characterise the toxicokinetics of the test substance. The notified chemical was primarily excreted in the urine with approximately 70% of the administered radioactivity detected after 24 hours in the high and low dose groups, mostly as a glycine conjugate of 6-CPA (66% for the low dose and 59% for the high dose), with small amounts excreted as the unconjugated 6-CPA (4% for the low dose and 8% for the high dose). There were also small amounts detected in the urine of a taurine conjugate of 6-CPA in the low and high doses, and approximately 1% of the parent compound in the high dose only. The main difference between the low and high doses was the amount of radioactivity detected in the urine at the 12 hour collection points and was markedly lower in the high dose (34.5%), than at the low dose (62.3%), which may indicate that the high dose has exceeded the limit of renal clearance for the notified chemical, although the study authors attributed this to slow absorption of the notified chemical. Faecal excretion accounted for approximately 22% at the low dose and 16% at the high dose. Almost all the administered dose was excreted by 72 hours as less than 1% remained in the carcass at 72 hours, with the liver and remaining carcass representing the highest amounts in both dose groups.

	Males	
	25 mg/kg bw	*250 mg/kg bw*
	(% of administered radioactivity at 12 hours)	
Urine and cage rinse	62.33	34.49
Faeces	-	-
Total	62.33	34.49
	(% of administered radioactivity at 24 hours)	
Urine and cage rinse	71.86	68.94
Faeces	20.12	12.80
Total	91.98	81.74
	(% of administered radioactivity at 48 hours)	
Urine and cage rinse	74.99	81.03
Faeces	20.96	15.46
Total	95.95	96.49
	(% of administered radioactivity at 72 hours)	
Urine and cage rinse	77.06	84.14
Faeces	21.55	16.04
Tissue	0.77	0.64
Total	99.38	100.82

The amount recovered in the urine of rats at 24 hours was slightly higher than in mice (80.5% and 78.0% for rats dosed with 1 and 60 mg/kg bw, respectively). Additionally, rats excreted a higher proportion of the

administered radioactivity as unconjugated 6-CPA, consequently they excreted less of the glycine conjugate in urine.

There were no histopathological observations in organs of the groups of mice administered single gavage doses at 0, 25 or 250 mg/kg bw of unlabelled test substance.

CONCLUSION
the notified chemical is rapidly absorbed and excreted in mice following oral administration, with approximately 99% of the administered dose excreted after 72 hours, and less than 1% remaining in the carcass. The glycine conjugate of 6-CPA is the major metabolite found in urine, with small amounts of 6-CPA and a taurine conjugate of 6-CPA. The main observable difference between mice and rats was that rats excrete a higher proportion of the unconjugated 6-CPA in urine compared to mice that excrete more of the conjugated glycine form.

TEST FACILITY Dow (1998)

B.38. Toxicokinetic

TEST SUBSTANCE Notified chemical

METHOD Non-guideline study

STUDY DESIGN AND OBJECTIVE
Two separate studies were conducted to evaluate the excretion of the notified chemical in a single dog (Redemann and Clark, 1967) and in two rats (Redemann *et al.*, 1966).

In the dog study, a single dog was fed unlabelled test substance in the diet at 40 ppm for three weeks before one week of feeding with radiolabelled test substance. Urine and faeces were collected twice daily. Radioactivity was detected using an unstated method. The glycine conjugate of 6-CPA present in the urine was identified using UV and IR absorption spectroscopy with a standard.

In the rat study, two rats were fed a single dose of radiolabelled test substance at 100 ppm. The animals were housed in metabolism cages and excreta were collected daily. The urine was analysed using paper chromatography, then the metabolites were identified using UV spectroscopy.

RESULTS
Approximately 80% of the administered dose was found in the urine of dogs, although no time data were provided. A glycine conjugate of 6-CPA only was identified.

The proportion of administered dose excreted in urine was not specified for the rat study. However, 6-CPA and the 6-CPA glycine conjugate were detected in urine. The study authors hypothesised that 6-CPA is an intermediate in the conversion of the notified chemical to the conjugate and that the dog has a greater ability to conjugate the notified chemical.

CONCLUSION
The notified chemical was excreted in the urine (80%) in the dog as a 6-CPA glycine conjugate but was excreted as both 6-CPA and a 6-CPA glycine conjugate in the rat.

TEST FACILITY Redemann *et al.* (1966)
Redemann and Clark (1967)

B.39. Toxicokinetic

TEST SUBSTANCE 6-chloropicolininic acid

METHOD No guideline stated
Similar to OECD TG 417 Toxicokinetics

STUDY DESIGN AND OBJECTIVE
The aim of this study was to determine the distribution, excretion and metabolism of 6-CPA in rats. Three

males and three females were administered a single oral (assumed to be gavage) 10 mg/kg bw dose of ^{14}C carboxy-labelled 6-CPA. All rats were placed in metabolism cages to collect urine, faeces and expired air. Urine and faeces were collected at 8, 24 and 48 hours after which the animals were sacrificed and the carcasses analysed. All radioactivity analyses were conducted using LSC. The urine samples were then pooled and analysed by thin-layer chromatography and mass spectrometry for metabolites.

Another group of six rats (3 male and 3 female) were administered 10 mg/kg bw of radiolabelled 6-CPA to determine plasma concentration over time, measured at 1, 2, 4, 8, 12, 16, 24, 36 and 48 hours post dose from orbital sinus puncture.

RESULTS
Target concentrations for the excretion and metabolism study was 11.0 mg/kg bw and for the plasma concentration study was 9.3 mg/kg bw. The total recovered radioactivity was between 95-105% and therefore the results are acceptable. Cage washings were less than 1% and were not included in the totals.

Urine was the primary route of excretion accounting for 97.64% of the administered dose at 48 hours, with small amounts excreted in the faeces (1.48%) and expired air (1.30%). The carcass accounted for 0.20%, with small amounts found in the liver and kidneys. The raw data for excretion at 8 and 24 hours were not provided. The compounds detected in urine were the parent compound and a glycine conjugate, with 30% of the radioactivity detected in urine at 8 hours due to 6-CPA and the remainder due to the glycine conjugate of 6-CPA. There were no notable differences between the sexes.

The raw plasma concentration data were not provided. The maximum detected concentration appeared after 1 hour and the concentration was below quantitation after 8 hours. The study authors concluded that the plasma concentration data indicate first-order rate constant for the clearance of 0.64 hr^{-1}. The half-life for plasma clearance was calculated to be 1.1 hours.

CONCLUSION
Based on the limited data available in the study, 6-CPA appears to be rapidly absorbed and excreted with 97% of the administered dose excreted via the urine within 48 hours as either the parent, with small amounts excreted in the faeces and expired air.

TEST FACILITY Dow (1973)

B.40. Dermal absorption

TEST SUBSTANCE Notified chemical

METHOD OPPTS 870.7600 Dermal Penetration – Draft

STUDY DESIGN AND OBJECTIVE
The aim of this study was to determine the *in vivo* dermal absorption of the notified chemical in rats. Two groups of four male Fischer 344 rats were administered an occlusive dermal dose of ^{14}C radiolabelled notified chemical at 1 mg/cm^2 to a 10 cm^2 shaved area on the back of the rat. The test substance was administered in a dipropylene glycol monoethyl ether vehicle and was administered at a volume of 10 $\mu L/cm^2$ of skin (equivalent to 100 g/L concentration of the notified chemical). The test substance was occluded using a Stomahesive skin barrier, covered and bandaged onto the rat for 24 hours. Animals were housed in Roth-type metabolism cages and excreta were collected and analysed by LSC.

The first group of rats were sacrificed directly after the 24 hours dosing period and the skin was washed just prior to sacrifice. The skin barrier, bandages and cover, and skin wash were analysed by LSC. The skin site was excised and the remaining skin, blood, kidneys, liver and carcass were all analysed by LSC. The second group were maintained until 72 hours with excreta collected every 24 hours. The dose site was washed at 24 hours and then re-occluded until the 72 hour sacrifice point, after which the animals were treated as described for the first group.

RESULTS
The total radiation recovered in the study was 90% for the first group and 84% for the second. However, because the test substance is considered to be volatile these values are within acceptable limits according to OECD (2011) Guidance Document. The achieved concentration was 91.2% (equivalent to 91.2 g/L). The study

authors excluded an animal from group 1 because the test substance was leaking from the bandage, which was supported by the low total recovery of 61% for this animal.

The unabsorbed portions (bandage, covering, frame, skin wash and final cage wash) were similar between the two groups, indicating consistency in the procedures between groups. The absorbed portion increased as expected following longer observation indicating that the test substance continues to be absorbed from the skin compartment. The urine was the major route of excretion with minor amounts excreted in faeces.

While excretion of the test substance decreased over 72 hours, the end of elimination was not reached, which indicates that absorption of the test substance from the skin compartment (total % absorbable in the Table below) past the 72 hour observation point is likely and therefore the absorbable dose (dosed and remote skin) is considered to be potentially absorbed for the notified chemical. The portion of dose considered to be potentially absorbed was 44% for group 1 and 39% for group 2. The higher value of 44% will be used for risk assessment of the notified chemical.

	*Group 1**	*Group 2*
	24 hr sacrifice	*72 hr sacrifice*
Bandage	7.97 ±7.50	8.77 ±9.77
Covering	2.74 ±0.74	4.68 ±2.66
Frame	27.28 ±3.91	24.28 ±5.32
Skin wash	4.87 ±1.67	3.65 ±0.61
Final cage wash	3.17 ±0.90	2.50 ±1.93
Total % unabsorbed	**46.03 ±3.39**	**43.87 ±7.94**
Dosed skin	17.83 ±3.79	4.13 ±2.08
Remote skin	1.63 ±0.02	0.57 ±0.26
Total % absorbable	**19.46 ±3.79**	**4.70 ±2.26**
Blood	0.14 ±0.06	0.02 ±0.01
Carcass	3.59 ±1.53	0.48 ±0.16
Kidneys	0.17 ±0.05	0.06 ±0.02
Liver	0.62 ±0.21	0.25 ±0.09
(Faeces 24 hr)	(0.86 ±0.21)	(0.83 ±0.30)
(Faeces 48 hr)	-	(1.72 ±0.64)
(Faeces 72 hr)	-	(0.71 ±0.23)
Faeces total	0.86 ±0.21	3.25 ±0.86
(Urine 24 hr)	(19.19 ±4.88)	(15.93 ±5.05)
(Urine 48 hr)	-	(10.66 ±3.79)
(Urine 72 hr)	-	(3.96 ±2.16)
Urine total	19.19 ±4.88	30.55 ±9.58
Total % systemic absorption	**24.58 ±6.43**	**34.61 ±10.65**
Total % recovery	90.07 ±4.03	83.61 ±10.65
Total % potentially absorbed	**44.04 ±7.43**	**39.30 ±10.53**

Date presented is mean % of administered radioactivity ± standard deviation. Separate faecal and urinary excretion presented for group 2, only total faeces and total urine included in total systemic absorption.
( ), indicates subtotals of faecal or urinary excreta.
*One animal excluded for leakage outside bandage.

CONCLUSION
Approximately 44 % of the notified chemical was absorbed when applied at 100 g/L concentration to the skin of rats.

TEST FACILITY Dow (1997b)

B.41. Mode-of-action

TEST SUBSTANCE Notified chemical

STUDY DESIGN AND OBJECTIVE
The aim of this study was to retrospectively evaluate proliferation and apoptosis of hepatocytes from a previously conducted two-week study in B6C3F1 mice (not evaluated in this report), and whether the proliferative and apoptotic responses were associated with liver weight increases.

In the two week dietary study, mice were administered the notified chemical at 0, 200 or 400 mg/kg bw/day. Paraffin-embedded blocks of liver tissue were analysed for 5 males and 5 females per dose. Proliferating cell nuclear antigen (PCNA) was used as an endogenous marker of hepatocellular proliferation and *in situ* end labelling (ISEL) was used as a marker of hepatocellular apoptosis.

PCNA is a cofactor protein involved in DNA replication and is only present during proliferation. In the study, tissue sections were incubated with a 1:400 dilution of monoclonal anti-PCNA 19A2 for 1 hour at room temperature, followed by incubation with a 1:200 dilution of biotinylated goat anti-mouse IgM. The tissue was then incubated with diaminobenzidine and counter stained with haematoxylin. The PCNA positive cells were microscopically identified by the presence of dark red/brown, nuclear and cytoplasmic precipitate. A proliferation index was then determined (percentage of PCNA positive cells). This was conducted for two left lateral sections, two right middle lobe sections and one right lateral lobe section.

The ISEL technique identifies fragmented DNA (indicative of apoptosis) based on the specific binding of terminal deoxynucleotidyl transferase (TdT) specifically binding to 3'-OH ends of fragmented DNA, with biotinylated deoxyuridine (dUTP-biot) incorporation at the breakage sites. In the study, TdT and dUTP-biot were incubated with the deparrafinised sections (which had been incubated with proteinase K at room temperature for 30 minutes) for 37°C for one hour, then terminated using TB buffer and covered with bovine serum albumin for 10 minutes. The sections were then incubated with avidin-biotin-horseradish peroxidase conjugate for 30 minutes at 37°C. Apoptotic cells were identified by the presence of reddish chromagen. The cells were analysed microscopically, with a positive control slide (mouse duodenum) used to identify apoptotic cells, and the apoptotic index was determined (number of apoptotic cells per three sections of liver).

RESULTS

There were statistically significant increases in centrilobular proliferation index in males treated at 200 and 400 mg/kg bw/day and in the periportal proliferation index in 400 mg/kg bw/day males (see following Table). Proliferation indices in female groups were similar to controls. The non-statistically significant increase in proliferation index in the periportal region in females treated at 400 mg/kg bw/day was considered by the study authors to be treatment related and biologically significant. There were non-statistically significant increases in apoptotic index in males (and females) but the study authors did not attribute these to treatment.

	Males (mg/kg bw/day)			*Females (mg/kg bw/day)*		
	0	*200*	*400*	*0*	*200*	*400*
Proliferation index (% PCNA positive cells)						
centrilobular	0.03	0.50*	3.16*	0.38	0.20	0.27
periportal	0.15	0.12	1.33*	0.37	0.49	0.75
Apoptotic index (no. of apoptotic hepatocytes/three sections of liver)						
group mean	2.0	4.2	3.8	1.0	1.0	2.0

*Statistically significant compared to control (P<0.05)

The report also presented the histopathology results from the livers in the two-week toxicity study. Slight single cell necrosis was observed in males treated at 400 mg/kg bw/day. Hypertrophy was observed in both sexes at both dose levels. Effects in females were less severe and there were no necrotic effects. The study authors attribute the proliferative response to cytotoxicity, based on the necrosis in males (i.e., a compensatory response to replace dying hepatocytes). Additionally, the study authors suggest that based on the results of this study, apoptosis does not play a significant role in the pathogenesis of liver enlargement.

CONCLUSION

The study indicated that a significant amount of hepatocellular proliferation occurred in males treated at 200 and 400 mg/kg bw/day and in females treated at 400 mg/kg bw/day, following treatment with the notified chemical for 2 weeks.

TEST FACILITY Dow (1996)

B.42. Mode-of-action

TEST SUBSTANCE Notified chemical

STUDY DESIGN AND OBJECTIVE

The aim of the study was to investigate potential modes-of-action contributing to liver tumour formation in mice treated with the notified chemical. In this study, male B6C3F1/Crl mice were administered dietary doses of the notified chemical at 0, 75, 250 or 400 mg/kg bw/day for 7 or 14 days (6/dose/time period, with the exception of the 14 day 400 mg/kg bw/day group, where 9 were used), with additional recovery groups (6/dose) treated for 14 days followed by a 21 day recovery period.

The animals were subject to cage-side observations, body weights, feed consumption, clinical chemistry (total bilirubin, total protein, albumin, globulin, cholesterol, triglycerides, gamma glutamyl transpepsidase, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase), liver weights, histopathology (duodenum and liver), in addition to targeted gene expression analysis to examine possible metabolic pathways, and hepatocellular proliferation.

5-Bromo-2'-deoxyuridine (BrdU) was used a surrogate marker for cellular proliferation. The BrdU was incorporated for 7 days prior to sacrifice for each group using a mini-osmotic pump implanted subcutaneously into the lumbar or intrascapular region on the dorsal region of each mouse to deliver a continuous dose of BrdU. Immunohistochemical staining for the BrdU-labeled nuclei as a measure of hepatocellular proliferation, based on the interpretation of the nuclear staining intensity.

RNA was extracted (Qiagen RNeasy kit) and analysed using TaqMan Gene Expression Assays (Applied Biosystems) by real-time polymerase chain reaction (Applied Biosystems 7500 Real-Time Polymerase Chain Reaction System). The transcription level of the following genes were examined: Cyp1a1 [aryl hydrocarbon receptor (AhR) response gene], Cyp2b10 [constitutive androstane receptor (CAR) response gene], Cyp3a11 [pregnane X receptor (PXR) response gene] and Cyp4a10 [peroxisome proliferator activated receptor alpha (PPARα) response gene]. The results of a non-concurrent positive control group (exposed to 150 mg/kg bw/day phenobarbital for 7 days) were included in the analysis.

Protein measurement for the Cyp2b10 activation was conducted by western immunoblotting. An activity analysis of the Cyp2b10 enzyme was conducted using 7-pentoxy-resorufin O-deethlyation (PROD) liver activity. Additionally, total hepatic cytochrome p450 (Cyp450) content was determined for the 14 day and recovery groups, by spectrophotometric comparison of the reduced form of Cyp450 (carbon monoxide exposed) to the unreduced form.

Group	*Nominal dose (mg/kg bw/day)*	*Achieved* dose (mg/kg bw/day)*		
		7-day	*14-day*	*Recovery*
control	0	0	0	0
low dose	75	65.7	58.3	55.6
mid dose	250	198	207	187
high dose	400	294	313	335

*Time-weighted average doses

RESULTS

There were no mortalities or treatment related cage side observations noted during the study. There were no statistically significant decreases in body weight gain (although slight decreases in body weight gains in each group treated at 400 mg/kg bw/day). There was no treatment related effect on feed consumption.

Cholesterol levels were statistically decreased in males treated at 250 and 400 mg/kg bw/day in both the 7 and 14 day studies, but not in the recovery groups. There was a statistically significant increase in AST noted in the 400 mg/kg bw/day groups at 7 days but not the 14 day or recovery groups. Additionally, there was a statistically significant increase in triglyceride levels in the 7 day study at 400 mg/kg bw/day (↑77%), with only a slight non-statistically significant increase in the 14 day study (↑23%), and no increase in the recovery group.

There were statistically significant increases in absolute and relative liver weights noted in groups treated at 250 and 400 mg/kg bw/day for the 7 and 14 day studies, but not in the recovery groups. Histopathological changes were observed in the liver (hypertrophy, inclusion bodies, mitotic alterations, vacuolisation and necrosis) and duodenum (hypertrophy and vacuolisation) in the 7 and 14 day studies (see following Table), but these changes were not noted in the recovery groups. The liver changes tended to occur in animals treated at 250 and 400 mg/kg bw/day with frequency and severity increasing with dose. The histopathological findings in the duodenum (hypertrophy and vacuolisation) were noted at all treatment levels with a dose-response relationship evident.

	Males (mg/kg bw/day)			
	0	*75*	*250*	*400*
Liver				
hypertrophy				
7-day	0/6	0/6	6/6 (1.7)	6/6 (2.0)
14-day	0/6	0/6	6/6 (1.8)	9/9 (3.0)
inclusion-body				
7-day	0/6	0/6	5/6 (1.0)	6/6 (2.0)
14-day	0/6	0/6	5/6 (1.0)	9/9 (2.0)
mitotic alteration				
7-day	3/6 (1.0)	2/6 (1.0)	6/6 (1.2)	6/6 (2.0)
14-day	0/6	0/6	4/6 (1.0)	7/9 (1.4)
necrosis, individual cell				
7-day	0/6	0/6	1/6 (1.0)	3/6 (1.0)
14-day	0/6	0/6	0/6	1/9 (1.0)
vacuolisation				
7-day	0	0	3/6 (1.0)	6/6 (1.0)
14-day	0	0	5/6 (1.0)	9/9 (1.0)
Duodenum				
hypertrophy				
7-day	0/6	3/6 (1.0)	6/6 (1.2)	6/6 (1.3)
14-day	0/6	2/6 (1.0)	6/6 (1.2)	9/9 (1.6)
vacuolisation				
7-day	0/6	6/6 (1.0)	6/6 (2.5)	6/6 (3.0)
14-day	0/6	4/6 (1.0)	6/6 (1.8)	9/9 (3.0)

( ), Average severity of affected animals for histopathological parameters: 1=very slight, 2=slight, 3=moderate.

The liver proliferation scores were mostly increased in animals treated at 400 mg/kg bw/day at 7 and 14 days, with some statistically significant increases at 250 mg/kg bw/day (see following Table). In contrast, the 400 mg/kg bw/day recovery group showed lower levels of proliferation compared to the concurrent controls, which was interpreted by the study authors as a recovery response.

	Males (mg/kg bw/day)			
	0	*75*	*250*	*400*
BrdU liver proliferation				
7-day study				
centrilobular	1.0	0.66	1.07	1.86
midzonal	1.0	0.51	1.46	3.02*
periportal	1.0	0.55	3.27*	8.02*
combined	1.0	0.57	1.94	4.34*
14-day study				
centrilobular	1.0	1.03	2.12	1.19
midzonal	1.0	0.75	1.70	3.80*
periportal	1.0	1.11	3.82*	11.19*
combined	1.0	0.95	2.43*	4.90*
recovery study				
centrilobular	1.0	1.09	1.00	0.39
midzonal	1.0	1.04	1.19	0.33
periportal	1.0	0.94	1.16	0.32*
combined	1.0	1.02	1.13	0.35

Data described as fold-change compared to concurrent control (score in treated group ÷ score in control group). *Statistically significant compared to control ($P<0.05$).

In the 7 day study, there was a clear dose related increase CAR Cyp2b10 activation (see following Table), which was similar to phenobarbital (stated by the study authors to have been administered at a carcinogenic concentration). However, unlike the phenobarbital group, PXR Cyp3a11 activation was similar among control and treated groups. There was a slight increase in AhR Cyp1a1 activation at 250 and 400 mg/kg bw/day, similar to the phenobarbital group. Additionally, there was a more noticeable increase in PPARα Cyp4a10 activation in the 250 and 400 mg/kg bw/day groups, with no response in the phenobarbital group. Similar changes were observed in the 14 day study. While the recovery groups showed almost complete recovery, there

were still slight increases in CAR Cyp2b10 activation.

The study authors comment that these gene expression data provide evidence that the hepatocellular tumours are likely to have a threshold dose. Furthermore, the results indicate that the test substance activates the CAR nuclear receptor and that ligand-mediated activation of a nuclear receptor has been identified in publications describing the mode-of-action for rodent hepatocellular carcinogenesis.

	Males (mg/kg bw/day)				
	0	*75*	*250*	*400*	*150 (PB)*
Gene expression					
7-day study					
Cyp1a1 (AhR)	1.00	1.11	1.96	2.03	2.06
Cyp2b10 (CAR)	1.00	4.05	351.02	761.04	807.43
Cyp3a11 (PXR)	1.00	0.86	1.38	1.51	6.93
Cyp4a10 (PPARα)	□.00	1.32	6.75	5.19	1.13
14-day study					
Cyp1a1 (AhR)	1.00	1.16	1.67	1.87	-
Cyp2b10 (CAR)	1.00	4.45	389.59	1092.3	-
Cyp3a11 (PXR)	1.00	0.69	1.12	1.19	-
Cyp4a10 (PPARα)	1.00	1.23	4.22	2.91	-
recovery study					
Cyp1a1 (AhR	1.00	1.02	0.92	1.04	-
Cyp2b10 (CAR)	1.00	1.60	2.91	2.70	-
Cyp3a11 (PXR)	1.00	1.08	1.23	0.88	-
Cyp4a10 (PPARα)	1.00	1.21	1.57	1.33	-

Data described as fold-change. *Statistically significant compared to control (P<0.05). PB, phenobarbital.

There were no statistically significant differences in total Cyp450 content, but there were slight non-statistically significant increases at 250 and 400 mg/kg bw/day in the 14 day (↑28% and ↑31%, respectively) and recovery studies (↑46% and ↑51%, respectively).

Results of the protein measurement assay were consistent with the gene expression analyses for Cyp2b10 in the 14 day and recovery groups (7 day groups not tested).

There were no statistically significant changes in Cyp2b10 activity (tested using PROD activity) in the 14 day or recovery groups. In contrast, the phenobarbital showed a statistically significant increase in activity relative to the control. The lack of elevated PROD activity was attributed by the study authors to mechanism-based (suicide) inhibition of the enzyme.

CONCLUSION
The proposed mode-of-action involves activation of the CAR nuclear receptor followed by hepatocellular proliferation. The study authors also suggest that the detected endpoints in this study show dose-responses, thus further supporting a threshold-based mechanism of carcinogenic activity.

TEST FACILITY Dow (2010a)

B.43. Mode-of-action

TEST SUBSTANCE Notified chemical

STUDY DESIGN AND OBJECTIVE
The aim of this study was to obtain information on the potential mode-of-action related to liver effects in mice treated with the notified chemical. This was done by assessing the hepatocellular proliferation in liver tissues from a previously conducted 3-month study in mice (Dow, 1995). Formalin fixed and paraffin embedded liver samples were obtained from mice administered 0, 200 or 400 mg/kg bw/day (10/sex/dose). Centrilobular, midzonal and periportal regions of the hepatic lobule were analysed separately for each animal by counting 1000 hepatocytes for each region and the percentage proliferation was determined. Cell proliferation was assessed by Ki67 immunohistochemical staining.

The sections were deparaffinised then hydrated using deionised water, before antigen retrieval, treatment

withof hydrogen peroxidase, and subsequently blocked with avidin and biotin blocking agents. The primary rat monoclonal antibody was added to each slide, which was then incubated for 30 minutes, before addition of the biotinylated anti-rat secondary antibody and incubation for a further 30 minutes. Streptaverdin/HRP conjugate was added and incubated for 30 minutes at room temperature, before adding Vector ImmPact DAB chomagen for 3-6 minutes at room temperature. The slides were then stained with haematoxylin. Slides with known proliferative activity were used as positive controls.

RESULTS

There were statistically significant increases in the percentage of Ki-67 positive cells in males treated at 400 mg/kg bw/day with some marginal non-statistically significant increases at 200 mg/kg bw/day (see following Table). The only statistically significant increase in females was for the centrilobular region in the 200 mg/kg bw/day group, but the values corresponding to the other female groups were generally increased compared to the controls.

	Males (mg/kg bw/day)			*Females (mg/kg bw/day)*		
	0	*200*	*400*	*0*	*200*	*400*
Proliferation index (% PCNA positive cells)						
centrilobular	0.02	0.06	0.11	0.01	0.13*	0.03
midzonal	0.02	0.04	0.12*	0.04	0.04	0.20
periportal	0.03	0.01	0.24*	0.05	0.18	0.11
combined	0.02	0.04	0.16*	0.03	0.12	0.11

*Statistically significant compared to control (P<0.05)

The study authors comment that these data indicate that treatment with the notified chemical results in increased hepatocyte mitogenesis in both sexes and that the observed increases are likely the result of treatment related hepatocellular single cell necrosis.

CONCLUSION

The study provides evidence of hepatocellular proliferation in male and female mice treated with the test substance.

TEST FACILITY Dow (2010b)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Bioaccumulation

TEST SUBSTANCE	Notified chemical
METHOD	163.62-11(d), 163.165-3 and 163.165-4, Fish Accumulation, EPA, published in the Federal Register July 10, 1978 and modified October 3, 1980
Species	Bluegill sunfish (*Lepomis sp.*)
Exposure Period	Exposure: 30 days Depuration: 14 days
Auxiliary Solvent	Acetone (≤0.24 mL/L)
Concentration Range	Nominal: 0.05 and 0.5 mg/L Actual: Not provided
Analytical Monitoring	Radiochemical assay by thin layer chromatography (TLC); HPLC/UV for the identification of ^{14}C labelled metabolites in fish (verified by TLC).
Remarks - Method	The test substance was ^{14}C-notified chemical, radiolabelled in the 2 and 6 positions of the pyridine ring with a specific activity of 10.2 mCi/rnmole, mixed with unlabelled material. The specific activity of the mixture was 5060 dpm/µg. Test fish (60 for each test level) were exposed to the test substance in a flow-through system with 3 volume replacements daily at 16 °C. The fish were vigorous and actively feeding at the beginning of exposure to the test substance. A solvent control was established. Sampling was conducted at days 3, 7, 10, 14, 21 and 28 during the uptake phase and at days 1, 3, 7, 10 and 14 during the depuration phase. Whole test fish at each sampling time were combusted in a Harvey Biological Materials Oxidizer then analysed by scintillation counting to calculate an approximate BCF.
RESULTS	
Bioconcentration Factor	<85
CT50	<1 day
Remarks - Results	Test conditions were stable for the duration of the test. Concentrations in fish peaked at around 21 days during the uptake phase. Lipid content of the samples was not measured. The test substance concentration in edible portions was between a quarter to a half of the concentration found in the viscera (head and intestine). The BCF of the test substance at the 0.05 mg/L and 0.5 mg/L treatment level was 85 or less for whole fish on day 21. The BCF for total ^{14}C of 230 and 50 were determined for whole fish at 0.05 mg/L and 0.5 mg/L, respectively, on day 30. The depuration rate constant was not calculated. However, the results indicate that total ^{14}C concentration in whole fish decreases to below half the steady state concentration within 1 day of depuration. The major metabolite in this study was identified to be 6-CPA. All other metabolites were either un-extractable or at too low levels to establish positive identification.
CONCLUSION	The notified chemical and its metabolite are slightly concentrating but are not expected to be bioaccumlative.
TEST FACILITY	Dow (1982)

C.1.2. Aerobic degradation in water-sediment systems

TEST SUBSTANCE	Notified chemical (2,6-^{14}C-nitrapyrin, 98.5% radiochemical purity) and a metabolite of the notified chemical (2,6-^{14}C-6-CPA; 100% radiochemical purity)
METHOD	Pesticide Assessment Guidelines, Subdivision D: Product Chemistry, Section 162-4 Conducted in accordance with the principles of good laboratory practice
Duration	Test 1 for notified chemical: 30 days; samples analysed at 0, 0.25, 1, 3, 7, 10, 15, 21 and 30 days after treatment Test 2 for the metabolite, 6-CPA: 32 days; samples analysed at 0, 1, 4, 7, 14, 21 and 32 days after treatment
Water:sediment ratio	2:1 (100 mL water to 50 g dry weight sediment)
Application	Test 1: nominal 0.37 µg/L of notified chemical Test 2: nominal 0.25 µg/L of 6-CPA Applied drop-wise evenly across water surface using positive displacement pipette
Solvent	0.1% acetonitrile
Controls	A sterile control was run concurrently with Test 1
Analytical monitoring	Water – concentration of test substance determined by direct analysis by liquid scintillation counter (LSC) and high performance liquid chromatography (HPLC) Sediment – extraction with 90:10 methanol : 2 N NaOH and analysis by LSC and HPLC, assayed sediment by oxidative combustion; extraction efficiency was >100% for the notified chemical in sand and >95% for 6-CPA in sandy loam Volatile trap – foam cut in half, one half assayed by oxidative combustion the other half extracted with acetonitrile and assayed by LSC CO_2 trap – LSC counting Metabolites – identification by co-chromatography with standards and liquid chromatography-mass spectrometry (LC-MS)
Remarks – Method	The behaviour of radiolabelled notified chemical and its metabolite, 6-CPA, in aquatic environment were each studied in a water/sediment system under aerobic conditions in the dark at 25 ºC ± 2 ºC. The closed test systems (under oxygen) consisted of two-chambered biometer flasks (one containing the water/sediment, the other containing 0.2 N NaOH for the collection of CO_2). The test for the notified chemical also included a trap for volatiles with a polyurethane foam plug inserted in the bridge for the collection of volatile organics. Duplicate samples were assayed at each time point. Surrogate samples (without the test substance) were used to determine the pH, dissolved oxygen and redox potential at each time point. These results indicate that for both test substances, the water columns were aerobic, while the sediment layers were anaerobic. Water and sediment samples were collected from Suffolk, Virginia, USA. The full characteristics of the test systems are reported in Table 3. A tiered approach was adopted to calculate the dissipation rates of the test substances in the test systems in accordance with FOCUS (2006). The kinetics data were fitted to both simple first order (SFO) and first order multi-component (FOMC) models. If the SFO model resulted in a better fit than the FOMC model, no further modelling was undertaken. Otherwise, the data was fitted to a double fist-order in parallel (DFOP) model, and used when a better fit than the FOMC model (except where parameter errors indicated poor fit). SFO models were also reported for use as modelling endpoints.

Table 3 Physico-chemical characterisation of aerobic water/sediment systems

Parameter	*Test 1 – notified chemical*		*Test 2 – 6-CPA*	
	Water	*Sediment*	*Water*	*Sediment*
Textural class[a]		Sand		Sandy loam
% Sand/silt/clay[a]		95/5/0		72/17/11
CEC [meq/100 g]		7.89		6.0
% Organic carbon		0.2		2.1
pH[c]	6.7	6.0	7.3	5.6
Redox potential E_h7 (mV)[b]	183 / 370	-240 / -41	245 / 476	-35 / 79
Oxygen content (mg/L)[b]	4.7 / 5.0		4.2 / 16.8	
Total suspended solids (ppm)	8		10	
Hardness ($CaCO_3$) (ppm)	20		71	
Conductivity (mmhos/cm)	0.11		0.19	b
Biomass (µg/g dry basis)[b]	13.6 / 10.3	13.6 / 10.3	14.2 / 21.5	14.2 / 21.5

[a] USDA Classification system.
[b] Format A/B, where A and B are the values determined at the beginning and end of the test respectively.

RESULTS

Table 4 Test 1: Material balance expressed as percent of applied radioactivity of the notified chemical (%AR) in a sand water-sediment system

Days after treatment (DAT)	*Water phase*	*Sediment extract*	*Sediment: non-extractable residues*	*CO_2 Trap (NaOH)*	*Organic Volatiles Trap*	*Material balance*
0	97.6	Not tested	Not tested	Not tested	Not tested	97.6
	101.4	Not tested	Not tested	Not tested	Not tested	101.4
0.25	75.0	13.3	5.3	0.1	4.1	97.8
	73.9	10.8	4.7	0.1	2.5	92.0
1	56.8	16.4	14.1	0.0	8.2	95.5
	55.8	19.0	11.9	0.1	4.6	91.4
3	34.8	25.8	22.7	0.1	14.7	98.1
	40.8	25.6	20.9	0.1	11.7	99.1
7	33.4	23.3	32.2	0.2	9.2	98.3
	31.3	25.5	31.3	0.1	10.1	98.3
10	27.2	25.1	33.4	0.1	11.5	97.3
	27.1	25.1	32.5	0.1	9.7	94.5
15	32.4	23.8	31.4	0.2	11.2	99.0
	29.0	26.1	33.2	0.4	9.6	98.3
21	31.5	25.4	33.4	0.4	8.9	99.6
	29.2	24.3	31.2	0.4	6.2	91.3
30	27.2	25.2	37.6	0.6	7.0	97.6
	25.9	24.5	36.1	0.5	6.5	93.5

Table 5 Test 1: Biotransformation of the notified chemical, expressed as percent of applied radioactivity (%AR), in a sand water-sediment system under aerobic conditions

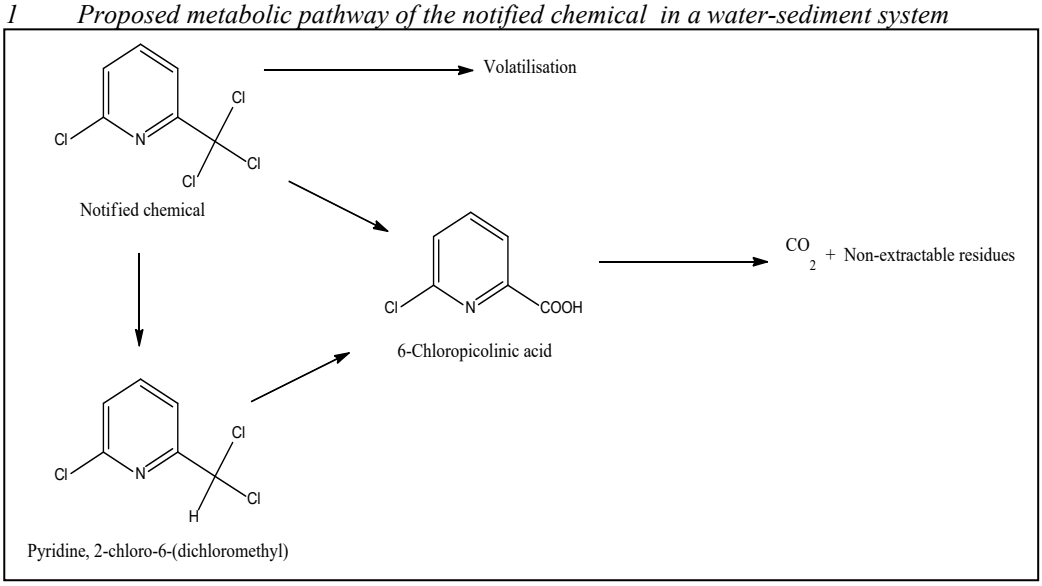
Days after treatment (DAT)	Aqueous Phase (%AR)				Sediment Extract (%AR)				Total System (%AR)			
	6-CPA	Unknown metabolite	DCMPyr	Notified chemical	6-CPA	Unknown metabolite	DCMPyr	Notified chemical	6-CPA	Unknown metabolite	DCMPyr	Notified chemical
0	1.8	0.0	0.0	95.1					1.8	0.0	0.0	95.1
	3.8	0.0	0.0	96.9					3.8	0.0	0.0	96.9
0.25	5.5	0.0	3.2	65.9	3.7	0.2	2.3	5.0	9.2	0.2	5.5	70.9
	3.2	0.0	1.8	68.7	3.1	0.4	2.1	3.9	6.3	0.4	3.9	72.6
1	7.2	0.9	5.7	42.1	8.4	0.9	2.3	3.1	15.6	1.8	8.0	45.2
	9.5	0.0	4.4	40.1	7.5	1.9	3.1	4.3	17.0	1.9	7.5	44.4
3	14.2	1.4	7.8	9.9	14.4	1.9	2.2	1.5	28.6	3.3	10.0	11.4
	16.6	1.2	7.6	14.5	13.7	1.9	3.6	2.5	30.3	3.1	11.2	17.0
7	26.9	2.4	1.6	0.0	14.9	2.2	1.3	0.0	41.8	4.6	2.9	0.0
	21.7	2.5	3.3	0.5	15.2	2.3	0.6	0.0	36.9	4.8	3.9	0.5
10	22.0	0.9	0.9	0.0	16.5	3.2	0.6	0.0	38.5	4.1	1.5	0.0
	20.4	2.4	1.0	0.0	15.3	3.1	0.4	0.0	35.7	5.5	1.4	0.0
15	25.8	2.6	0.0	0.0	15.6	3.5	0.0	0.0	41.4	6.1	0.0	0.0
	25.5	1.2	0.4	0.4	16.4	2.7	0.7	0.4	41.9	3.9	1.1	0.8
21	28.9	1.2	0.0	0.0	15.7	2.1	0.2	0.3	44.6	3.3	0.2	0.3
	26.2	1.4	0.0	0.0	14.3	2.1	0.2	0.0	40.5	3.5	0.2	0.0
30	23.9	1.9	0.0	0.2	15.6	2.3	0.2	0.3	39.5	4.2	0.2	0.5
	22.2	1.9	0.0	0.0	16.1	2.2	0.0	0.2	38.3	4.1	0.0	0.2
Maximum:	28.9	2.6	7.8	96.9	16.5	3.5	3.6	5	44.6	6.1	11.2	96.9

6-CPA = 6-CPA; DCMPyr = 2-chloro-6(dichloromethyl)pyridine.

Table 6 Test 1: Characterisation of non-extractable residues in notified chemical treated samples 15 days after treatment

Non-Extractable Residues (%AR)	% in Fraction			%AR in Fraction		
	Fluvic acid	Humic acid	Humin	Fluvic acid	Humic acid	Humin
33.4	34.5	23.2	42.3	11.5	7.7	14.2
32.5	33.3	21.7	45.0	10.8	7.1	14.6

Figure 1 Proposed metabolic pathway of the notified chemical in a water-sediment system



Remarks - Results

Test 1: Notified chemical

Notified chemical overall material balance (Table 4) ranged from 91.3% to 101.4% (96.7% ± 3.0%) of the applied radioactivity (%AR) throughout the test. Residues in the aqueous phase decreased to approximately 23%AR at study termination. Extractable ^{14}C-residues in sediment reached 25%AR at 3 days after treatment (DAT) and remained at this level for the remainder of the study. Non-extractable ^{14}C-residues in sediment reached a maximum of 37%AR at study termination. From 7 DAT until study termination at 30 DAT, the majority of the applied radioactivity was associated with the sediment compartment (ranging 55.2-62.8%AR). At the end of the study, less than 1%AR was present in the CO_2 trap, indicating limited mineralisation of the notified chemical. An average of 8.5%AR (ranging 2.5% to 14.7%AR) was recovered in the foam trap throughout the study. The organic volatile was identified as the notified chemical.

The notified chemical decreased from 97%AR to less than 1%AR in the aqueous phase and total system by 7 DAT (Table 5). The major transformation product in water was 6-CPA which reached a maximum concentration of 28.9%AR at 21 DAT. This was also the major transformation product in sediment with a maximum concentration of 16.5%AR at 10 DAT. The minor transformation product found in both water and sediment extract was 2-chloro-6-(dichloromethyl)pyridine, formed at a maximum of 7.8%AR (at 3 DAT) and 3.6%AR at (3 DAT), respectively. The maximum total system concentrations for the major and minor metabolites were 44.6% (at 21 DAT) and 11.2%AR (at 3DAT, respectively. Unidentified ^{14}C ranged from from 1% to 11%AR and was composed of multiple low level degradates that did not individually comprise of more than 5%AR.

The notified chemical dissipates from the system by volatilisation to atmosphere, hydrolysing to 6-CPA or degrading to 2-chloro-6-(dichloromethyl)pyridine. 2-Chloro-6-(dichloromethyl)pyridine degrades to 6-CPA. This further degrades to CO_2 and non-extractable residues. The proposed metabolic pathway is shown in Figure 1 above.

Table 7 Test 2: Material balance expressed as percent of applied radioactivity (%AR) of 6-CPA in a sandy loam water-sediment system

Days after treatment (DAT)	*Water phase*	*Sediment extract*	*Sediment: non-extractable residues*	*CO_2 Trap (NaOH)*	*Material balance*
0	94.4	3.3	0.1	Not tested	97.8
	95.8	1.7	0.0	Not tested	97.5
1	73.2	24.7	1.4	0.0	99.3
	70.5	25.1	1.6	0.0	97.2
4	49.6	44.1	4.3	0.1	98.1
	52.0	43.2	4.5	0.1	99.8
7	45.7	46.8	4.8	0.3	97.6
	43.9	48.1	5.4	0.2	97.6
14	35.7	55.4	6.9	0.5	98.5
	33.5	57.2	7.0	0.9	98.6
21	32.2	56.7	9.1	0.9	98.9
	35.8	53.8	7.9	1.3	98.8
32	39.1	50.8	7.3	1.8	99.0
	35.4	53.6	7.2	1.8	98.0

Table 8 Test 2: Biotransformation of 6-CPA expressed as percent of applied radioactivity (%AR) in a sandy loam water-sediment system under aerobic conditions

Days after treatment	Aqueous Phase (%AR)		Sediment Extract (%AR)		Total System (%AR)	
(DAT)	6-CPA	Other	6-CPA	Other	6-CPA	Other
0	94.4	0.0	3.2	0.2	97.6	0.2
	95.8	0.0	1.5	0.2	97.3	0.2
1	73.2	0.0	24.7	0.0	97.9	0.0
	70.5	0.0	24.9	0.2	95.4	0.2
4	49.6	0.0	43.7	0.4	93.3	0.4
	52.0	0.0	43.0	0.1	95.0	0.1
7	45.7	0.0	46.1	0.6	91.8	0.6
	43.9	0.0	48.1	0.0	92.0	0.0
14	35.7	0.0	55.0	0.3	90.7	0.3
	33.5	0.0	56.9	0.3	90.4	0.3
21	32.2	0.1	56.3	0.4	88.5	0.5
	35.8	0.0	53.6	0.3	89.4	0.3
32	39.0	0.1	50.6	0.2	89.6	0.3
	35.4	0.0	53.4	0.2	88.8	0.2

Remarks - Results

Test 2: 6-CPA

6-CPA overall material balance (Table 7) ranged from 97.2% to 99.8% (98.3% ± 0.8%) of the applied radioactivity (%AR) throughout the test. Residues in the aqueous phase decreased to approximately 37%AR at study termination. Extractable ^{14}C-residues in sediment reached 56%AR at 14 DAT and declined to approximately 52%AR at study termination. Non-extractable ^{14}C-residues in sediment accounted for less than 10%AR for the study duration. Less than 2%AR was recovered from the caustic traps at experimental termination.

6-CPA concentrations in the total water-sediment system (Table 8) declined by less than 10%AR through the study duration. 6-CPA accounted for the majority of applied radiation in both the water column and sediment with no metabolites observed in either phase.

Dissipation rates of the notified chemical and 6-CPA

The notified chemical dissipation rates were only calculated using a single compartment model for total system, water column and sediment phase. Time 0 was either 0 DAT or, where the concentration of the analyte initially increases in the compartment, the DAT with the maximum concentration of the analyte. The dissipation rates for the notified chemical and the major and minor metabolite are reported in Table 9 below. The half-life (DT50) of the notified chemical in the water column and total system by the best fit model were 0.7 and 0.8 days, respectively. However, the SFO half-life of 0.9 and 0.8 days for whole systems and the water column, respectively are used for environmental fate modelling. The notified chemical reached a maximum of 5%AR in the sediment at 0.25 DAT and fell to below 1%AR by 7 DAT. The notified chemical sediment dissipation rate could not be determined due to the low concentrations (less than 5%).

The dissipation half-life (Table 9) for 6-CPA was 6 days for the water column and greater than 1000 days for total system. A t-test was performed which demonstrated that the slope of the 95% confidence interval was non-zero, indicating that degradation occurred. However, the rate of degradation was much slower than the duration of the test. As the DT50 for the total system significantly exceeds the test duration, the results should be treated with caution.

Table 9 Dissipation rates of the notified chemical and its metabolites

Compartment / *Chemical*	*Best fit model dissipation rates*				*Modelling endpoint dissipation rates*			
	Model Type	*DT50 (days)*	*DT90 (days)*	R^2	*Model Type*	*DT50 (days)*	*DT90 (days)*	R^2
Test 1: notified chemical treated system								
Water								
n.c.	DFOP	0.7	3	0.9978	SFO	0.8	3	0.9769
DCMPyr	Top-down SFO	2	8	0.9634	Top-down SFO	2	8	0.9634
6-CPA	NA	NA	NA	NA	NA	NA	NA	NA
Sediment								
n.c.	NA	NA	NA	NA	NA	NA	NA	NA
DCMPyr	NA	NA	NA	NA	NA	NA	NA	NA
6-CPA	NA	NA	NA	NA	NA	NA	NA	NA
Entire system								
n.c.	DFOP	0.8	4	0.9976	SFO	0.9	3	0.9839
DCMPyr	SFO	3	9	0.9464	SFO	3	9	0.9464
6-CPA	SFO	173	575	0.9841	SFO	173	575	0.9841
Test 2: 6-CPA treated system								
Water	FMOC	6	>1000	0.9779	DFOP	5	520	0.9814
Sediment	NA	NA	NA	NA	NA	NA	NA	NA
Entire system	FMOC	>1000	>1000	0.9378	SFO	243	807	0.7759

n.c. = notified chemical; DCMPyr = 2-chloro-6(dichloromethyl)pyridine; 6-CPA = 6-chloropicolinic acid; NA = Not applicable. A dissipation rate could not be calculated because the test substance or metabolite concentration had not appreciably declined by study termination; SFO = Simple first-order; FMOC = First-Order Multi-Compartment; DFOP = Double First-Order in Parallel.

CONCLUSION

The half-life of the notified chemical in a water-sediment system was 0.9 days using simple first order modelling. The half-life of the notified chemical in the water column was 0.8 days. The notified chemical is readily degradable in water-sediment systems. The major metabolite is 6-CPA.

The half-life of the major metabolite, 6-CPA, in a water-sediment system could not be reliably determined within the duration of the study.

TEST FACILITY

Dow (2009)

C.1.3. Aerobic degradation in soil of a metabolite

TEST SUBSTANCE

6-Chloropicolinic acid (6-CPA)

METHOD

US EPA Pesticides Assessment Guidelines, Subdivision N, Section 162-1 (now OPPTS 835.4300)
Conducted in accordance with the principles of good laboratory practice

Duration and sampling interval

180 days; samples analysed at 0, 3, 7, 15, 31, 60, 90, 137 and 180 days

Test substance application

Direct application using a positive displacement pipette (610 µg/L, containing 23.3 µg of test substance dissolved in water : acetonitrile at 9:1 v/v) to the soil surface with a measured treatment rate of 0.35 kg/ha (91.7% of the nominal treatment rate of 0.38 kg/ha; the nominal rate is equivalent to soil concentration of 0.253 mg/kg soil (dry weight) assuming equal distribution in the upper 10 cm soil layer and a soil bulk density of 1.5 g/cm^3.

Analytical monitoring

Non-extractable soil residues – The radioactive content of the test substance in dried extracted soil residues were quantified by combustion of soil aliquots followed by liquid scintillation counting (LSC).
Extractable soil residues – Soils were extracted with acetonitrile : water (4:1 v/v) followed by acetonitrile : water : formic acid (8:2:0.5 v/v/v).

Extraction efficiency ranged from 86 to 96%AR as determined on Day 0 samples. Radioactivity in extracts was determined by LSC. Analysis for quantification and characterisation of extracts was by HPLC. Selected extracts were further analysed by LC/MS/MS to confirm structural identity. Further extraction using an alkaline solution (0.1M sodium hydroxide) was conducted on days 0 and 180 to provide information on the bound residue, but was not used to calculate total extractable residue.
Traps – Radioactivity was determined by LSC. The composition of the volatile radioactivity in the CO_2 trap was confirmed to be >99.8% CO_2 (as determined in barium carbonate precipitation test on day 180).

Remarks – Method

The rate of degradation of ^{14}C-6-CPA was investigated in three soils as detailed in Table 10. The soils were incubated under aerobic conditions at 25 ºC ± 1 ºC in the dark at a moisture content equivalent to 75% of $^1/_3$ bar. The test substance was applied to the soil surface at a nominal application rate of 0.38 kg/ha and the soil samples were incubated for up to 180 days. The test system consisted of straight-sided, conical, soil flasks connected to a series of trap vessels (ethylene glycol trap for organic volatiles followed by two containing 2 M KOH for the collection of CO_2). Duplicate samples were tested at nine time-points. Moisture content was maintained by the addition of deionised water as required. Biomass was determined on four untreated flasks at the beginning and end of the test.

The DT50 and DT90 values for 6-CPA were determined using a SFO model. The kinetic evaluations and statistical calculations for the quality checks were implemented by a numerical software package (Matlab, 2005).

Table 10 Properties of study soils

Soil identity and name	*07/041 Georgia*	*07/042 Michigan*	*07/043 Nebraska*
Textural class	Sandy loam	Sandy clay loam	Silt loam
% Sand/silt/clay	79/8/13	51/23/26	15/58/27
Moisture content (% w/w)	6.93	14.01	11.050
Moisture content at 1/3 Bar (% w/w)	7.2	17.1	29.0
Moisture content at 75% of 1/3 Bar (% w/w)	5.4	12.8	21.8
Organic matter (%)	1.1	2.7	3.0
Cation exchange capacity (meq/100 g)	5.1	12.4	15.2
pH (1:1 soil to water ratio)	6.1	7.9	7.4
Base saturation data			
Cation ppm			
Calcium	374 (36.8%)	1980 (79.7%)	1760 (57.7%)
Magnesium	64 (10.5%)	136 (9.1%)	327 (17.9)
Sodium	8 (0.7%)	8 (0.3%)	35 (1.0%)
Potassium	64 (3.2%)	89 (1.8%)	485 (8.2%)
Hydrogen	25 (48.8%)	11 (9.0%)	23 (15.2%)
Bulk density (disturbed; g/cm^3)	1.36	1.11	1.12
Microbial biomass (mg C/ 100 g soil)			
initial	5.57	23.26	29.67
final	8.61	15.90	23.26

RESULTS

Table 11 Distribution and material balance of radioactivity in soil 07/0471 Georgia sandy loam incubated at 25 ºC expressed as percent of applied radioactivity (%AR)

Incubation time (days)	*Extract 1[a]*	*Extract 2[b]*	*Total extracted*	*Carbon Dioxide[c]*	*Non-extractable residues*	*Material balance*
0	83.38	12.25	95.62	0.00	10.56	106.65[d]
	83.69	11.88	95.57	0.00	9.92	106.01[d]
3	72.83	15.13	87.96	2.32	13.08	103.35
	73.14	15.04	88.18	1.31	12.27	101.76
7	65.55	12.17	77.72	5.65	11.87	95.24
	65.72	11.92	77.64	5.07	12.57	95.28
15	62.70	12.22	74.92	12.04	10.49	97.46
	63.58	11.46	75.04	9.49	10.67	95.20
31	21.59	7.66	29.25	18.45	47.02	94.71
	22.17	8.06	30.23	19.39	48.75	98.38
60	15.67	6.97	22.64	31.34	43.79	97.77
	15.73	7.46	23.19	28.24	44.18	95.62
90	5.42	3.65	9.07	48.24	31.29	88.60
	12.22	7.50	19.72	49.03	36.64	105.38
137	10.64	6.47	17.11	42.49	36.21	95.82
	11.12	6.65	17.77	47.02	36.30	101.09
180	7.31	6.11	15.31[d]	44.47	31.66	91.45
	11.34	8.72	22.43[d]	39.78	35.54	97.75

[a] Extract 1 was acetonitrile/water (4:1, v/v)
[b] Extract 2 was acetonitrile/water/formic acid (8:2:0.5, v/v/v)
[c] Radioactivity detected in the ethylene glycol traps was ≤0.01%AR
[d] Does not equal the sum of its components (unclear whether these results include %AR in alkaline extract)

Table 12 Distribution and material balance of radioactivity in soil 07/0472 Michigan sandy clay loam incubated at 25 ºC expressed as percent of applied radioactivity (%AR)

Incubation time (days)	*Extract 1[a]*	*Extract 2[b]*	*Total extracted*	*Carbon Dioxide[c]*	*Non-extractable residues*	*Material balance*
0	85.90	9.97	95.87	0.00	8.91	105.53[d]
	86.40	9.77	96.17	0.00	8.64	105.46[d]
3	79.34	12.04	91.38	1.13	10.22	102.72
	81.18	10.28	91.47	1.15	9.85	102.46
7	80.56	7.09	87.65	2.08	9.88	99.61
	81.30	9.29	90.59	1.05	7.27	98.91
15	77.27	8.44	85.71	1.67	9.94	97.32
	77.24	9.20	86.44	4.00	8.94	99.38
31	35.48	9.07	44.55	10.15	41.34	96.04
	36.59	9.11	45.71	10.20	42.18	98.09
60	30.39	7.97	38.35	17.88	39.98	96.22
	30.72	7.99	38.72	18.28	40.12	97.12
90	22.45	11.88	34.33	30.86	31.73	96.92
	28.12	14.99	43.11	30.40	32.48	106.00
137	10.02	6.87	16.89	48.87	25.52	91.28
	9.85	6.83	16.68	50.12	26.92	93.72
180	11.07	7.53	18.60	44.16	27.58	89.81[d]
	8.89	5.91	14.80	49.57	24.04	91.28[d]

[a] Extract 1 was acetonitrile/water (4:1, v/v)
[b] Extract 2 was acetonitrile/water/formic acid (8:2:0.5, v/v/v)
[c] Radioactivity detected in the ethylene glycol traps was ≤ 0.01%AR
[d] Does not equal the sum of its components (unclear whether these results include %AR in alkaline extract)

Table 13 Distribution and material balance of radioactivity in soil 07/0473 Nebraska silt loam incubated at 25 ºC expressed as percent of applied radioactivity (%AR)

Incubation time (days)	Extract 1[a]	Extract 2[b]	Total extracted	Carbon Dioxide[c]	Non-extractable residues	Material balance
0	75.19	12.75	87.93	0.00	7.28	95.48[d]
	72.28	12.43	84.71	0.00	7.11	92.08[d]
3	69.62	12.39	82.02	0.30	12.25	94.57
	70.33	12.46	82.79	0.31	11.36	94.46
7	80.87	10.70	91.57	0.85	7.88	100.30
	82.06	11.63	93.70	0.46	6.63	100.79
15	65.71	12.01	77.72	0.63	11.33	89.68
	69.82	14.73	84.55	2.29	11.53	98.38
31	15.49	8.07	23.56	33.90	40.10	97.55
	14.50	7.51	22.01	34.23	37.99	94.23
60	10.35	6.04	16.39	45.85	32.64	94.88
	10.69	6.26	16.95	41.32	31.84	90.09
90	3.88	3.13	7.00	56.69	26.28	89.97
	4.87	4.39	9.26	55.29	27.51	92.06
137	1.05	0.62	1.68	61.24	31.37	94.29
	6.66	8.28	14.95	47.62	30.89	93.45
180	3.04	2.10	5.13	74.86	21.49	102.23[d]
	1.78	0.6	2.38	64.02	19.90	86.63[d]

[a] Extract 1 was acetonitrile/water (4:1, v/v)
[b] Extract 2 was acetonitrile/water/formic acid (8:2:0.5, v/v/v)
[c] Radioactivity detected in teh ethylene glycol traps was ≤ 0.01%AR
[d] Does not equal the sum of its components (unclear whether these results include %AR in alkaline extract)

Figure 2 Proposed degradation pathway of 6-CPA in aerobic soils

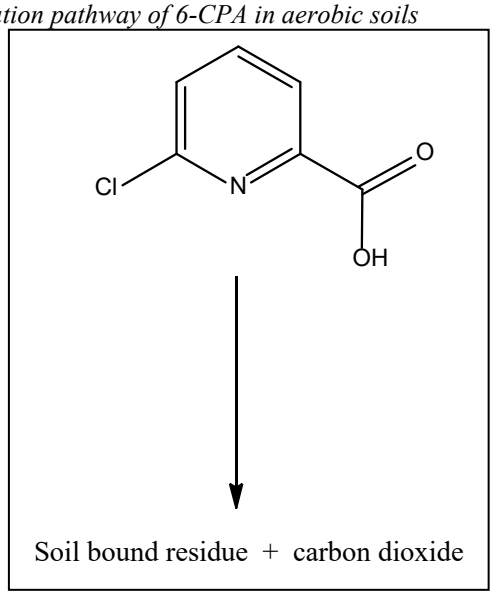


Table 14 Aerobic soil degradation rates of 6-CPA

Soil	DT50 (days)	DT90 (days)	Minimum chi^2 error (%)[a]	Significance of a single-sided t-test[b]
07/041 (Sandy Loam)	25.9	85.9	15.35	1.2×10^{-6}, > 99.9%
07/042 (Sandy Clay Loam)	47.6	158.0	11.55	4.4×10^{-8}, > 99.9%
07/043 (Silt Loam)	23.2	77.0	17.86	3.3×10^{-6}, > 99.9%
Average	31.9	107.0		

[a] A scaled error (chi^{2}Error%) of less than 15% is considered a good fit to the model
[b] A t-test error of <0.05 with >95% parameter significance is considered sufficiently small.

Remarks - Results The material balance for the replicate samples ranged from 86.6% to 106.7% of the %AR for the three soils (Table 11 to Table 13).

The major product formed in each soil was carbon dioxide, accounting for up to 70%AR. No major metabolites (>10%) were observed, while a number of minor metabolites were observed (all ≤2%) but did not warrant identification. Non-extractable residues accounted for up to 48%AR.

The distribution of radioactivity was broadly similar for all three soils. There was a steady increase in the carbon dioxide production in all three soils over 180 days, reaching a maximum of 49% in Soils 07/041 and 07/042, and 70% in Soil 07/043. Virtually no volatile organic products were detected throughout the study.

The half-life (DT50; Table 14) of 6-CPA in three soils in the aerobic soil study ranged from 23.2 to 47.6 days.

CONCLUSION

The notified chemical metabolite, 6-CPA, is fairly degradable in soils and is not expected to be persistent in the soil compartment.

TEST FACILITY

Batelle (2008)

C.1.4. Soil dissipation model

TEST SUBSTANCE

Notified chemical in a formulation
Microencapsulated notified chemical in a formulation

METHOD

Not a GLP study

Remarks – Method

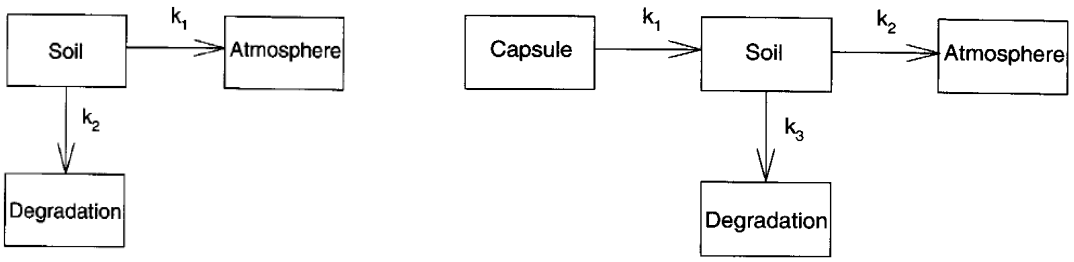
The study hypothesis is that net dissipation rate of microencapsulated notified chemical once applied to soils will be the sum of the vapour release rate from the capsules and its aerobic soil metabolism degradation rate (reported as $t_{½}$ = 11-18 days; test study not provided). As a result of the use of the microencapsulated form, it was anticipated that the notified chemical concentration peaks are likely to be attenuated in environmental compartments. Further, it was anticipated that residual notified chemical within capsules in soil residues will be less bioavailable.

The reported laboratory data (test studies not provided) for volatility loss rates from sand treated with formulations containing the notified chemical and the microencapsulated form of the notified chemical are substantially different: the reported times to achieve a 50% loss of the two substances were approximately 1 and 10 days, respectively.

Environmental fate modelling was conducted using simple box models incorporating first-order rate constants (Figure 3). The conceptual models used for the two test substances are shown in Figure 3. The laboratory volatility data indicating 1-day and 10-day half-lives for the notified chemical and its microencapsulated form, respectively, were used to approximate the volatility and capsule release constants of 0.693 per day and 0.0693 per day, respectively. The soil degradation rate of the notified chemical was calculated from a half-life of 25.1 days (the 90th upper percentile of the two half-lives reported and used in the US EPA fate modelling; US EPA, 2004a) to be 0.0276 per day.

The models were implemented in a spreadsheet with an input of unit mass at Time 0 and incorporation after 1 day for the notified chemical and after 10 days in the microencapsulated form. The simulation was run over a period of 60 days. It was assumed that: 1) incorporation was 100% efficient and that there were no further volatility loss following incorporation; 2) there was no direct ransfer from capsules to atmosphere; and, 3) constant temperature near laboratory conditions.

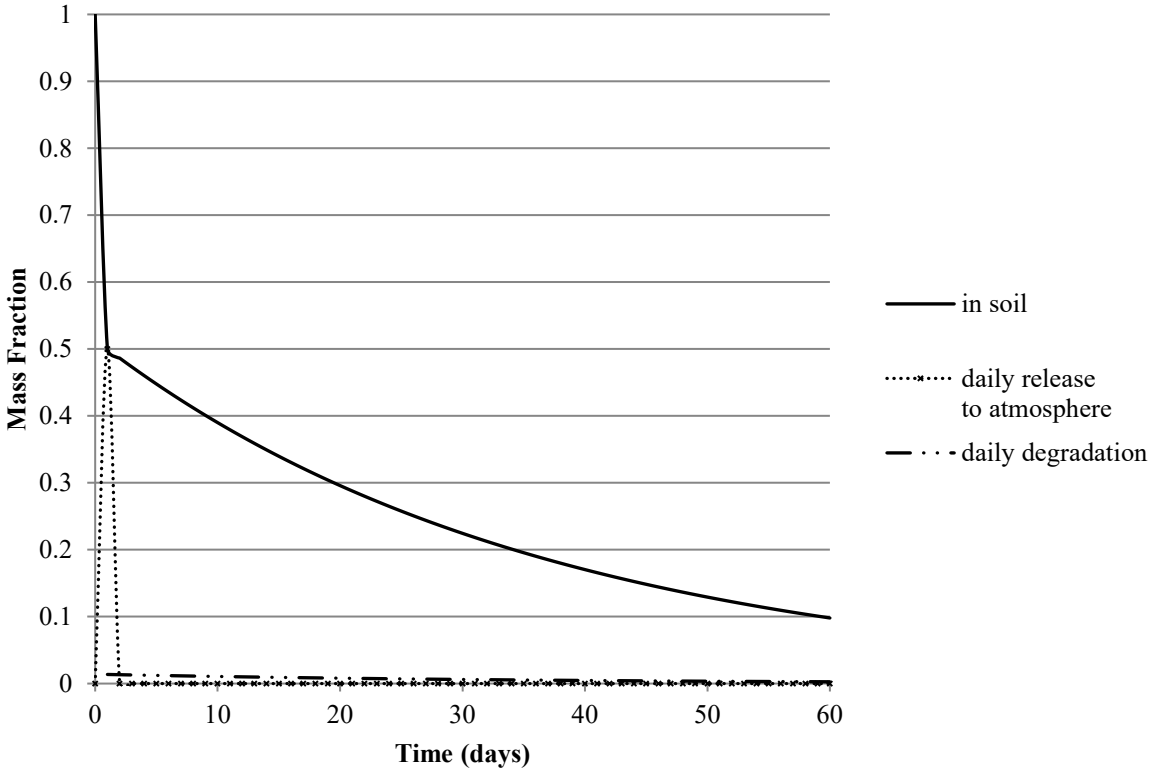
Figure 3 Box models of dissipation for a formulation containing
a) notified chemical; and, b) microencapsulated notified chemical



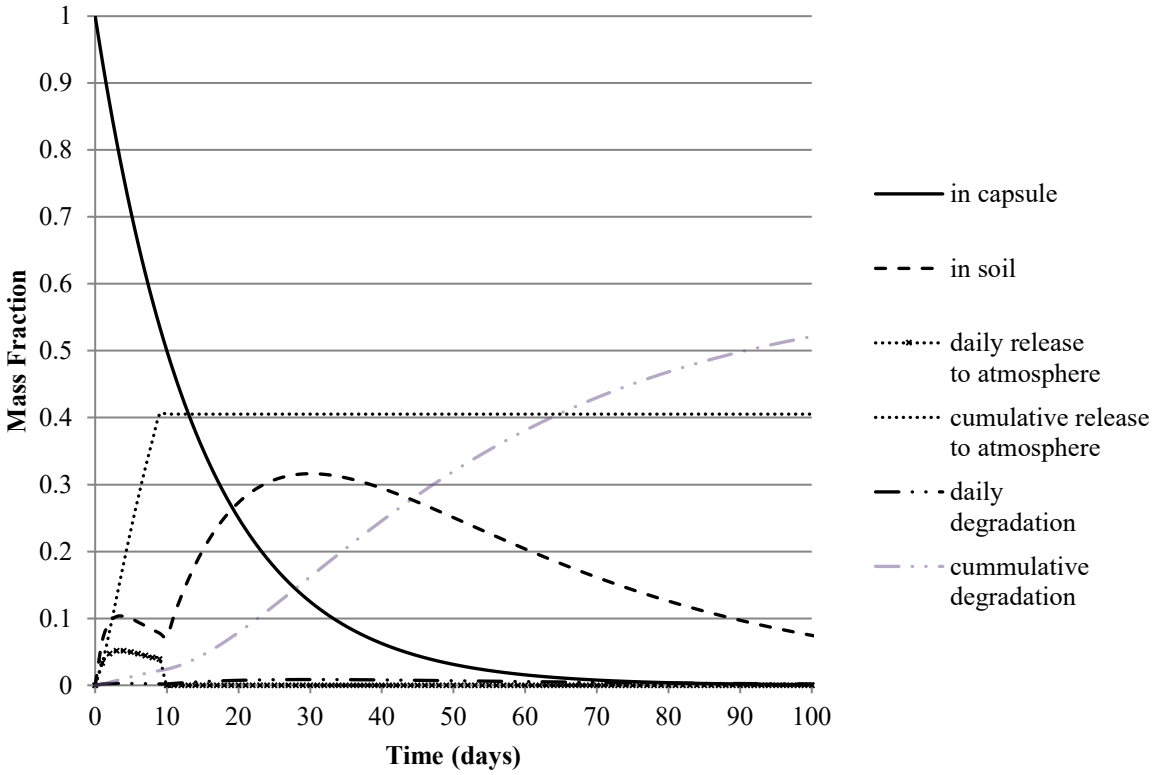
$k_1 = 0.693\ d^{-1}$, $k_2 = 0.0276\ d^{-1}$ $k_1 = 0.0693\ d^{-1}$, $k_2 = 0.693\ d^{-1}$, $k_3 = 0.0276\ d^{-1}$

RESULTS

Figure 4 – Modelled distribution of notified chemical mass following pre-emergence use of a formulation containing
a) free notified chemical with incorporation on Day 1; and,



b) microencapsulated notified chemical with incorporation at Day 10



c) microencapsulated notified chemical with immediate incorporation (Day 0)

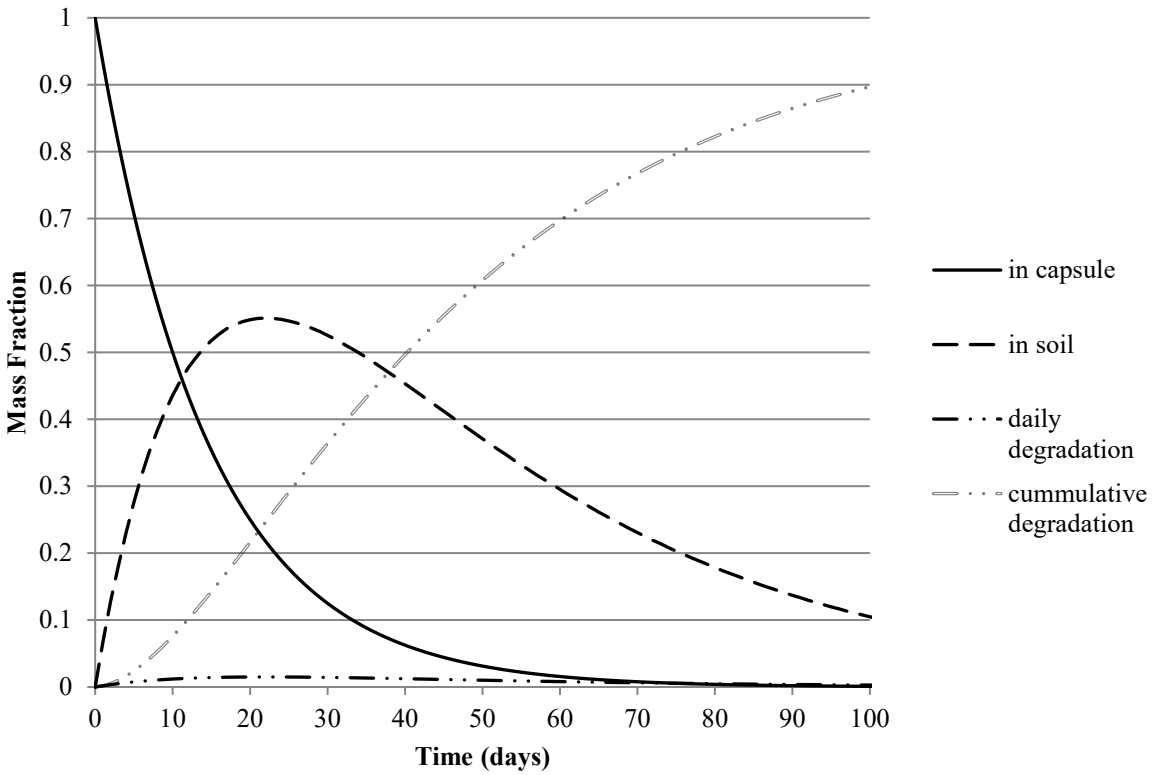


Table 15 - Comparison of peak mass fractions and soil dissipation

Test substance (day of incorporation)	*Volatility*[a]		*In soil*			*In capsule*	
	Peak daily mass fraction	*Day*	*Peak mass fraction*	*Day*	*DT50*[b]	*Peak mass fraction*	*Day*
Nitrapyrin (Day 1)	0.5	1	0.47	1	25	-	-
Microencapsulated nitrapyrin (Day 10)	0.05	4	0.29	31	41	0.93	1
Microencapsulated nitrapyrin (Day 0)	-	-	0.55	22	40	0.93	1

[a] daily release to atmosphere
[b] Where Time 0 = day of peak mass fraction

Remarks - Results

Incorporation of the microencapsulated form of the notified chemical 10 days after application is the worst-case scenario for release to the atmospheric compartment for the microencapsulated form of the notified chemical. Volatile peak daily mass fractions are lower for the microencapsulated form of the notified chemical with incorporation occurring 10 days after application, 0.05 [Table 15; Figure 4 (b)], when compared with the notified chemical with incorporation after 1 day, 0.5 [Table 15; Figure 4 (a)].

The effective half-life in soil of the microencapsulated form of the notified chemical was reported to be extended from 25 days to 41 days due to the contribution of mass released from the capsule over time [Table 15]. The peak mass fraction in soil of the microencapsulated form of the notified chemical of 0.29 was less than that calculated for the free notified chemical of 0.47.

In addition to the above results reported in the study, cumulative release to atmosphere was calculated [shown in Figure 4 (b)]. For the microencapsulated form of the notified chemical with incorporation after 10 days, the cumulative mass fraction released to the atmospheric compartment is 0.41.

Additionally, the model was used to calculate a worst case soil concentration for the microencapsulated form of the notified chemical, assuming that incorporation occurred immediately after application, with no loss due to volatilisation. The results are shown in Table 15 and Figure 4 (c). The worst case peak mass fraction in soil for the microencapsulated form of the notified chemical with immediate incorporation was calculated to be 0.55 on Day 22, with an effective half-life in soil of 40 days.

The models were also extended to 110 days to show when notified chemical residues in soil are expected to fall below 90%.

CONCLUSION

When the notified chemical is applied to agricultural soils in the microencapsulated form the expected worst case:
1) peak daily mass fraction of the notified chemical in the atmospheric compartment is 0.05;
2) peak mass fraction of the notified chemical in the terrestrial compartment is 0.55; and,
3) peak mass fraction of the free notified chemical on the soil surface is 0.104 following release from capsules;
4) the residual mass fraction of notified chemical in soils assuming immediate incorporation is 0.139 after 90 days; and,
4) the effective half-life of the notified chemical in soil is 41 days.

TEST FACILITY

Dow (2007a)

C.1.5. Run-off (model)

TEST SUBSTANCE: Notified chemical
Microencapsulated notified chemical

METHOD: DSEWPaC Run-off model

Remarks – Method: Run-off is highly dependent on several factors, some of which are location specific and others event specific. The most important are rainfall and its intensity, infiltration of soil (in turn related to moisture content of soil), the slope, type of soil, type of drainage, crop type, amount of trash on soil and cultivation (Mensink *et al.*, 1996). Other influences include mobility and persistence of the chemical, formulation type and formulation placement (Grover, 1989).

Although there are inherent complexities in modelling run-off as an environmental transport process for agricultural chemicals, a reasonable estimation of the amount of chemical in run-off water may be made using a modified sub-model of the REXTOX model proposed by the OECD (Probst *et al.*, 2005). The model considers rainfall and run-off water, topography of the land (slope), degradation of the chemical, mobility of the chemical and buffer zones. In addition to the REXTOX sub-model, heterogeneity of fields, interception and retention of the chemical by crops for foliar applications, and in certain cases, transport of the chemical attached to eroded sediment are considered (MORAG, 2010).

The following equation is used to calculate the percentage of the application dose of a chemical available as dissolved substance in run-off water ($L\%_{run\text{-}off}$):

Equation 1: $L\%_{run\text{-}off} = (R/P) \times Crsoil\_surface \times f1slope \times f2bufferzone \times f3foliar\_application \times heterogeneity\_factor \times 100$

Table 16 – Explanation of terms in Equation 1

Parameter	*Definition*	*Input values*
$L\%_{run\text{-}off}$	The percentage of the application dose of a chemical available as dissolved substance in run-off water	Determined by calculation
R	The daily quantity of run-off water in millimetres (mm/day)	20 mm/day (fixed)
P	The daily precipitation in millimetres (mm/day)	100 mm/day (fixed)
Crsoil_surface	Concentration reduction factor based on the environmental fate of the chemical in soil	1 (at Tier 1 level). Chemical specific values calculated in higher tier assessments with a separate equation.
f1slope	Proportion of chemical in run-off water dependent on the slope of field to which the chemical is applied	0.5 (default, can be application specific, 1 for very steep slopes, 0.1 for flat scenario)
f2bufferzone	Parameter representing reduction of chemical in run-off water due to buffer zones measured in metres (m)	1 (default – no effect)
f3foliar_application	The amount of foliar applied chemical available for run-off	Application specific value calculated at Tier I level and above based on standard values for interception by various crops
heterogeneity_factor	Factor based on effective portion of field contributing to run-off	0.5 (fixed)

A tiered approach is used for calculating the predicted environmental concentration (PEC) of chemicals in run-off water from agricultural fields. Initially, the concentration of the chemical in run-off water at the

edge of the agricultural field is calculated considering application specific factors (Tier 1). If an unacceptable risk is shown during the risk characterisation, chemical specific factors are taken into account (Tier 2). The PEC may be further refined by consideration of dilution of the "edge-of-field" run-off water in an environmental water body (Tier 3) (MORAG, 2010).

Based on Australian climate and catchment data, the model gives consideration to a rainfall event of 100 mm/day (P) resulting in a maximum of 20 mm/day of run-off water (R) in its worst-case scenario (Tier 1). The potential mitigating effects on the concentrations of a chemical in run-off resulting from the partitioning and transformation of the chemical in soil are not considered at Tier 1 and hence the parameter, Crsoil_surface, is set equal to unity for calculations at this level.

The PEC of a chemical in run-off water at the edge-of-field is the parameter used to characterise the risks from off-field transport of chemicals by run-off at Tier 1 and 2 levels. This parameter is the quotient of the mass of chemical present in run-off water from an agricultural field (area measured in hectares) and the volume of run-off water (in litres) from the same field occurring in a single run-off event as shown below.

$PEC_{run\text{-}off}$ = Mass of chemical in run-off water/ha ÷ Volume of run-off water/ha

The volume of run-off water in the model is calculated based on the default rainfall event which gives rise to a maximum of 20 mm/day of run-off water. This run-off rate would give rise to up to 200 m^3/ha of run-off water per day (= 0.02 m × 10^4 m^2/ha), which is equivalent to 200,000 L/ha (= 200 m^3/ha × 10^3 L/m^3).

The mass of chemical available for run-off per hectare is the product of the fraction of the application dose of a chemical available as dissolved substance in run-off water (= $L\%_{run\text{-}off}$ ÷ 100) and the mass application rate of the notified chemical per hectare (mg notified chemical/ha), as shown below:

Mass of chemical in run-off water/ha = ($L\%_{run\text{-}off}$ ÷ 100) × Mass of chemical applied per hectare

Based on the relationships defined above, the equation for calculating $PEC_{run\text{-}off}$ can be rewritten as follows:

Equation 2: $PEC_{run\text{-}off}$ = (($L\%_{run\text{-}off}$ ÷ 100) × Mass of chemical applied per hectare (mg/ha)) ÷ 200,000 L/ha

The PEC of a chemical in run-off water after entering an existing environmental water body is the parameter used to characterise the risks from off-field transport of chemicals by run-off at Tier 3 levels. Consideration is given to a 1500 m^3 water body of environmental significance. This represents a 1 ha (15 cm deep) pond or a low flow (~ 0.03 – 0.06 m/sec; ~ 0.1-0.2 km/hr) primary stream with 1500 m^3 per day flow having approximate dimensions of ~ 2 m wide and ~ 25 cm deep (based on SPDEFTP, 2003).

In a worst-case scenario this water body is considered to be fed entirely by the largest likely field to be 100% treated at the maximum rate with the chemical of interest. The considered field size is 10 ha (US EPA, 2004c). The concentration in the water body may be calculated assuming that 200 m^3 of water contaminated with chemical from each hectare, for a total of 10 ha flows into the 1500 m^3 water body, resulting in a total water body of 3500 m^3.

Remarks - Results

Chemicals in solution is the major form of transportation during run-off,

with only chemicals with a water solubility of < 1 mg/L being transported primarily by sediment (Grover, 1989). This is due to the volume of run-off water greatly outweighing the mass of sediment transported in a run-off event (ibid.; Afyuni *et al.,* 1997).

The notified chemical is moderately water soluble with medium mobility in soil and has the potential for transport dissolved in water during a run-off event. However, due to the microencapsulated form of the notified chemical, a limited proportion of the notified chemical is freely available in soils.

The notified chemical in soils in microencapsulated form is considered more likely to behave as an undissolved particulate and have the potential for sediment transport during a run-off event.

The formulation placement, such that the notified chemical is expected to be incorporated into soils within 10 days of application, further reduces the amount of applied notified chemical (both freely available in soils or in the microencapsulated form) that is likely to be mobilised during a run-off event.

Sediment phase
A calculation of $PEC_{run-off}$ in the water phase is not the primary exposure route for microencapsulated notified chemical which is expected to have limited solubility. Therefore, the PEC is calculated for the notified chemical transported in the sediment phase and diluted in an environmental water body (assume 1500 m^3 existing volume and 2000 m^3 of run-off water) with a volume of 3 500 000 L).

For sediment transport, it is assumed that 400 kg/ha or 0.3% of top soil is eroded in a worst-case scenario (based on Afyuni *et al.*, 1997; Mensink *et al.*, 1996). For a worst case run-off event from 10 ha treated land the total amount of the microencapsulated form of the notified chemical transported by sediment would be 15 g (500 g/ha x 0.3% x 10 ha). Therefore, the calculated $PEC_{run-off}$ (15 g ÷ 3 500 000 L) would be 4.3 µg/L

This is a worst case calculation in the event of a run-off event immediately following application as it assumes no loss of the notified chemical due to diffusion from capsules into soils and its subsequent volatilisation from the soil surface. Further, it assumes that the entire amount of applied microencapsulated notified chemical is present in the top soil. However, it is expected that incorporation into soils will significantly reduce its potential for run-off.

Water phase
The $PEC_{run-off}$ is now calculated for the free notified chemical in soils using the model described above. The worst-case scenario is to assume that the entire amount of applied notified chemical is freely available on the soil surface. However, this is an unrealistic scenario that does not accurately account for the modified-release dose-form of the notified chemical, its high volatility from the soil surface in its free form and reduced potential for run-off following its incorporation into soils.

The peak mass fraction in soils is highest following incorporation [refer to C.1.4, Figure 4 (b), (c)]. However, it is assumed that the incorporation reduces the potential for runoff. Therefore, in the present situation, the Crsoil_surface parameter is modified by a factor of 0.104, which is the peak mass fraction of the applied notified chemical that is expected to be freely available on the soil surface before incorporation [refer to C.1.4,

Figure 4 (b)]. In other words, the worst case assumes that 10.4% of the applied amount of free notified chemical is available on the soil surface for run-off.

Tier 1
The notified chemical may be applied pre-emergent. For weeds and bare soil the amount of intercepted chemical retained by foliage (F_{ret}) is zero. Consequently, in the present situation f3foliar_application is assumed to equal to 1 (f3foliar_application = 1 - F_{ret}; where F_{ret} = F_{int} × 0.5; F_{int} = foliar interception, assumed to be zero for worst case pre-emergent application).

Substituting the above parameter values into Equation 1, together with the default input values listed in Table 16, results in $L\%_{run\text{-}off}$ of 0.52% [(20/100) × 0.104 × 0.5 × 1 × 1 × 0.5 × 100]. Substituting this value into Equation 2, with an application rate of 500 g/ha, results in a PEC of 0.013 mg/L at edge of field [(0.52% × 500 000 mg/ha) ÷ 200,000 L/ha].

Tier 2
The model is further refined by applying mitigating factors that consider the fate of the chemical and are incorporated within the Crsoil_surface parameter.

The model usually assumes that in a worst-case scenario, the runoff event occurs three days after the application of the pesticide. In the current situation, it is not appropriate to apply a factor for degradation on the soil surface over three days. This is because a factor of 0.104 was applied to this parameter in the Tier 1 model which accounts for release, degradation and environmental partitioning of the notified chemical.

However, the mobility of chemical in soils may also be taken into account. The fraction of the chemical available for run-off due to mobility is related by the following equation: Crsoil_surface = 1 ÷ (1 + Kd).

K_d values are generally only used in this equation if they are available for the specific soil(s) of interest. Otherwise an estimation is used from the formula K_d = Koc × % organic carbon ÷ 100. The formula assumes that the only mechanism for adsorption of the pesticide to soil is via the organic carbon content. In most cases this provides a reasonable estimate of Kd.

The adsorption Kd of the notified chemical in five US soils showed correlation with organic carbon content. Therefore to extrapolate these results to Australian soils, the Koc is used with an assumed organic carbon content of 1.0% (ANRA, 2001). For a worst-case scenario for run-off, the lowest experimental adsorption Koc value, 256 mL/g, is used.

Using the above information [1 ÷ (1 + 256 × 1%)] results in a Crsoil_surface value of 0.28. Applying a mitigating factor for mobility derived for Crsoil_surface (0.52% × 0.28) gives a revised $L\%_{run\text{-}off}$ of 0.14%. Substituting this value into Equation 2 [(0.14% × 500 000 mg/ha) ÷ 200,000 L/ha] results in a PEC of 3.64 µg/L.

Tier 3
When diluted in an environmental water body, assuming a treated area of 10 ha, 200 m^3 of water per hectare and a 1500 m^3 water body, the PEC is calculated [3.64 µg/L × (2000 ÷ 3500)] to be 2.08 µg/L.

CONCLUSION

The $PEC_{run\text{-}off\ (capsules)}$ of the microencapsulated form of the notified chemical on the soil surface that is transported in the sediment phase, when diluted in an environmental water body, is 4.3 µg/L.

The $PEC_{run\text{-}off\ (free)}$ of the free notified chemical on the soil surface that is transported in the water phase, when diluted in an environmental water body, is 2.08 µg/L.

C.2. Ecotoxicological Investigations

C.2.1. Toxicity to Avian Species

a. Dietary toxicity to bobwhite quail (a pilot study)

TEST SUBSTANCE	Notified chemical (93.6%) and 6-chloropicolinic acid (6-CPA) (99%)
METHOD	Procedure for Evaluation of Acute Toxicity to Fish and Wildlife, 14 December 1964
Species	Bobwhite quail (three weeks of age on experimental day zero; hatching eggs purchased from the Georgia Quail·Farm, Georgia)
Exposure Period	8 days (5 days exposure to treated dietary followed by 3 days maintained on toxicant-free diet)
Test levels – notified chemical	10, 100, 1000 ppm (nominal)
Test levels - metabolite	10, 100, 1000ppm (nominal)
Auxiliary Solvent	Not reported
Remarks – Method	One replicate of seven birds was established per treatment group.

The body weights were taken on experimental days 0, 5 and 8, the mortality was recorded daily, and the feed consumption was recorded for day 0-5 and 6-8 periods.

Observations for incidence of cataracts, tremors, etc., were made daily. Birds were placed directly on medicated diets on day zero continuing through to day 5 at which time all medicated diets were removed and replaced with non-medicated diets. Diets were prepared with Cohoon's Elevator Turkey Starter.

RESULTS

Test group (mg/kg diet)	*Mortality (%)*	*Mean feed consumption (g feed/bird)*		*Mean body weight (g/bird)*		*Effect on day 0-8 body weight gain compared to control (%)*
		during days 1 - 5	*during days 6 - 8*	*on day 0*	*on day 8*	
Control	14.3	235	154	30	49	N/A
Notified chemical						
10	0	271	196	31.9	44.7	-14.2
100	0	257	193	29.7	47.1	-2.9
1000	42.9	171	106	32.6	40.8	-23.4
Metabolite						
10	0	259	224	32.1	50.4	-3.9
100	14.3	295	246	31.3	52.7	3.1
1000	14.3	288	206	34.3	58.7	4.8

LC50	>1000 ppm (nominal) for both the notified chemical and the metabolite
NOEC	100 ppm (nominal) for the notified chemical and 1000 ppm for the metabolite.
Remarks – Results	Mortality of 14.3% (1 out of 7) was observed in the control.

For the notified chemical, birds fed at 10 and 100 ppm suffered no mortality but had 42.9% mortality at the 1,000 ppm level. Average body weight gains were severely retarded at the 1,000 ppm level. The LC50 was determined to be >1000 ppm, and the NOEC = 100 ppm.

With the metabolite of the notified chemical, no clinical effects were reported at all levels for the test. A mortality of 14.3% was observed in the

	treatment groups 100 and 1000 ppm. No biologically significant effects on body weight gain and feed consumption were observed at all the test levels with the metabolite. Therefore, the LC50 was determined to be >1000 ppm (nominal), and the NOEC was determined to be 1000 ppm (nominal). The notified chemical is considered to be slightly toxic to bobwhite quail given 42.9% mortality was observed at 1000 ppm. Considering no dose related mortality was observed at 1000 ppm in this study, and at 4640 ppm in the previous study for duck, the metabolite is considered to be potentially practically nontoxic to bobwhite quail on a dietary basis based on the test results.
CONCLUSION	The notified chemical is slightly toxic and the metabolite is practically non-toxic to bobwhite quail.
TEST FACILITY	Dow (1967b)

b. Dietary toxicity to Japanese quail

TEST SUBSTANCE	Notified chemical (9.36%) and 6-chloropicolinic acid (6-CPA) (99%)
METHOD	Procedure for Evaluation of Acute Toxicity to Fish and Wildlife, 14 December 1964
Species	Japanese quail (5 -7 days of age; The Dow Chemical Company's Japanese Quail Stock colony)
Exposure Period	8 days (5 days exposure to treated dietary followed by 3 days maintained on toxicant-free diet)
Test levels – notified chemical	100, 300, 400, 500, 600, 700, 800, 900, 1000, 3000 ppm (nominal)
Test levels – metabolite	300, 500, 700, 900, 1000, 3000, 5000 ppm (nominal)
Statistical analysis	Litchfield-Wilcoxin (1949)
Remarks – Method	The study was performed based on the previous pilot test. One replicate of 15 birds was established per treatment group. Feeding schedule was two days on non-medicated feed followed by five days of medicated diets, followed by three days of non-medicated feed. A formulation of one of the biologically inert but dietitious attapulgite group of carriers containing 25% the notified chemical or the metabolite was prepared and then premixed with a portion of the final feed mix. The basal diet used in these studies was Cohoon's Elevator Turkey Starter. Watering was done with quail fount gravitational type waterers, individually by pen. A reference control test was performed using 1,1,1-trichloro-2,2-bis (p-chlorophenyl) (p,p' isomer, DDT) at levels from 100 – 1000 ppm. Feed consumption was measured for each time period. Body weights were measured by pen as a group on experimental day -2, 0, 5 and 8. Mortality and adverse effects such as tremoring, cataracts, etc. were observed and recorded daily. The concentrations of test substances in the diet were not measured throughout the test period.

RESULTS

Table 17 – Dietary toxicity to bobwhite quail results for the notified chemical

Test group (mg/kg diet)	*Mortality (%)*	*Mean feed consumption (g feed/bird)*		*Mean body weight (g/bird)*		*Effect on day 0-8 body weight gain compared to control (%)*
		during days 1 - 5	*during days 6 - 8*	*on day 0*	*on day 8*	
Control	0	7.94	8.59	19.8	38.6	N/A
100	0	9.99	9.53	20.7	40.1	-1.2
300	0	9.39	8.87	18.9	40.3	19
400	0	8.24	9.71	19.7	41.7	17.6
500	0	7.77	6.24	17.4	25.2	-52.8
600	0	6.52	8.04	18.5	35.8	-1.5
700	27	4.72	6.76	19.4	27.5	-56
800	67	5.93	9	20.7	35.6	24.1
900	40	7.28	9.44	21	34.8	-30.8
1000	67	4.84	5.94	20.3	28.2	-59
3000	100	5.93	-[a]	17.8	-[a]	-[a]

[a] All tested birds dead by day 5

Table 18 – Dietary toxicity to bobwhite quail results for the metabolite

Test group (mg/kg diet)	*Mortality (%)*	*Mean feed consumption (g feed/bird)*		*Mean body weight (g/bird)*		*Effect on day 0-8 body weight gain compared to control (%)*
		during days 1 - 5	*during days 6 - 8*	*on day 0*	*on day 8*	
Control	0[a]	8.41	10.38	22.9	41.8	N/A
300	0	9.19	10.18	22	41.6	7.9
500	0	10.97	12.31	21.6	37.2	-12.5
700	7	9.28	11.28	20.7	36.6	-6.9
900	0	10.53	10.35	24.1	39.5	-22.6
1000	0	11.68	10.51	23.5	40.3	-13.3
3000	0	10.32	10.4	22.1	44.7	23.9
5000	0	10.53	10.33	23.1	39.1	-16.1

[a] There was one death out of 15 on day 2 due to causes unrelated to treatment

LC50	820 ppm for the notified chemical; and, >5000 ppm for the metabolite (nominal)
NOEC	400 ppm for the notified chemical; and, 300 ppm for the metabolite (nominal)
Remarks – Results	The LC50 for the reference control was determined as 470 ppm. For the notified chemical, the LC50 was determined by the study author to be 820 (752.3 – 893.8) ppm. Significant adverse effects on body weight were observed at 500 ppm. No adverse effects on body weight were observed at levels below 400 ppm inclusive. Therefore, the NOEC (for body weight) for the notified chemical is determined as 400 ppm. For the metabolite, one death out of 15 was observed in the 700 ppm level. Since no mortality was observed at all other levels, this is not considered to be test substance related. Therefore, the LC50 is determined to be >5000 ppm. Significant adverse effects on body weight were observed at levels 500, 900, 1000, and 3000 ppm. This is considered to be test substance related although the effects were not clearly dose responsive. Therefore, the NOEC (for body weight) is determined to be 300 ppm.

CONCLUSION The notified chemical is considered to be slightly toxic, and the metabolite is practically nontoxic to Japanese quail on a dietary basis.

TEST FACILITY Dow (1968a)

c. Acute oral toxicity to turkey poults and white leghorn cockerel chicks

TEST SUBSTANCE: Notified chemical (93.6%)

METHOD: Not provided

Species: Turkey poults (aged 4-6 weeks)
White leghorn cockerel chicks (aged five weeks)

Test levels: 100, 126, 158, 200, 252, 316, 398, 500, 630, 795, 1000 mg/kg bw (nominal)

Statistical analysis: Not provided

Remarks – Method: Following a range finding test, five birds for each test level were dosed with pure chemical in gelatin capsules or pipetted directly into crop with liquids that cannot be capsuled. Birds selected for the test were withdrawn from feed 24 hours prior to dosing, and were maintained on non-medicated diets at all times. Immediately after dosing the birds are returned to feed and water *ad lib*.

The mg/kg bw chemical dose for the bird was based on the "starved" body weight.

Body weights were measured on experimental day 0, 3 or 4, 7 and 14. Mortality and adverse effects such as CNS disturbances, regurgitation of feed or capsules, disorders of the eye, general condition and voidings were observed and recorded on a daily basis.

The concentrations of test substances in the diet were not measured throughout the test period. No control tests were established.

RESULTS

Table 19 – Dietary toxicity to turkey poults aged 6 weeks

Test group	*Mortality on*	*Mean body weight (g/bird)*				*Weight gain between*
(mg/kg bw)	*day 14 (%)*	*on day 0*	*on day 3*	*on day 7*	*on day 14*	*day 1 - 14 (%)*
32	0	1906.8	2102	2356.4	2669.4	40
63	0	2532	2662	2990.2	3582.8	41.5
126	60	2396.5	2349	2295	2686	12.1
252	100	2628	2522	-	-	

Table 20 – Dietary toxicity to leghorn cockerel chicks aged 5 weeks (test 1)

Test group	*Mortality on day 7 (%)*	*Mean body weight (g/bird)*		
(mg/kg bw)		*on day 0*	*on day 3*	*on day 7*
32	0	505.2	610.6	698.6
63	0	517.8	619.2	707.2
126	0	556.4	639	738.6
252	60	526	549	624.5

Table 21 – Dietary toxicity to leghorn cockerel chicks aged 5 weeks (test 2)

Test group	Mortality on	Mean body weight (g/bird)				Weight gain between
(mg/kg bw)	day 14 (%)	on day 0	on day 3	on day 7	on day 14	day 1 - 14 (%)
126	0	647.2	743.8	834.4	965.2	49.1
252	40	669	697	809.7	967.3	44.5
504	100	-	-	-	-	
1008	100	-	-	-	-	

LD50: 118 (95% CL 85 – 164) mg/kg bw for turkey poults and
235 (95% CL 169 - 328) mg/kg bw for leghorn cockerel chicks.

NOEC: Not applicable since no blank control was performed.

Remarks – Results: All body weights were based on survived birds only. All the mortality occurred on day 2-4.

Information regarding mortality and adverse effects were not provided in the study report. The study author has calculated the LD50s for both birds.

Details about the calculation were not provided in the study report. The calculations are considered acceptable since they are close to the observed value ranges according to the test data. NOEC values have not been established since not blank control was performed. Although no blank control was conducted, the results are considered reliable since dose responses were observed in the study.

The notified chemical is considered to be moderately toxic to both turkey poults and leghorn cockerel chicks on an acute basis based on the test results.

CONCLUSION The notified chemical is considered to be moderately toxic to birds on an acute basis.

TEST FACILITY Dow (1968b)

a. Dietary toxicity of a metabolite to mallard duck

TEST SUBSTANCE 6-chloropicolinic acid (6-CPA)

METHOD Not provided
Species Mallard ducks aged 14 days hatched and maintained at 37.5 °C
Exposure Period 8 days (5 days exposure to treated dietary followed by 3 days maintained on toxicant-free diet)
Test levels - test group 215, 464, 1000, 2150, 4640 ppm (nominal)
Test levels - dieldrin control 100, 159, 251, 398, 631 ppm
Auxiliary Solvent Corn oil
Statistical analysis Mortality was analysed statistically by the method of Litchfield
Remarks – Method Five pens, each containing 10 ducks were established for each of the control and test groups. A blank control and a dieldrin control (purity 88% HEOD) control were conducted. Prior to initiation of the study and during the eight-day test, the basal diet was a standard game bird starter ration. Game bird diet and water were available *ad libitum* throughout the study.

Body weights were recorded by pen at initiation and termination of the study. Food consumption was recorded by pen during the five-day exposure period. Food consumption was measured accurately, but is presented as an estimate due to the unavoidable wastage by the birds.

Symptoms of toxicity and mortality were recorded daily throughout the study.

The concentrations of test substances in the diet were not measured throughout the test period.

RAC

Test group (mg/kg diet)	*Control*	*215*	*464*	*1000*	*2150*	*4640*
Mortality (%)	0.0	0.0	0.0	0.0	0.0	0.0
Mean feed consumption during days 1 to 5 (g feed/bird/day)	669	630	715	775	795	615
Mean body weight on day 0 (g/bird)	192.6	205	225	210	242	192
Mean body weight on day 8 (g/bird)	366	365	412	365	425	392
Effect on body weight gain compared to control (%)	N/A	-6.3	-3.6	-8.5	-7.6	7.5
Clinical signs	None	None	None	None	Yes	Yes

LC50 >4640 ppm (nominal)
NOEC 1000 ppm (nominal)
Remarks – Results The test study did not specify which test guideline was followed, if any.

	The LC50 for the dieldrin control was determined to be 174.9 ppm (95% CL 137.6 – 222.3 ppm). The test substance caused depression and a reduced reaction to external stimuli (sound and movement) at 2150 and 4640 ppm. There were no symptoms of toxicity or behavioural abnormalities noted at the 215, 464, or 1000 ppm dosage levels. Therefore, the NOEC was determined to be 1000 ppm. The test substance is considered to be practically non-toxic to mallard duck on a dietary basis based on the test results.
CONCLUSION	The metabolite, 6-CPA is practically non-toxic to mallard duck on a dietary basis.
TEST FACILITY	Truslow (1974)

C.2.2. Acute toxicity to fish

a. Bluegill (*Lepomis macrochirus*)

TEST SUBSTANCE	Notified chemical (92.4%)
METHOD	U.S. Environmental Protection Agency. Test Guidelines 40CFR.797.1300 and 40CFR.797.1400, Federal Register, Vol. 50, No. 188, Washington, D.C., 1985 Committee on Methods for Toxicity Tests with Aquatic Organisms. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. U.S. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-009, 1975 American Society for Testing and Materials, ASTM Standard E729-80, Standard Practice For Conducting Acute Toxicity Tests With Fishes, Macroinvertebrates and Amphibians, Philadelphia, PA, 1980 OECD TG 203 Fish, Acute Toxicity Test – Flow-through
Species	*Lepomis macrochirus*
Exposure Period	96 h
Auxiliary Solvent	N,N-dimethyl formamide
Water Hardness	81 mg $CaCO_3$/L
Analytical Monitoring	HPLC/UV
Remarks – Method	After a preliminary range finding test, a definitive test was conducted in accordance with the OECD guideline above and in compliance with GLP standards and principles. There were no reported deviations from the test protocol. A blank and a solvent control (0.1 mL/L) were run in conjunction with 6 test concentrations following a geometric progression with a factor of 1.67. Ten fish were exposed at each treatment level and control (5 fish per replicate, 2 replicates per concentration). Based on the average of analyses on days 0 and 4, the fish were exposed to measured concentrations of 1.3, 1.5, 2.3, 4.0, 5.5 and 9.3 mg/L. Test conditions were: 21.5-22.6 °C; pH 7.1-7.7; 8.1-8.8 mg O_2/L; 16 h light/8 h dark.

RESULTS

Measured Concentration (mg/L)	*Number of Fish*	*Mortality*			
		24 h	*48 h*	*72 h*	*96 h*
Control (<5 ppm)	10	0	0	0	1
Solvent control (<5 ppm)	10	0	0	0	0
1.3	10	0	0	0	0
1.5	10	0	0	0	0
2.3	10	0	0[a]	0[a]	0[a]
4.0	10	0[a]	4[a]	7[a]	8[a]
5.5	10	8[a]	9[a]	10	10
9.3	10	10	10	10	10

[a] Surviving fish exhibited loss of equilibrium, surface swimming, and lethargy.

LC50 (95% Confidence interval)	5.0 (4.0-9.2) mg/L at 24 hours (non-linear interpolation) 4.2 (3.3-4.9) mg/L at 48 hours (Probit method; Finney, 1971) 3.5 (2.3-5.5) mg/L at 72 hours (non-linear interpolation) 3.4 (2.3-5.5) mg/L at 96 hours (non-linear interpolation)
NOEC	1.5 mg/L at 96 hours
Remarks – Results	The OECD validity criteria were met. In accordance with the OECD test guideline the results are based on the mean of the measured concentration as the measured concentration deviated from the nominal concentration by more than 20%. The statistical analysis of the data by non-linear interpolation (95% confidence interval calculated using the binomial method; Johnson, 1969), yielded a 96 hour LC50 of 3.4 mg/L with a 95% confidence interval of 2.3-5.5 mg/L. The 96 hour mortality threshold concentration was 2.3 mg/L. Sub-lethal effects, such as lethargy, surface swimming, and loss of equilibrium, were not noted below the 2.3 mg/L dose levels. Therefore, the NOEC was 1.5 mg/L.

CONCLUSION The notified chemical is toxic to bluegill on an acute basis.

TEST FACILITY Dow (1991a)

b. Rainbow trout (*Oncorhynchus mykiss* Walbaum)

TEST SUBSTANCE Notified chemical (92.6%)

METHOD U.S. Environmental Protection Agency. Test Guidelines 40CFR.797.1300 and 40CFR.797.1400, Federal Register, Vol. 50, No. 188, Washington, D.C., 1985
Committee on Methods for Toxicity Tests with Aquatic Organisms. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. U.S. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-009, 1975
American Society for Testing and Materials, ASTM Standard E729-80, Standard Practice For Conducting Acute Toxicity Tests With Fishes, Macroinvertebrates and Amphibians, Philadelphia, PA, 1980.
OECD TG 203 Fish, Acute Toxicity Test – Static

Species	*Oncorhynchus mykiss* Walbaum
Exposure Period	96 h
Auxiliary Solvent	acetone
Water Hardness	72 mg $CaCO_3$/L
Analytical Monitoring	HPLC/UV
Remarks – Method	After a preliminary range finding test, a definitive test was conducted in accordance with the OECD guideline above and in compliance with GLP standards and principles. There were no reported deviations from the test protocol.

A blank and a solvent control (0.5 mL/L) were run in conjunction with 6 test concentrations following a geometric progression with a factor of 1.67. Ten fish were exposed at each treatment level and control (5 fish per replicate, 2 replicates per concentration). Based on the analysis on day 0, the fish were exposed to measured concentrations of 0.9, 1.0, 1.6, 2.9, 4.9 and 10.8 mg/L. Test conditions were: 12.2-12.5 °C; pH 7.3-8.0; 7.2-10.8 mg O_2/L; 16 h light/8 h dark.

RESULTS

Measured Concentration (mg/L)	*Number of Fish*	*Mortality*			
		24 h	*48 h*	*72 h*	*96 h*
Control (< 5ppm)	10	0	0	0	1
Solvent Control (<5 ppm)	10	0	0	0	0
0.9	10	0	0	0	0
1.0	10	0	0	0	0
1.6	10	0	0	0	0[a]
2.9	10	0[a]	0[a]	0[a]	1[a]
4.9	10	0[a]	2[a]	2[a]	7[a]
10.8	10	10	10	10	10

[a] Surviving fish exhibited loss of equilibrium, surface swimming, and lethargy.

LC50	7.3 (4.9-10.8) mg/L at 24 hours (non-linear interpolation)
(95% Confidence interval)	6.3 (2.9-10.8) mg/L at 48 hours (non-linear interpolation) 5.7 (4.4-7.9) mg/L at 72 hours (Probit method; Finney, 1971) 4.0 (3.1-5.4) mg/L at 96 hours (Probit method; Finney, 1971)
NOEC	1.0 mg/L at 96 hours
Remarks – Results	In accordance with the OECD test guideline the results are based on the measured concentration at 0 hours as the measured concentration deviated from the nominal concentration by more than 20%. Samples were also taken for analysis at 96 hours but the data is not available due to equipment breakdown. However, the stability of the test substance in the test solutions was demonstrated in the static range-finding test. The stability after five days was found to be 99% at 0.5 mg/L and 119% at 5.0 mg/L. Therefore, the OECD validity criteria were met. Even though testing was conducted at concentrations below the water solubility of the test substance, incomplete solubility was evident at all concentrations during initial dosing. Further observations throughout the test were not made. It is unlikely that the observed toxicity is due to physical effects of the undissolved test substance as incomplete solubility was observed at all test concentrations. Further, the results are based on the measured concentrations and not the nominal concentrations. The statistical analysis of the data by Probit method (Finney, 1971), yielded a 96 hour LC50 of 4.0 mg/L with a 95% confidence interval of 3.1-5.4 mg/L. The 96 hour mortality threshold concentration was 1.6 mg/L. Sub-lethal effects, such as lethargy, surface swimming, and loss of equilibrium, were not noted below the 1.6 mg/L dose levels. Therefore, the NOEC was 1.0 mg/L.
CONCLUSION	The notified chemical is toxic to rainbow trout on an acute basis.
TEST FACILITY	Dow (1991b)

c. Tidewater silverside (*Menidia beryllina*)

TEST SUBSTANCE	Notified chemical (91.2%)

METHOD In house

Species	*Menidia beryllina*
Exposure Period	96 h
Auxiliary Solvent	Dimethyl formamide
Water Salinity	20-22‰
Analytical Monitoring	HPLC/UV
Remarks – Method	After a preliminary range-finding test, a definitive test was conducted in compliance with GLP standards and principles. Although the study report did not state that the test was conducted in accordance with recognised test methods, the test protocol was largely consistent with OECD TG 203 Fish, Acute Toxicity Test – Flow-Through. The dilution water was filtered natural seawater with a salinity of 20 to 22‰. A blank and a solvent control (94.1 µL/L) were run in conjunction with 5 test concentrations following a geometric progression with a factor of 1.67. Twenty fish were exposed at each treatment level and control (no replicates). Based on the analyses from samples collected on days 0, 2 and 4, the fish were exposed to measured concentrations of 1.26, 2.24, 3.15, 5.82 and 10.4 mg/L. Test conditions were: 21.2-22.2 °C; pH 8.2-8.5; ≥3.2 mg O_2/L (≥42% saturation); 16 h light/8 h dark. There were two reported deviations from the test protocol. The first deviation was that the volume additions per day was only 4.4; the protocol specified a minimum of 5 turnovers per day. The second deviation occurred as a result of dissolved oxygen concentrations falling below 60 percent of saturation. Although dissolved oxygen concentration in the dilution water (control) remained greater than 93 percent of saturation during the 96-hour exposure, dissolved oxygen concentrations in the solvent control and all five test concentrations decreased below 60 percent of saturation during the test. The lower dissolved oxygen concentrations in the test concentrations appeared to result from an increased bacterial presence due to the solvent.

RESULTS

Concentration (mg/L)		*Number of Fish*	*Mortality*			
Nominal	*Measured*		*24 h*	*48 h*	*72 h*	*96 h*
Control	<0.20	10	0	0	0	0
Solvent control	<0.20	10	0	0	0	0
1.3	1.26	10	2	2	2	2
2.2	2.24	10	0	0	0[e]	0[e]
3.6	3.15	10	0[a]	0[c]	0[c]	0[g]
6.0	5.82	10	2[b]	3[d]	12[f]	20
10	10.4	10	20	20	20	20

[a] Four fish were dark.
[b] Six fish were lethargic and twelve were dark.
[c] Six fish were dark.
[d] Four fish were lethargic and eight were dark.
[e] One fish was dark.
[f] One fish was lethargic and all fish were dark.
[g] Five fish were dark.

LC50	7.31 (5.82-10.4) mg/L at 24 hours
(95% Confidence interval)	7.14 (5.82-10.4) mg/L at 48 hours 5.39 (3.15-10.4) mg/L at 72 hours 4.28 (3.15-10.4) mg/L at 96 hours
LOEC	1.26 mg/L at 96 hours
Remarks – Results	The OECD validity criteria for oxygen concentration for not less than 60% of the air saturation value (equivalent to 4.6 mg O_2/L) were not met

for the solvent control and test solutions. The solvent control exhibited lower dissolved oxygen concentrations (3.2-4.1 mg O_2/L) than any of the test solutions. The lowest observed concentration in the test solutions of 3.8 mg O_2/L was for the 3.15 mg/L test solution on day 4. All other test solutions remained at 4.3 mg O_2/L or higher for the duration of the test. As the validity criteria were not met, the results should be treated with caution.

The results are based on the mean measured concentration; the measured concentrations were within 20% of the nominal concentration for the duration of the test.

The statistical analysis of the data by non-linear interpolation (with the 95 percent confidence limits determined by binomial probability), yielded a 96 hour LC50 of 4.28 mg/L with a 95% confidence interval of 3.1-10.4 mg/L. Mortality or sub-lethal effects, such as lethargy and discolouration were observed at all tested concentrations. Therefore, the NOEC could not be determined.

CONCLUSION The notified chemical is toxic to tidewater silverside on an acute basis.

TEST FACILITY Toxikon (1991a)

d. Rainbow trout and bluegill

TEST SUBSTANCE Notified chemical (92.6%)

METHOD Committee on Methods for Toxicity Tests with Aquatic Organisms. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. U.S. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-009, 1975. (Static)

Species	*Salmo gairdneri* Richardson *Lepomis macrochirus* Rafinesque
Exposure Period	96 h
Auxiliary Solvent	Acetone
Water Hardness	128 mg $CaCO_3$/L
Analytical Monitoring	Nominal concentrations were not verified
Remarks – Method	The definitive tests were conducted in accordance with the guideline above. There were no reported deviations from the test protocol but the study report did not include all relevant parameters in the guideline. The test solutions were prepared from natural filtered (activated carbon) water sourced from Lake Huron (dissolved oxygen: 7.4 mg O_2/L; pH 8.0). Ten fish were exposed at each treatment level (unknown number of replicates). The report indicates that a control was conducted but does not state whether a solvent control was included and does not record the solvent concentration in the test solutions. Test conditions were: 12 ºC ± 1 ºC (Rainbow trout) and 22 ºC ± 1 ºC (Bluegill); 16 h light/8 h dark.

RESULTS

Rainbow Trout (*Salmo gairdneri* Richardson)			
Nominal Concentration (mg/L)	*Number of Fish*	*Mortality*	
		72 h	*96 h*
4.9	10	0	0
5.6	10	2	2
6.5	10	0	0
7.5	10	0	0
8.7	10	1	1
10	10	8	10
11.5	10	9	NR

Bluegill (*Lepomis macrochirus* Rafinesque)			
Nominal Concentration (mg/L)	*Number of Fish*	*Mortality*	
		72 h	*96 h*
2.4	10	NR	1
3.2	10	NR	0
4.2	10	0	1
5.6	10	1	1
7.5	10	0	2
10	10	10	10

NR = not reported

	Rainbow trout	Bluegill
LC50 (95% Confidence interval)	9.6 (9.0-10.4) mg/L at 72 hours 9.1 (8.9-9.4) mg/L at 96 hours	8.7 (8.6-8.7) mg/L at 72 hours 7.9 (7.4-8.5) mg/L at 96 hours

Remarks – Results

Mortality of the control fish did not exceed 10% for any of the test species. However, there was no reported analytical monitoring of the test substance or dissolved oxygen concentrations in the test solutions over the duration of the test. Therefore, the results should be treated with caution.

There were no observations for effects other than mortality throughout the test. The NOEC and LOEC were not determined.

The statistical analysis of the data was done by moving average method (Thompson, 1947). Mortality results were not reported unless they influenced the statistical analysis. The 24 and 48-hour data for both test species was not reported because of significant lack of fit for the moving average model. The 96-hour LC50 values of the two test species are: rainbow trout, 9.1 (8.9-9.4) mg/L and for the bluegill, 7.9 (7.4-8.5) mg/L.

CONCLUSION

The notified chemical is, at best, toxic to rainbow fish and bluegill on an acute basis.

TEST FACILITY

Dow (1978)

e. Rainbow trout, channel catfish and goldfish

TEST SUBSTANCE

Notified chemical (technical grade), two notified chemical formulations (N-Serve 24 and N-Serve 24E), 6-chloropicolinic acid (6-CPA)

METHOD

USDI, Procedure for Evaluation of acute toxicity of pesticides to fish and wildlife, December 14, 1964. (Static) Standard Methods for the Examination of Water and Wastewater, 12th Edition, 1964, pp 556-558

Species	Rainbow trout (*Salmo gairdneri* Richardson) Channel catfish (*Ictalurus punctatus* Rafinesque) Goldfish (*Carassius auratus* Linnaeus)
Exposure Period	96 h
Auxiliary Solvent	Acetone
Water Hardness	50 mg $CaCO_3$/L
Analytical Monitoring	Unknown

Remarks – Method

The definitive tests were stated to be in compliance with the guidelines above. There were no reported deviations from the test protocol.

A summary study report was provided for a static water test method of the three fish species to the four test substances. The test solutions were prepared from aerated distilled water with a hardness of 50 mg $CaCO_3$/L. Ten fish were exposed at each treatment level but the number of test

solutions, nominal and/or measured concentrations and number of replicates were not reported. A blank control and toxicity reference (ρ,ρ`-DDT) was run with each species of fish. Reported test conditions were: 60 °F (Rainbow trout, Channel catfish) and 80 ºF (Goldfish).

RESULTS

Test substance and species	*Number of fish per test concentration*	*LC50 mg test substance/L (as mg notified chemical/L)*			
		24 h	*48 h*	*72 h*	*96 h*
Notified chemical (technical grade)					
Rainbow trout	10	11.8	7.8	7.0	7.0
Channel catfish	10	11.8	10.0	10.0	8.0
Goldfish	10	11.0	8.7	7.6	7.6
Notified chemical formulation (N-Serve 24)					
Rainbow trout	10	13.4 (3.3)	13.4 (3.3)	13.0 (3.2)	12.4 (3.1)
Channel catfish	10	15.2 (3.7)	12.15 (3.0)	11.75 (2.9)	11.75 (2.9)
Goldfish	10	13.3 (3.3)	12.4 (3.1)	11.1 (2.7)	10.0 (2.5)
Notified chemical formulation (N-Serve 24E)					
Rainbow trout	10	7.2 (1.7)	6.95 (1.7)	6.95 (1.7)	6.95 (1.7)
Channel catfish	10	13.8 (3.4)	13.4 (3.3)	13.4 (3.3)	12.9 (3.1)
Goldfish	10	12.4 (3.0)	11.8 (2.9)	11.0 (2.7)	11.0 (2.7)
Metabolite (6-CPA)					
Rainbow trout	10	42.2	40.5	40.5	40.5
Channel catfish	10	100.0	100.0	100.0	100.0
Goldfish	10	239.0	239.0	239.0	239.0

Remarks – Results

The raw data reporting mortalities for each test solution were not available. Mortality of the control fish did not exceed 10% for the channel catfish, but exceeded 10% for both the rainbow trout (ranged from 10% to 20%) and goldfish (ranged from 65% to 73%). There was no reported analytical monitoring of the test substance or dissolved oxygen concentrations in the test solutions over the duration of the test. There were no results for effects other than mortality. The NOEC and LOEC were not reported. The method used for the statistical analysis to determine the endpoints was not reported. Therefore, the results should be treated with caution.

The 96 hour LC50 values for the notified chemical were 6.97 mg/L for rainbow trout, 8.0 mg/L for channel catfish, and 7.6 mg/L for goldfish. The potential for toxic effect of formulation solvent systems can be seen by comparing the LC50 values (as mg/notified chemical) for the formulations with the technical grade. The metabolite, 6-CPA, is less toxic to fish than the notified chemical having a 96 hour LC50 of 40.5 mg/L for rainbow trout, 100 mg/L for channel catfish, and 239 mg/L for goldfish.

CONCLUSION

The notified chemical is, at best, toxic to rainbow fish, channel catfish and goldfish on an acute basis.

The metabolite is, at best, harmful to rainbow trout and channel fish and is unlikely to be harmful to goldfish on an acute basis.

TEST FACILITY

The (1971b)

f. Channel catfish, fathead minnow and rainbow trout

The following additional endpoints for fish were reported for the notified chemical (US Department of the Interior, 1968). The test protocol was not reported and study reports were not available.

Test species	*LC50 (mg/L)*	
	24 h	*96 h*
Channel catfish	7.75	5.80
Fathead minnow	11.0	10.2
Rainbow trout	8.9	6.45

C.2.3. Acute toxicity to aquatic invertebrates

a. Daphnia (test 1)

TEST SUBSTANCE	Notified chemical (96%)
METHOD	Committee on Methods for Toxicity with Aquatic Organisms, 1975. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. Ecological Research Series, EPA-660/3-75-009
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	Acetone (maximum 0.5 mL/L)
Water Hardness	100 mg $CaCO_3$/L
Analytical Monitoring	Nominal test concentrations were not verified
Remarks - Method	The notified chemical was evaluated at nominal levels of 3.7, 5.6, 7.5, 10.0, 13.5 and 18.0 mg/L, for acute toxicity to an aquatic invertebrate species, *Daphnia magna* Straus, using a flow-through test method at 25 ºC. The test solution was prepared in dechlorinated Lake Huron water (dissolved oxygen 7.7 mg/L, pH 7.6). A blank control using dechlorinated Lake Huron water and a solvent control containing the largest amount of solvent used in any concentration were set. This value is determined using Finney's probit analysis method with a computer program (Finney, 1952).

RESULTS

Nominal Concentration mg/L	*Percentage Immobilised (%)*	
	24 h	*48 h*
3.7	3	30
5.6	27	37
7.5	37	60
10.0	53	87
13.5	97	100
18.0	100	

LC50 (95% confidence interval)	5.8 (5.0 – 6.5) mg/L at 48 hours
NOEC	Not determined
Remarks - Results	The control daphnids exhibited a mortality of 10% during the test. It is not reported if this was for the blank or solvent control. OECD TG 202 for acute toxicity test with daphnia recommends a validity criterion of ≤10% mortality in the (blank) control test. Therefore, the test result is considered to meet the criteria of OECD TG202. The notified chemical is considered to be moderately toxic to daphnia on an acute basis. Dissolved oxygen and concentrations were not verified for the duration of the test. Therefore, the results should be treated with caution.

CONCLUSION The notified chemical is toxic to daphnia on an acute basis.

TEST FACILITY Dow (1977)

b. Daphnia (test 2)

TEST SUBSTANCE Notified chemical (92.4%)

METHOD U.S. Environmental Protection Agency. Test Guidelines 40CFR797.1300 and 40CFR797.1400, Federal Register, Vol. 50, No. 188, Washington, D.C., 1985
Committee on Methods for Toxicity with Aquatic Organisms, 1975. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians. Ecological Research Series, EPA-660/3-75-009
American Society for Testing and Materials, ASTM Standard E729-80, Standard Practice For Conducting Acute Toxicity Tests With Fishes, Macroinvertebrates and Amphibians, Philadelphia, PA, 1980
Test Guideline No. 202, Daphnia sp., Acute Immobilization Test and Reproduction Test – Flow-through

Species	*Daphnia magna* Straus (less than 24-h old)
Exposure Period	48 hours
Auxiliary Solvent	N,N-dimethyl formamide (DMF)
Water Hardness	188 mg $CaCO_3$/L
Analytical Monitoring	HPLC for measurement of the test concentrations
Remarks - Method	The notified chemical was evaluated at nominal levels of 0.78, 1.3, 2.1, 3.6, 6.0 and 10.0 mg/L, for acute toxicity to *Daphnia magna* Straus, using a flow-through test method at 19.4 – 20.7 ºC. The test solution was prepared in Lake Huron water, which was limed and flocculated with ferric chloride, and further sand-filtered, pH-adjusted with CO_2, carbon-filtered, and UV irradiated prior to use (dissolved oxygen 9.0 – 9.4 mg/L, pH 6.7 – 8.3). A blank control and a solvent control containing the DMF were established. All test groups were set up in triplicate and 10 animals were used for each replicate. Analysis of the concentration/response data was performed using Statistical Analysis System (SAS)® computer software. Specifically, Dunnett's T-test and Analysis Of Variance (ANOVA) were used to determine the NOEC.

RESULTS

Concentration (mg/L)		*Percentage Immobilised (%)*	
Nominal	*Actual*	*24 h*	*48 h*
Blank control	N/A	0	10
Solvent control	N/A	0	7
0.78	0.95	0	13
1.3	1.5	0	27
2.1	2.57	30	57
3.6	4.0	77	77
6.0	7.57	97	100
10.0	9.64	100	100

LC50 (95% confidence interval)	2.2 (1.86 – 2.57) mg/L at 48 hours
NOEC	Not determined
Remarks - Results	The 48-hour LC50 for the notified chemical was determined to be 2.20 mg/L with a 95% confidence interval of 1.86 - 2.57 mg/L. Due to mortality at the lowest test concentration, the 48-hour mortality threshold concentration was less than 0.95 mg/L. Based on statistical analysis of the concentration/response data, the NOEC was calculated to be 1.50 mg/L. The test is considered to meet the validity criteria of OECD TG202. The

notified chemical is considered moderately toxic to daphnia on an acute basis.

CONCLUSION The notified chemical is toxic to daphnia on an acute basis.

TEST FACILITY Dow (1991)

c. Eastern oyster

TEST SUBSTANCE Notified chemical (91.2%)

METHOD U.S. Environmental Protection Agency. 1982; Pesticide Assessment Guidelines - Subdivision E - Hazard Evaluation: Wildlife and Aquatic Organisms, EPA 540/9-82-024, 72-3 Acute Toxicity Test for Estuarine and Marine Organisms, pp.72-76
U.S. Environmental Protection Agency. 1985. Standard Evaluation Procedure: Acute Toxicity Test for Estuarine and Marine Organisms (Mollusc 96-hour Flow-Through Shell Deposition Study), EPA-540/9-85-011, 10 pp

Species	Eastern Oyster (*Crassostrea virginica*, 24.5 mm ± 2.4 mm umbo-distal valve edge length)
Exposure Period	96 hours
Auxiliary Solvent	Dimethyl formamide
Water Salinity	28-30‰
Analytical Monitoring	Gas chromatograph equipped with an electron capture detector.
Remarks - Method	After a range finding test, a definitive test in accordance with the guideline above and in compliance with GLP standards and principles. There were no significant deviations to the protocol.

A blank control and solvent control (0.7 µL/L) were run in conjunction with 5 test concentrations in a geometric series with a factor of 1.6. Twenty oysters were exposed at each treatment level and control under flow-through conditions. Based on the mean of the analyses on days 0, 2 and 4, the oysters were exposed to measured concentrations of 0.16, 0.26, 0.43, 0.72 and 1.2 mg/ mg/L. The dilution water was unfiltered natural saltwater with salinity of 28-30‰. Test conditions were: 22.6-25.2 °C; pH 7.8-8.0; 5.4-6.9 mg O_2/L (≥77% dissolved oxygen saturation), 16 h light/8 h dark.

The 96-hour EC50 and 95 percent confidence limits were calculated for reduction in new shell growth using mean measured concentrations of the test substance. The EC50 was calculated by a computer program using the method of inverse estimation (Neter, Wasserman & Kutner, 1983) following a fit of the line using least squares estimation. Statistical differences in growth between the solvent control oysters and the five the notified chemical exposure concentrations were determined by analysis of variance (ANOVA) and Dunnett's procedure for multiple comparisons of means with a control (Steel and Torrie, 1960). The NOEC was determined to be the highest test concentration which was not statistically, different from the control.

RESULTS

Concentration (mg/L)		*New Shell Growth*		
Nominal	*Actual*	*Mean (SD) (mm)*	*Difference (mm) from solvent control*	*Percent change from solvent control*
Blank control	<0.05	1.8 (1.2)	-	-
Solvent control	<0.05	1.5 (0.7)	-	-
0.16	0.16	1.2 (0.6)	0.3	-22
0.26	0.26	1.0 (0.6)	0.5	-32[a]
0.43	0.44	0.8 (0.5)	0.7	-48[a]
0.72	0.71	0.5 (0.5)	1.0	-67[a]
1.2	1.2	0.2 (0.4)	100	-88[a]

[a] The mean new shell growth in this group is significantly less than the solvent control mean at α=0.05.

EC50 (95% confidence interval)	0.56 (0-2.78) mg/L at 96 hours
NOEC	0.16 mg/L at 96 hours
Remarks - Results	There were no mortalities observed in any of the control or treatment solutions. New shell growth the control and solvent control were both less than 2 mm at the end of the test indicating reduced shell deposition in the control. Therefore, the results should be used with caution. The measured concentrations of the test substance in the test solution were within ± 20% of the nominal concentration for the duration of the test. Dissolved oxygen, pH, salinity and temperature were monitored throughout the test. Undissolved test substance was observed in the highest concentration test solution. The mean test temperature (23.8 °C) exceeded the current recommended test temperature of 20 °C for this species. However, previous guidelines allowed for higher temperatures when molluscs were collected from warmer waters. The variance in temperature throughout the test slightly exceeded the recommended variance of not more than 2 °C due to the inability of the water bath to compensate for large diurnal fluctuations in ambient water temperature. This deviation to the protocol was not considered to be significant. The 96-hour EC50 of the test substance to oysters, *Crassostrea virginica*, was calculated to be 0.56 mg/L with 95 percent confidence limits of 0 and 2.78 mg/L. The slope of the EC50 curve was -1.0696. The NOEC was 0.16 mg/L, the highest test concentration with no statistically significant difference in new shell growth as compared to the solvent control oysters.
CONCLUSION	The notified chemical is very toxic to oysters on an acute basis.
TEST FACILITY	Toxikon (1991b)

d. Grass shrimp

TEST SUBSTANCE	Notified chemical (91.2%)
METHOD	In house
Species	Grass shrimp (*Palaemonetes pugio*, Adults; 19 ± 1.4 rom rostrum-telson)
Exposure Period	96 hours
Auxiliary Solvent	Dimethyl formamide
Water Salinity	20-22‰
Analytical Monitoring	Gas chromatograph equipped with an electron capture detector
Remarks - Method	After a range finding test, a definitive test in accordance with the in house protocol above and in compliance with GLP standards and principles.

A blank control and solvent control (94 µL/L) were run in conjunction with 5 test concentrations in a geometric series with a factor of 1.67. Twenty shrimp were exposed at each treatment level and control under flow-through conditions (no replicates). Based on the mean of the analyses on days 0, 2 and 4, the oysters were exposed to measured concentrations of 0.16, 0.26, 0.43, 0.72 and 1.2 mg/L. The dilution water was filtered natural saltwater. Test conditions were: 21.2-22.2 °C; pH 8.2-8.3; 20-22‰; 4.2-5.4 mg O_2/L (55-71% dissolved oxygen saturation), 16 h light/8 h dark.

There were two deviations from the test protocol in the conduct of this study. The first deviation was that the volume additions per day was only 4.4; the protocol specified a minimum of 5 turnovers per day. The second deviation occurred as a result of dissolved oxygen concentrations falling below 60 percent of saturation. Although dissolved oxygen concentration in the dilution water (control) remained greater than 92 percent of saturation during the 96-hour exposure, dissolved oxygen concentrations in the solvent control and all five test concentrations decreased below 60 percent of saturation during the test. The lower dissolved oxygen concentrations in the test concentrations appeared to result from an increased bacterial presence due to the solvent. Test concentrations remained stable following the addition of aeration demonstrating the lack of volatility of the test substance. These protocol deviations were not significant and were not considered by the study author to affect the test results.

The LC50 values were estimated by a computer program (Wheat, 1989) using non-linear interpolation. Confidence limits for LC50 values determined by non-linear interpolation were calculated by binomial probability.

RESULTS

Concentration (mg/L)		*Number of Shrimp*	*Mortality*			
Nominal	*Measured*		*24 h*	*48 h*	*72 h*	*96 h*
Blank control	<0.05	20	0	0	0	0
Solvent control	<0.05	20	0	0	0	0
1.3	1.16	20	0	0	0	0
2.2	2.19	20	0	0	0	0
3.6	3.34	20	2	5	6	13
6.0	5.77	20	20	20	20	20
10.0	10.1	20	20	20	20	20

LC50	4.14 (3.34-5.77) mg/L at 24 hours
(95% confidence interval)	3.87 (3.34-5.77) mg/L at 48 hours
	3.78 (2.19-5.77) mg/L at 72 hours
	3.10 (2.19-5.77) mg/L at 96 hours
NOEC	2.19 mg/L at 96 hours
Remarks - Results	There was no mortality observed in the control solutions. The measured concentrations of the test substance in the test solution were within ± 20% of the nominal concentration for the duration of the test. Dissolved oxygen, pH, salinity and temperature were monitored throughout the test.

Initial dissolved oxygen concentrations in the solvent control and all five test solutions were 4.2 to 5.4 mg/L (55 to 71 percent of saturation). Dissolved oxygen concentrations in the solvent control and the five test solutions decreased over the first 48 hours of the test to between 2.7 and 4.4 mg/L (28 to 58 percent of saturation). Increases in the diluter cycling rate failed to offset reductions in dissolved oxygen concentrations. To prevent possible losses of shrimp due to jumping, gentle aeration was initiated on test day 2 and continued for the remainder of the test.

Dissolved oxygen concentrations remained ~ 3.4 mg/L (~ 45 percent of saturation) in all test solutions during the remainder of the test (possible that low oxygen levels may slow metabolism and therefore lower dose/uptake of the test substance).

The 96-hour LC50 based on mean measured the notified chemical concentrations was 3.10 mg/L with 95 percent confidence limits of 2.19 to 5.77 mg/L (Table 3). The slope of the toxicity curve was 9.3 as calculated by logit analysis. The NOEC was 2.19 mg/L, based upon the lack of mortality and sublethal effects at this concentration.

CONCLUSION The notified chemical is toxic to grass shrimp on an acute basis.

TEST FACILITY Toxikon (1991c)

C.2.4. Algal growth inhibition test

TEST SUBSTANCE Notified chemical (purity not reported)

METHOD OECD TG 201 Alga, Growth Inhibition Test

Species	Green alga (*Selenastrum capricornutum*)
Exposure Period	72 hours
Concentration Range	Nominal: control, solvent control (0.1 mL/L), 0.56, 1.0, 1.8, 3.2, 5.6 and 10 mg/L Mean measured: <0.03, <0.03, 0.38, 0.66, 1.1, 2.1, 3.2 and 6.3 mg/L Concentration of 6-chloro-picolinic acid at 72 hours: <0.07, <0.07, 0.07, 0.1, 0.15, 0.26, 0.45, 0.93.
Auxiliary Solvent	Acetone
Water Hardness	15 mg $CaCO_3$/L
Analytical Monitoring	Liquid chromatography with UV detection
Remarks - Method	After a range finding test, a definitive test in accordance with the guideline above and in compliance with GLP standards and principles. There were no reported deviations to the protocol.

A blank control and solvent control (0.1 mL/L; six replicates) were run in conjunction with 6 test concentrations (triplicate) in a geometric series with a factor of 1.8. Test conditions were: pH 7.5-8.0; 24.3 °C; 8200 Lux.

The results were statistically analysed using one-way analysis of variance and Dunnett's procedure (Dunnett, 1964) to identify significant difference (P=0.05) from the solvent control. The median effect concentration and 95% confidence interval were estimated by linear regression.

RESULTS

Biomass		*Growth*	
E_bC50 *mg/L at 72 h*	*NOEC* *mg/L at 72 h*	*E_rC50* *mg/L at 72 h*	*NOEC* *mg/L at 72 h*
0.92 (0.30-2.8)	0.66	1.7 (0.91-3.1)	0.66

Remarks - Results The test guideline validity criteria were met.

The measured concentration of the test substance ranged from 72% to 88% of nominal at the beginning of the test and declined to 39% to 50% of nominal value after 72 hours. Part of the disappearance was accounted for by the known metabolite 6-CPA, representing from 5% to 10% of the nominal test substance concentration. No attempt was made to confirm the fate of the unaccounted for test substance which may have been

caused by adsorption to glass or further degradation. The results are based on the mean measured concentrations which ranged from 61% to 68% of the nominal value.

The area under the growth curve at the lowest test concentration (0.56 mg/L nominal) was found to be significantly lower than that of the solvent control. However, no statistical difference was obtained for the next test concentration and therefore the result was considered spurious. The no significant (P=0.05) effect concentration (NOEC) for biomass and growth rate was 0.66 mg/L. The lowest concentration at which significant effects (LOEC) was observed for these parameters was 1.1 mg/L. The median effect concentration for biomass (EC_b50) was 0.92 mg/L with a 95% confidence interval of 0.3 to 2.8 mg/L. The median effect concentration for growth rate (E_rC50) was 1.7 mg/L with a 95% confidence level of 0.91 to 3.1 mg/L.

CONCLUSION The notified chemical is toxic to green algae

TEST FACILITY Brixham (1985a)

C.2.5. Acute toxicity to earthworms

TEST SUBSTANCE Notified chemical (technical grade, purity not reported)

METHOD OECD TG 207 (1984); EPA-OPPTS 850.6200
EEC guidelines "C(Ll)4 Toxicity for Earthworms Artificial Soil Test, Revision 6" prepared for Annex V of EEC Directive 79/831 (Ref DG XI/128/82)
OECD Principles of Good Laboratory Practice (revised in 1997), Paris, ENV/MC/CHEM(98) 17.

Species	Earthworms (*Eisenia foetida*), adults >2 months old that had been cultured in pig manure
Exposure Period	15 days
Test levels	0,100, 180, 320, 560 and 1,000 mg/kg dry soil (nominal)
Light intensity	750 lux ± 250 lux
Amount of soil/test vessel	750 g wet weight
Observations	Mortality and behavioural effects after 7 and 15 days were recorded. Each batch of 10 earthworms was weighed just before the test and at termination.
Test soil	70% fine silica sand, 20% kaolinite clay, 10% sedge peat. Calcium carbonate was incorporated into the soil (5 g/kg). Soil moisture: 35%.
Vehicle	Acetone
Statistical analysis	LC50 calculated by logit method (weighted regression of logit percent mortalities on log doses). Simple binomial probability was used where only one dose has non zero or hundred percent mortality logit analysis is unable to produce confidence limits. Percent weight losses were analysed as a one way analysis of variance, with between treatment variations tested against within treatment variation by an F-test.
Remarks – Method	Following a range finding test, earthworms were exposed to the test substance that was evenly incorporated into an artificial soil at 20 ºC ± 2 ºC. Four replicates of 10 earthworms each were used at each test concentration. Solutions of the test substance were prepared in acetone and made up to 25 mL for application to 2.5 kg (dry weight equivalents) of artificial soil. 25 mL of acetone alone was used in the preparation of the control soil. Acetone in the treated soil was allowed to evaporate (1-2 hours) before test.

Chloroacetamide was incorporated into the soil in the same way as the notified chemical to give nominal concentrations of 32, 56 and 100 mg/kg dry soil for toxic standard treatment.

RESULTS

Test concentration (mg/kg dry soil)	*Mortality at Day 7 (%)*	*Mortality at day 15 (%)*	*Weight at day 0 (g/batch)*	*Weight at day 15 (g/batch)*	*Weight change by day 15 (%)*
Solvent control	0	0	5	4.4	-12
100	0	0	4.68	4.05	-13.4
180	0	2.5	4.98	4.14	-16.9
320	12.5	100	4.83	-	-
560	100	100	4.85	-	-
1000	100	100	4.88	-	-

LC50 (95% confidence limits): 209 (180-320) mg/kg dry soil at 15 days

NOEC: Not established

Remarks – Results: The 15 day LC50 value and (95% confidence limits) for 2-chloroacetamide was 91 (56-100) mg/kg dry soil which is consistent with results obtained in tests at this laboratory since 1982, when the standard was first adopted. This indicated that the earthworms were reacting normally under the test conditions (15 day LC50 value 91 mg/kg dry soil).
The 15 day LC50 value was 209 (95% confidence limits 180-320) mg/kg dry soil. Biologically significant effects on weight have been observed at the lowest test level of 100 mg/kg dry soil, although the study author claimed no statistically significant reduction in bodyweight at concentrations up to 180 mg/kg ($p>0.05$) compared to the control. Therefore, the NOEC is <100 mg/kg dry soil and not be established. The differences between the treatments up to 180 mg/kg and the control were not statistically significant ($P>0.05$).

CONCLUSION: The notified chemical is slightly toxic to earthworms on an acute basis.

TEST FACILITY: Brixham (1985b)

C.2.6. Nitrification in soil

TEST SUBSTANCE: Notified chemical (N-Serve formulation)

METHOD: In house

Remarks – Method: The effects of the test substance on the nitrification in soil was determined in 6 soils collected from surface (0-15 cm) samples in Iowa selected to obtain a range in pH (5.1-8.1), texture (9-60% sand, 12-49% silt, 20-42% clay), organic carbon content (1.2-4.2%) and $CaCO_3$ equivalent (0%-4.1%). The soil profile details are provided in Table 22. 20 g of soil were treated with water containing 4 g of nitrogen as $(NH_4)_2SO_4$ or urea and different amounts of the test substance and stored at 20 ºC, 25 ºC and 30 ºC for up to 42 days. After incubation each sample was analysed for NH_4-N, NO_3-N and NO_2-N. Each test concentration/incubation combination was performed in triplicate. A control was established in order to calculate the percent inhibition of the test substance.

RESULTS

Table 22 Characteristics of soils

Soil	*pH*	*Organic Carbon (%)*	*Total Nitrogen (g/kg)*	*Sand/Silt/Clay (%)*	*$CaCO_3$ equivalent (g/kg)*
Harps	7.7	4.2	5.0	9/49/42	41
Okoboji	6.1	2.9	2.2	19/47/34	0
Webster	6.1	3.3	2.4	31/39/30	0
Nicollet	5.1	2.1	1.8	39/31/30	0
Clarion	6.0	1.4	1.3	43/37/20	0
Storden	8.1	1.2	0.5	60/12/28	15

Table 23 Percent (%) inhibition on nitrification in soils of the notified chemical incubated at 25 ºC

Concentration	*Harps*		*Okoboji*		*Webster*		*Nicollet*		*Clarion*		*Storden*	
(mg/kg soil)	*21 d*	*42 d*	*21 d*	*42 d*	*21 d*	*42 d*	*21 d*	*42 d*	*21 d*	*42 d*	*21 d*	*42 d*
0.1	0	0	5	0	10	0	15	0	13	0	3	0
0.5	18	0	11	2	22	20	24	10	40	17	79	14
1.0	24	0	22	20	46	30	27	13	50	35	82	43
5.0	71	21	47	39	68	55	56	49	88	82	98	94
10	66	48	59	50	72	48	72	58	88	82	98	94

Table 24 Influence of temperature on the percent (%) inhibition on nitrification in soils of the notified chemical 21 days after treatment

Concentration	*Harps*			*Webster*			*Storden*		
(mg/kg soil)	*20 ºC*	*25 ºC*	*30 ºC*	*20 ºC*	*25 ºC*	*30 ºC*	*20 ºC*	*25 ºC*	*30 ºC*
1.0	37	24	0	57	46	6	87	82	13
5.0	71	68	42	76	72	34	97	95	83

Remarks – Results

The results in Table 23 show that inhibition of nitrification in soils may be correlated with organic carbon in soils with increasing inhibition observed with decreasing organic carbon content. For example, the Harps soil with a 4.2% organic carbon (%oc) showed greater than 25% inhibition of nitrification only at the highest test concentration of 10 mg/kg soil after 42 days. This compares with soils of ≤2.1%oc where the test substance at concentrations of 1 mg/kg soil and above show greater than 25% inhibition of nitrification after 42 days.

The results in Table 24 show that the effect of the test substance on the inhibition of nitrification in soils is dependent on temperature with decreased inhibition observed with increased temperature. Thus, the inhibition of nitrification is greater than 46% after 21 days at 25 ºC in soils with ≤2.1%oc, whereas nitrification is less than 13% after 21 days in the same soils at 30 ºC.

CONCLUSION

The notified chemical is not expected to have a long-term effect on nitrogen transformation in soils at concentrations at or below 0.5 mg/kg soil.

TEST FACILITY

McCarty and Bremner (1990)

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