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May 2008

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

4,4'-methylenebis[3-chloro-2,6-diethylbenzenamine] ("Lonzacure M-CDEA")

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

For the purposes of subsection 78(1) of the Act, this Full Public Report may be inspected at our NICNAS office by appointment only at 334-336 Illawarra Road, Marrickville NSW 2204.

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

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Director
NICNAS

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FULL PUBLIC REPORT

4,4'-methylenebis[3-chloro-2,6-diethylbenzenamine] ("Lonzacure M-CDEA")

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
International Sales and Marketing Pty Ltd (ABN 36 467 259 314) of 262 Highett Road, Highett, VIC 3190
and
Rema Tip Top Industrial Australia Pty Ltd (ABN 92 110 697 624) of Unit 9-10, 332-550 Edgar St, Bankstown NSW 2200

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:
Purity, Hazardous Impurities, Identity of recipients, Import volume and Use details.

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

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
EU, Canada, US, Philippines

2. IDENTITY OF CHEMICAL

CHEMICAL NAME
Benzenamine, 4,4'-methylenebis[3-chloro-2,6-diethyl-

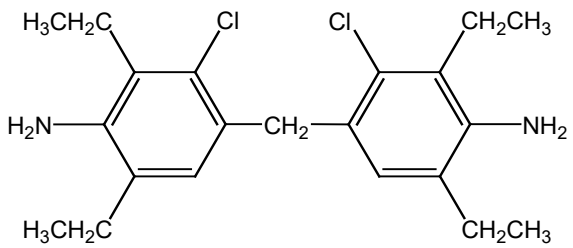
OTHER NAME(S)
4,4'-Methylene-bis(3-chloro-2,6-diethylaniline)
Bis(4-amino-2-chloro-3,5-diethylphenyl)methane
M-CDEA

CAS NUMBER
106246-33-7

MARKETING NAME(S)
Lonzacure M-CDEA
Luvocure MC-FP
P5367

MOLECULAR FORMULA
$C_{21}H_{28}Cl_2N_2$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
379 Da

ANALYTICAL DATA
Reference ^{1}H-NMR, FT-IR, GC, and UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY >97%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20ºC AND 101.3 kPa
Off-white, crystalline powder

Property	Value	Data Source/Justification
Melting Point	89-90.4ºC	Measured
Boiling Point	>300ºC at 101.3 kPa	Measured
Density	1244 kg/m^3 at 22ºC	Measured
Vapour Pressure	≤0.013 kPa at 81.7ºC	Measured
Water Solubility	0.000025 g/L at 20ºC	Measured
Fat Solubility	165 g/kg standard fat at 37ºC	Measured
Hydrolysis as a Function of pH	Not determined	See Appendix A
Partition Coefficient (n-octanol/water)	log P_{ow} = 6.3 at (22 ± 1)ºC	Measured
Surface Tension	72.32 mN/m	Measured
Adsorption/Desorption	log K_{oc} = 5.79	Estimated
Dissociation Constant	pKa = 3.2 to 3.6	Estimated
Particle Size	Inhalable fraction (<100 µm): ~62% Respirable fraction (<10 µm): 8.55% MMAD* = ~91.5 µm	Measured
Flash Point	Not determined	Low vapour pressure solid
Flammability	Not highly flammable	Measured
Autoignition Temperature	>(90 ± 2)ºC	Measured
Explosive Properties	Not explosive	Measured
Oxidising properties	Not oxidising	Measured

* MMAD = Mass Median Aerodynamic Diameter

DISCUSSION OF PROPERTIES
The notified chemical is water-insoluble, but soluble in fat. It is unlikely to be significantly ionised in the normal environmental pH range. It is comprised of a high proportion of inspirable particles (<100 µm), and contains a smaller proportion of respirable particles (<10 µm). For full details of tests on physical and chemical properties, please refer to Appendix A.

Reactivity
The notified chemical is non-volatile, non-oxidising, non-flammable and not explosive. It is therefore expected to be stable under normal ambient conditions.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be imported by sea, as both a pure compound (imported in both powdered and pelleted form) and as a formulated liquid product (<15% notified chemical).

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	1-10	1-10	1-10	1-10	1-10

PORT OF ENTRY
The notified chemical will be imported through the ports of Melbourne and Sydney.

IDENTITY OF RECIPIENTS
Industrial formulators and manufacturers in the eastern states of Australia.

TRANSPORTATION AND PACKAGING
The solid notified chemical will be imported in 25 kg plastic-lined paper bags, palleted in mixed containers. These will be transported by truck between the port and the warehouse, and between the warehouse and the site of elastomer manufacturing.

The liquid product containing the notified chemical is a Dangerous Good (Class 8 Corrosive) according to the ADG6 (FORS, 1998), due to the formulation. It will be imported in palleted 200 L drums, and these will be transported between the port and the coatings formulation site by Dangerous Goods-licensed trucks.

USE
The notified chemical is a curing agent for polyurethane/polyurea elastomers and coatings. The imported pure compound will be combined with reactive polyurethane systems for the manufacture of moulded, solid elastomeric components. The imported liquid product, containing the notified chemical, will be used for the formulation of sprayed elastomer coatings for high-impact applications in the mining, manufacturing, and building and construction industries (e.g. coatings on hoppers, silos, truck beds, railway wagons, or dredging shovels).

OPERATION DESCRIPTION
Manufacture of solid elastomers
The content of individual bags of notified chemical will be manually transferred from the original packaging to an agitated storage hopper of a polyurethane dispensing machine (at ambient temperature). The hopper will then be closed and sealed, blanketed with dry nitrogen, and agitated and heated to 100ºC (at which temperature the notified chemical is molten). Within the machine, the notified chemical will be dispensed automatically, mixed with a portion of reactive polyurethane pre-polymer from a second pre-heated feed line, and added to heated moulds. Once the mixture has cured into a solid elastomer, the moulded component will be manually removed from the mould and placed in an oven at ~130ºC for 16 to 48 hours to post-cure. After this time, the moulded item is ready to be packaged and sold.

The polyurethane dispensing machine does not require regular cleaning.

Laboratory technicians will also carry out in-house product development work. Typically, this will involve heating the solid notified chemical in a laboratory fume hood or in open air, then mixing it with a polyurethane prepolymer and casting it into heated moulds. These will be allowed to cool, then the moulds will be opened and the cast components will be post-cured in an oven.

End use of elastomeric coating formulations
The spraying of coatings will be carried out using manual spray equipment, applied by an operator. The liquid formulation containing the notified chemical will be mixed with a second component as it passes through the high-pressure spray equipment. The spray will be applied to metal surfaces, where it will cure within seconds at ambient temperature into an elastic material on the surface. Due to the reactivity of the two components, a post-curing process will not be required.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

NUMBER AND CATEGORY OF WORKERS

Category of Worker	*Number*	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Warehouse staff	3	4	30
Transport contractors	8	4	30
Laboratory technicians	2	1	30
End users (manufacturing and application)	20	6	100

EXPOSURE DETAILS

Manufacture of solid elastomers

The highest potential for exposure of manufacturing workers to the notified chemical would be during the addition of pure chemical (≥97%) to the agitated hopper. This activity has also been claimed to cause the highest worker exposure during the use of structurally related dianilines as curing agents (in similar applications to those proposed for the notified chemical), and these exposures have been well studied (ATSDR, 1994; RoC, 2005). Exposures of 4,4'-methylenebis(2-chloroaniline) (MBOCA; CAS 104-14-4) have been evaluated by monitoring the urine of exposed workers, in which concentrations as high as 49 mg/L have been recorded, although values ranging from <0.5 to 100 µg/L are more common. These values have been recorded even when the primary route of exposure was dermal and workers wore gloves during handling.

Indirect exposure to the notified chemical also should be considered. MBOCA levels of 0-15 µg/L have been recorded in the urine of family members of workers, who had no direct contact with it (ATSDR, 1994), suggesting that exposure to the notified chemical may occur even when the notified chemical is not directly handled. The potential for indirect exposure is likely to be highest where the powdered notified chemical is used, but while a likely exposure level cannot be estimated, it is likely to be lower than that experienced by workers who are directly handling the notified chemical.

Given that the notified chemical may be imported as a powder that contains respirable particles, its addition to the hopper may result in direct inhalation exposure of workers. Given dry manipulation of non-aggregating inspirable particles, EASE exposure modelling predicts that workers may be exposed to a worst-case airborne concentration of particulates of 2-5 mg/m^3 in the presence of LEV (EC, 2003). Given a 70 kg adult male worker (inhalation rate of 62 m^3/day with medium activity) exposed to dusts of the notified chemical for 6 hours/day, and 100% inhalation absorption (EC, 2003), a worst-case estimate of worker inhalation exposure to the notified chemical is calculated to be 1.1 mg/kg bw/day in the presence of LEV.

Vapours of the notified chemical may potentially result from the heating process applied; however, the vapour pressure of the notified chemical is low (measured near the processing temperature), and the heated chamber is described as "sealed" by the notifier.

Direct dermal and ocular exposure to the notified chemical may also occur during its addition to the hopper. Both of these routes of exposure may be increased by the presence of airborne particulates. Dermal exposure has been described as "the most important occupational exposure pathway" for related dianiline curing agents (ATSDR, 1994). The estimated reasonable worst case and typical case dermal exposure is 3000 mg and 900 mg respectively using measured data for the exposure scenario 'dumping of powders in a formulation facility' (EC, 2003). Therefore, for a 70 kg worker and a 100% dermal absorption, reasonable worst-case and typical case dermal exposure is estimated to be 42.9 mg/kg bw/day and 12.9 mg/kg bw/day respectively. Dermal absorption is likely to be less than 100% (see section 6.2).

Therefore, excluding oral exposure, a typical combined-route exposure during the handling of powdered notified chemical is estimated as 14 mg/kg bw/day. Worst-case exposures could be 44 mg/kg bw/day.

It is claimed that workers will wear personal protective equipment (PPE) to reduce their exposure during addition of the notified chemical to the hopper, including protective clothing, chemical resistant gloves, dust mask and chemical resistant goggles. The use of LEV in the vicinity of the polyurethane dispensing machine has been described, and this was inherent into the assumptions used above to estimate exposure. Assuming appropriate clothing and gloves (assume 90% protection), and provided that the dust mask used is properly fitted and adequate for respirable-sized particles (assume 10% protection), an exposure range of 2.3 - 5.3 mg/kg bw/day is calculated (EC, 2003).

The notifier has also advised that the imported pellets of the notified polymer are not friable, and so the level of airborne particulates and exposure to the notified chemical are expected to be lower when these are used, relative to the handling of powder.

Manufacturing workers will also experience dermal exposure to finished solid elastomeric components. Prior to post-curing, limited dermal exposure is possible during manual removal of partly cured solid components from their moulds. At this stage, although the majority of the notified chemical would have undergone reaction with the polymeric matrix, there may still be unreacted chemical remaining. The notifier has advised that these processes will be carried out in ventilated areas and that the workers involved will wear PPE including coveralls, boots, heat-resistant gloves and either a half-mask respirator and face shield, full-face respirator, or air-fed full-face mask and hood.

After post-cure, the notified chemical is expected to be fully covalently bound into a cured polymeric matrix and be unavailable to cause exposure.

Laboratory workers will likely experience similar types of exposures to manufacturing workers, but on a smaller scale and for shorter time periods. These workers are reported to wear high-temperature PPE, including coveralls, full foot protection, insulating gloves, face shield and half-face respirator (if necessary).

End use of elastomeric coating formulations

During spray application of two-part coating formulations containing the notified chemical (at <15%), workers will likely experience dermal, ocular, and inhalation exposure to sprayed droplets containing the notified chemical. Further dermal and ocular exposure may occur during cleaning and maintenance of high-pressure spray equipment.

Dermal exposures to spray paints while painting large areas (without the use of LEV) have been measured, with typical exposures of 3 mg/cm^2/scenario and worst-case exposures of 12 mg/cm^2/scenario (EC, 2003). Given an exposure frequency of five times daily to a concentration of 7.5% notified chemical, 100% dermal absorption and the equivalent of a two-hand exposure (840 cm^2) to a 70 kg adult, a typical dermal exposure of 13.5 mg/kg bw/day is estimated. Worst-case exposures (also assuming five times daily) may be as high as 54 mg/kg bw/day. Assuming the use of gloves and protective clothing (90% protection), a dermal exposure estimate of 1.4 – 5.4 mg/kg bw/day is calculated (EC, 2003). Spray application is expected to be carried out in a downdraft spray booth (or similar) under most circumstances. Performing spraying tasks in ventilated spray booths leads to a significant reduction in dermal exposure to paint overspray. Exposures resulting during cleaning of spray guns and equipment cannot be calculated, but are likely to be small due to the use of automated cleaning apparatus and dilution with solvent(s).

It is not currently possible to estimate the possible inhalation exposure to the notified chemical during the application of spray paints using EASE modelling (EC, 2003). Given the hazard of the sprayed components (e.g. isocyanates and corrosive components), spray application is expected to be carried out in a downdraft spray booth (or similar) under most circumstances. Only professional spray painters will use it in an industrial setting, and so these workers are expected to wear PPE including protective clothing and boots, chemical resistant gloves and goggles. Where engineering controls are not able to be used (e.g. in outdoor or in large applications) or are not effective to remove airborne droplets, suitable respirators (including the use of air-fed hoods) are expected to be utilised. The majority of these measures are described in the MSDS for the imported formulation, apart from the requirement for use in a spray booth. These measures, if implemented correctly, are expected to prevent most inhalation exposure to spray paints containing the notified chemical.

As the sprayed coatings cure rapidly upon surfaces (within seconds at ambient temperatures), dermal exposure to the notified chemical from coated surfaces is expected to be negligible. The notified chemical will be covalently bound into the cured polymeric matrix of the coating.

6.1.2. Public exposure

The notified chemical is intended for industrial use only, and will not be available to the public. Direct exposure would therefore not be expected. Indirect exposure from accidental spills or environmental sources may be possible, but are unlikely for the proposed use.

Members of the public may experience dermal exposure to moulded polyurethane components or cured coatings containing the notified chemical, but in these the notified chemical is expected to be covalently bound into a cured polymeric matrix and hence should be unavailable to cause exposure.

It has been reported that residual levels of MBOCA, a related dianiline curing agent, may remain in plastics and polyurethane foams that have been manufactured with it (RoC, 2005). However, there is no data available to describe any actual residual levels or any potential for public exposure. Residual levels of MBOCA in plastic articles are thought unlikely to result in significant public exposure (ATSDR, 1994). The same should be true for the notified chemical in equivalent applications.

6.2. Human health effects assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD_{50} >5000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD_{50} >2000 mg/kg bw; low toxicity
Rabbit, skin irritation	Non-irritating
Rabbit, eye irritation	Slightly irritating
Guinea pig, skin sensitisation – adjuvant test	No evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days	NOEL = 36 mg/kg bw/day (males) NOEL = 40 mg/kg bw/day (females)
E. coli, bacterial reverse mutation	Non-mutagenic
S. typhimurium bacterial reverse mutation	Non-mutagenic
In vitro chromosome aberration study (CHL cells)	Non-clastogenic
In vitro chromosome aberration study (human lymphocytes)	Non-clastogenic
In vivo/in vitro rat primary hepatocyte UDS assay	Non-mutagenic
In vivo mouse micronucleus test	Non-clastogenic

Toxicokinetics, metabolism and distribution

Absorption of the notified chemical across biological membranes is likely to be limited; while its molecular weight is favourable, its low water solubility and high $logP_{ow}$ are not (EC, 2003). Absorption from the gastrointestinal tract may be aided by micellar solubilisation of fat-soluble components. There is evidence supporting these predictions in the toxicological studies that have been conducted on the notified chemical. In particular, although different species may play some role, higher toxicity was evident after intraperitoneal injection of the notified chemical in the micronucleus test (by-passing absorption processes) than was seen in the acute oral and dermal toxicity studies. Some gastrointestinal absorption of the notified chemical must have occurred to cause the liver effects observed in the repeated dose oral toxicity study, although as this occurred only at the higher dose levels it is unclear to what extent absorption actually occurred. Given its lipophilicity and low molecular weight, the notified chemical would be expected to be absorbed across the lungs following inhalation of solids or vapours.

A structurally related dianiline, 4,4'-methylenebis(2-chloroaniline) (MBOCA; CAS 104-14-4) has been shown to be rapidly absorbed across skin and other biological membranes in both humans and various experimental models (HSDB, 2008). It should be noted that the MBOCA's $logP_{ow}$ of 3.91 is lower than that of the notified chemical, yet it is the outer limit of predicted favourable absorption. An application of dry MBOCA is rapidly and progressively absorbed across human skin models. However, it should be noted that the majority (~90%) of a dermal exposure of radiolabelled MBOCA in beagle dogs was found to remain in skin at the application site after 24 hours, suggesting a rapid uptake into the stratum corneum but slower percutaneous absorption. It is expected that the notified chemical would also be taken up into the stratum corneum, but its percutaneous absorption is likely to be slower than that of MBOCA, due to its greater lipophilicity.

As systemic effects were observed in toxicity studies on the notified chemical (emaciation and body weight loss), and from the tissues affected (e.g. adrenals and kidney), it is apparent that it is able to distribute to at least some tissues after absorption. MBOCA was found to distribute systemically after intravenous administration with the highest concentrations in the small intestine, liver, adipose, lung, kidney, skin, and adrenals (HSDB, 2008). The distribution of a 2,6-dialkyl-substituted dianiline, 4,4'-methylenebis(2-ethyl-6-methylaniline) (MMEA; CAS 19900-72-2), 20 hours after an oral dose, has been shown to be similar to that of MBOCA, except for MMEA's much lower levels in lung (Kugler-Steigmeier *et al*, 1989). MMEA perhaps more closely resembles the notified chemical in lipophilicity and reactivity than does MBOCA. It is noted that the distribution of MBOCA and MMEA includes all of the sites for which toxicity was observed in the repeat dose toxicity study on the notified chemical.

In addition, studies using radiolabelled MBOCA have shown that MBOCA (and/or its metabolites) accumulates in the liver after either acute or intermediate oral administration, with levels increasing more than 100-fold after 28 doses (HSDB, 2008). It is presumed that this accumulation is due to adduct formation (see below).

By analogy with MBOCA, the probable metabolic pathways for the notified chemical will be primarily oxidative (HSDB, 2008). *N*- and *o*-oxidation of MBOCA has been observed, but *o*-oxidation of the notified chemical would not be expected due to its substitution. Subsequent conjugation with sulfate and glucuronate would be expected. By the same analogy, a smaller degree of *N*-acetylation of the notified chemical would be expected.

Following metabolism of the notified chemical, it would be expected to be largely excreted into the urine. MBOCA and its metabolites are largely excreted in the urine and bile of both experimental animals and exposed humans (HSDB, 2008). MMEA was also found to be largely excreted in the urine, with 8% of an oral dose being excreted after 4 hours (Kugler-Steigmeier *et al*, 1989). The majority of urinary MBOCA is conjugated with sulfate or glucuronate (2-3 fold over unconjugated MBOCA levels), and a smaller proportion (<10% unconjugated MBOCA level) is acetylated (HSDB, 2008). After accidental dermal exposure of human workers to MBOCA, high levels were detected in the urine, and from this, the biological half-life of MBOCA was estimated in humans to be approximately 23 hours. Given the more lipophilic nature of the notified chemical, any that was absorbed might be hypothesised to have a longer half-life.

Acute toxicity, irritation and sensitisation

The notified chemical displayed low acute oral and dermal toxicity, with few signs of toxicity in the majority of animals. However, it is uncertain as to how much absorption actually occurred. Although mice might be expected to have a lower LD_{50} than rats (by analogy with MBOCA (RTECS, 2008)), the higher toxicity observed in mice following intraperitoneal injection (in the micronucleus test) at equivalent doses to those in rats in the oral acute study was suggestive of increased toxicity resulting from bypassing gastrointestinal absorption.

No data for the inhalation toxicity of the notified chemical has been provided. No analogue data is available. Given its low oral bioavailability, it might also be expected to display low acute inhalation toxicity (EC, 2003).

Application of dried notified chemical was not irritating to rabbit skin. The notified chemical was only slightly irritating to the rabbit eye (and this may have been a mechanical, abrasive effect). It was also not found to be a skin sensitiser in a guinea pig maximisation study.

Repeated dose toxicity (sub acute)

In the main, the notified chemical did not cause severe effects when dosed to rats in feed at up to 146 mg/kg bw/day in the 28-day repeated dose toxicity study. Treatment-related effects included hypertrophic changes in the liver, increased adrenal weights (without histopathological correlates), and increased platelet counts. The NOEL for these effects was 36 mg/kg bw/day (males) and 40 mg/kg bw/day (females). As the study did not include a recovery group, it is unknown if these effects were reversible. The hypertrophic changes in the liver cannot be presumed to be adaptive, due to their unknown reversibility. No other evidence of liver dysfunction was reported.

From the dose range-finding study, dosing at 1000 mg/kg bw/day for 14 days showed a more severe presentation of toxicity, including emaciation and death (50% had died after 14 days). Animals treated with 1000 mg/kg bw/day also showed stomach haemorrhages and bloody content of the urinary bladder.

None of these effects were sufficiently severe to classify the notified chemical as hazardous according to the NOHSC *Approved Criteria* (NOHSC, 2004).

Dianilines are potential retinotoxic agents and potential reproductive toxicants by analogy to MDA (US EPA, 1995). Histopathological examination of the eyes and reproductive organs was not carried out in the 28-day repeat dose toxicity study on the notified chemical.

Genotoxicity and carcinogenicity

Dianilines that are structurally related to the notified chemical have been classified as “reasonably anticipated to be human carcinogens” (RoC, 2005). Both 4,4’-methylenedianiline (MDA; CAS 101-77-9 and 13552-44-8) and MBOCA have been included in this group, and have both been investigated in multiple human and animal studies. These chemicals are positive in bacterial reverse mutation studies (with metabolic activation), UDS studies (*in vitro* and *in vivo*), and *in vitro* sister chromatid exchange studies (HSDB, 2008; RTECS, 2008). Studies on MBOCA have shown that, after metabolic activation (by *N*-oxidation) it is able to form adducts with DNA and other cellular structures—primarily in the liver, lung and urinary bladder wall (HSDB, 2008). Metabolic conjugation or acetylation abolishes this activity. In many chronic toxicity/carcinogenicity studies (88 weeks to 2 years in rats), MBOCA has induced hepatocellular, pulmonary, urinary bladder, mammary gland and other tumours.

A published study of various derivatives of MDA showed that alkyl substitutions *ortho*- to the amino group on both rings of the dianiline abolish its mutagenic activity in a bacterial reverse mutation study (Rao *et al*, 1982). This included a 2,6-diethyl substitution (like that of the notified chemical), and so would suggest that the notified chemical is less likely to be mutagenic as a result of its 2,6-diethyl substitution.

However, another published study contradicts this conclusion, using MBOCA and MMEA, the latter of which is 2,6-dialkyl-substituted like the notified chemical (Kugler-Steigmeier *et al*, 1989). In this study, these two chemicals were first tested in a modified bacterial reverse mutation study using *S. typhimurium* strains TA100 and TA98 with 20% liver homogenate in the S9 mix (slightly higher than that used to test the notified chemical).

MMEA showed a very weak mutagenic activity (relative to MBOCA), and only in TA100. Then, a wing-spot mutation test was carried out using *Drosophila*, in which MBOCA was positive but MMEA was negative. Finally, to determine their levels of *in vivo* DNA adduct formation, radiolabelled versions of the two chemicals were dosed by gavage into rats (~30 mg/kg bw). After 20 hours, their lung and liver DNA was purified and the radioactivity counted. In the liver DNA of MMEA-treated animals, 98% of the radioactivity was covalently bound to DNA (c.f. 75-87% for MBOCA). When converted to a specific activity of covalent adduct formation, MMEA was of equivalent activity to MBOCA in liver, but in lung MBOCA produced 30-50 times the adduct level of MMEA. This suggested that the *in vitro* test methods are unable to detect DNA binding by the 2,6-dialkyl-substituted dianilines, and that their DNA adduct forming capability may in fact cause mutations *in vivo*.

In contrast to study outcomes for MDA and MBOCA, five different studies on the genotoxicity of the notified chemical have all shown a negative outcome. Only two of these are assays that determine mutagenicity (the expected mode of action for the notified chemical), and neither is a stringent *in vivo* approach. For new dianilines, like the notified chemical, the US EPA considers a 2-year carcinogenicity bioassay in rats and mice to be the most appropriate test (US EPA, 1995).

It is unknown if the notified chemical would induce tumour development with longer durations of treatment. It does appear, however, that while its DNA adduct-forming ability in liver is likely to be high (by analogy with MMEA), it may be low in other tissues. This tissue-specific phenomenon is unexplained for MMEA, and the possibility that the notified chemical may be similar is an alternative interpretation for the absence of mutagenic activity in the two available studies (although one was performed on primary hepatocytes). Given the known properties of structurally related chemicals and the limited extent of available test data, the possibility that the notified chemical might be mutagenic and/or carcinogenic cannot be excluded.

Classification
Based on the available data, the notified chemical cannot be classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

6.3. Human health risk characterisation

6.3.1. Occupational health and safety

General description of risk
In general, the notified chemical was not found to be hazardous in any of the toxicological investigations that were available. The changes observed in the 28-day repeated dose study were, in general, not severe. However, the findings available for structurally related chemicals cast some doubt on the veracity of the available mutagenicity studies. It is quite possible that the hypertrophic changes observed in the 28-day study (which could not be considered to be adaptive in the absence of data demonstrating their reversibility) may present with greater severity after longer treatment periods. Therefore, in order to determine the likely risk of the notified chemical, the NOELs available from the 28-day study are considered appropriate in the absence of test data from studies of longer duration.

Manufacture of solid elastomers
Manufacturing workers involved in the addition of powdered notified chemical (≥97%) to the agitated hopper would experience the greatest exposure to the notified chemical, and therefore the greatest risk. However, given the potential for indirect exposure, other manufacturing workers may also experience some unknown but smaller risk.

Given the combined typical and worst-case exposure estimates of 14 and 44 mg/kg bw/day and the NOEL for the changes observed in the 28-day study of 36 mg/kg bw/day (for males), a margin of exposure (MOE) range of 0.8-2.6 is calculated. These MOE values indicate that the notified chemical, used as proposed, may present an unreasonable risk (demonstrated in an MOE <100), and that additional exposure control measures will be required during the handling of powdered notified chemical.

The notifier has indicated that workers will wear personal protective equipment (PPE) to reduce their exposure during addition of the notified chemical to the hopper, including protective clothing, chemical resistant gloves, dust mask and chemical-resistant goggles. Assuming appropriate clothing and gloves, and provided a dust mask adequate for respirable-sized particles is used, an MOE range of 6.8-15.6 is calculated. This indicates that even with the minimum PPE required, the notified chemical may still pose an unreasonable risk. Therefore, additional measures such as the use of a full-face respirator/hood or the use of pelleted forms of the notified chemical should be implemented.

The notifier has also advised that the imported pellets of the notified polymer are not friable, and so the level of airborne particulates and inhalation exposure to the notified chemical is expected to be lower when these are used, relative to the handling of powder. For MBOCA, the use of fused, hardened pellets was shown to

significantly reduce worker exposures, as measured by monitoring urine samples (ATSDR, 1994).

Given the likelihood of indirect exposure, adequate industrial hygiene practices (such as segregation of tasks, showering after work shifts, changing of attire) should be implemented to prevent exposure of workers who are not handling the notified chemical, and potentially the families of workers. As percutaneous absorption is expected to be slow this will also limit dermal exposure.

End use of elastomeric coating formulations

During spray application of two-part coating formulations containing the notified chemical (at <15%), workers will likely experience dermal, ocular, and inhalation exposure to sprayed droplets containing the notified chemical. Assuming the appropriate use of downdraft spray booths and/or respiratory protection (necessary from the known hazards of the paint formulations sprayed), the extent of respiratory exposure is assumed to be minor, in comparison to the potential for dermal exposure.

Dermal exposures of 1.4-5.4 mg/kg bw/day are estimated (five applications/day, without LEV, assuming the use of gloves and protective clothing), and compared with the NOEL of 36 mg/kg bw/day (for males), an MOE range of 6.6-26 is calculated. These MOE values indicate that the notified chemical, used in spray painting at the upper proposed concentration, may present an unreasonable risk, and that additional engineering control measures will be required. Spray application should be carried out in a downdraft spray booth where practicable. Poor technique and/or unfavourable use scenarios may result in an unreasonable risk from dermal exposure.

It should be noted that the paint formulations will contain more hazardous components than the notified chemical (e.g. corrosives or sensitisers), and so it is likely that workers will use the appropriate controls to prevent exposure.

6.3.2. Public health

The notified chemical is intended for industrial use only, and will not be available to the public. Therefore, the risk from the imported forms of the notified chemical is negligible in the absence of accidental spills or environmental sources.

Members of the public may experience dermal exposure to moulded polyurethane components or cured coatings containing the notified chemical. The notified chemical is expected to be reacted during the curing of these, and to become covalently bound within a polymeric matrix. Although small quantities of residual, unreacted chemical may be present, at the present state of knowledge these are thought to be unlikely to become available from cured materials. Therefore, the notified chemical is not expected to pose an unreasonable risk to public health.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE

Both the notified chemical and the blended product containing the notified chemical as a component will not be manufactured in Australia. Local operations will include transport, storage and reformulation. Release to the environment during shipping, transport and warehousing will only occur in the unlikely event of accidental spills or leaks from the packaging.

Release of the notified chemical from the elastomer manufacturing sites is not expected. The reaction of the notified chemical with a second component of the polyurethane system will occur at 100ºC within a sealed system, and so release of the notified chemical via evaporation during reaction is not expected (melting point of ~90ºC). Release of the blended product from the site is not expected, as the product will be used in a closed system.

RELEASE OF CHEMICAL FROM USE

Release of the notified chemical and blended product from use is not expected, as the notified chemical will be consumed in reaction, forming a solid elastomer product.

RELEASE OF CHEMICAL FROM DISPOSAL

Residues of the notified chemical in the import packaging are unlikely, as the powder form of the chemical will be readily emptied. Possible wastage is estimated at 0.04% of the import volume (4 kg per annum). It is

expected that the packaging containers will normally be disposed of to landfill.

For the blended product, about 0.5% will remain in emptied drums (50 kg notified chemical per annum). The drums will be disposed of according to local regulations (e.g. by incineration), in which case the residual notified chemical in the drums will not be expected to be released to environment.

7.1.2 Environmental fate

Both the notified chemical and blended product will be intended for industrial use only. After reaction to form coatings or solid elastomers, all components including the notified chemical will become inert. The notified chemical cannot be classed as ready biodegradable in this form.

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

7.1.3 Predicted Environmental Concentration (PEC)

No significant concentrations of the notified chemical are expected in the aquatic environment based on the limited possibility for release and the low water solubility of the notified chemical. The PEC for the notified chemical has therefore not been calculated.

7.2. Environmental effects assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	LC50 >1 mg/L	Test on concentrations higher than 5 mg/L could not be conducted due to the low water solubility of the notified chemical. No effects were observed at suspensions of 1 mg/L. Mortality observed with suspensions of 5 mg/L was considered as physical effects based on the formation of needles of the notified chemical.
Daphnia Toxicity	EC50 >0.1 mg/L	The notified chemical has no effect on the mobility of *Daphnia magna* at tested dosage <0.1 mg/L (i.e. up to the limit of solubility).
Algal Toxicity	EbC50 (72 h) > 7.4 mg/L ErC50 (0-72 h) > 7.4 mg/L NOEL < 7.4 mg/L	The notified chemical is at worst toxic to algae.
Inhibition of Bacterial Respiration	EC50 > 10 mg/L	Notified chemical is at worst harmful.

The ecotoxicology tests indicate that at concentrations below the solubility, the notified chemical has no observable effect on the aquatic environment.

7.2.1 Predicted No-Effect Concentration

The notified chemical will be converted into part of polyurethane elastomers at the production site and will not be released to environment in any significant quantity. On this basis, the calculation of a PNEC value has not been considered necessary.

7.3. Environmental risk assessment

The notified chemical will be converted into part of polyurethane elastomers at the production site and will not be released to environment in any significant quantity. On this basis, the environmental risk of the notified chemical is not considered to be unacceptable.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available data, the notified chemical cannot be classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

and

As a comparison only, the classification of the notified chemical using the Globally Harmonised System for the Classification and Labelling of Chemicals (GHS) (United Nations, 2003) is presented below. This system is not mandated in Australia and carries no legal status but is presented for information purposes.

	Hazard category	***Hazard statement***
Environment	Acute Category 2	Toxic to aquatic life

Human health risk assessment
Under the conditions of the occupational settings described, the notified chemical may pose an unacceptable risk to the health of workers. Appropriate protective equipment and appropriate engineering controls, minimising the potential for exposure, are required for the risk to workers to not be considered unacceptable.

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

Environmental risk assessment
The notified chemical is not considered to pose a risk to the environment based on its reported use pattern and low potential for exposure of aquatic organisms.

Recommendations

REGULATORY CONTROLS
Material Safety Data Sheet

- The MSDS for the powdered notified chemical provided by the notifier should be amended as follows:
  - *Include recommendations to avoid inhalation, skin and eye exposure.*
  - *Respiratory protection should also be recommended where powders are handled directly, including local exhaust ventilation and/or the use of appropriate respirators.*
  - *Include the following statement (or similar) in section 11:*
    Chemicals similar in structure have been found to cause oncogenicity in laboratory test animals.

CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following engineering controls to minimise occupational exposure to the notified chemical as introduced:
  - *Local exhaust ventilation should be applied at all sites where powdered notified chemical is handled.*
  - *Dust-free formulations (e.g. pelleted notified chemical) should be used wherever possible.*

- Employers should implement the following engineering controls to minimise occupational exposure to the notified chemical during spray painting operations:
  - *Spray painting should, wherever practicable, be carried out in a well-maintained downdraft (or equivalent) spray booth.*
  - *Adequate local and general ventilation should be available at the site.*

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the imported powdered notified chemical:
  - *Avoid the generation of airborne dusts.*

- *Maintain a good standard of cleanliness around hoppers and sites where the notified chemical is handled.*
- *Workers' tasks should be segregated to avoid indirect exposure of workers who do not handle the notified chemical.*
- *Workers should shower after work shifts and change their attire.*

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical in products intended for spray application:
  - *Spray painting should be carried out according to the NOHSC* National Guidance Material For Spray Painting *(NOHSC, 1999).*

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the powdered notified chemical, as introduced:
  - *Gloves and coveralls*
  - *Full face respirator or air-fed hood*

  Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the pelleted notified chemical, as introduced:
  - *Gloves, coveralls, and safety glasses*

  Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical used in spray painting applications:
  - *Gloves and coveralls*
  - *Safety glasses, goggles or face shield*
  - *An appropriate respirator or air-fed hood (as needed)*

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

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

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

Disposal

- The notified chemical should be disposed of by landfill.

Emergency procedures

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

Regulatory Obligations

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

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

(1) Under Section 64(1) of the Act; if

- further toxicological data becomes available.

or

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

- the function or use of the chemical has changed from a curing agent in plastics and coatings, or is likely to change significantly;
- the amount of chemical being introduced has increased from 3 tonnes, or is likely to increase, significantly;
- if the chemical has begun to be manufactured in Australia;
- additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

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

Material Safety Data Sheet

The MSDS of the notified chemical and product containing the notified chemical provided by the notifier were reviewed by NICNAS. The accuracy of the information on the MSDS remains the responsibility of the applicant.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point 89-90.4ºC

Method	EC Directive 84/449/EEC A1 Melting/Freezing Temperature.
Remarks	Capillary method used. The notified chemical began melting at 89ºC (wet point) and was fully melted at 90.4ºC.
Test Facility	Imperial Chemical Industries PLC (1987a)

Boiling Point >300ºC at 101.3 kPa

Method	EC Directive 84/449/EEC A2 Boiling Temperature.
Remarks	Heating of the notified chemical above 100ºC caused it to change from a colourless to a deep yellow liquid. No boiling was observed upon heating to 300ºC.
Test Facility	Imperial Chemical Industries PLC (1987a)

Density 1244 kg/m^3 at 22ºC

Method	EC Directive 84/449/EEC A3 Relative Density.
Remarks	The density was determined by displacement, using a capillary stoppered density bottle method. The original method was modified by the addition of 1% surfactant to the immersion liquid, to allow for adequate wetting of the particles of notified chemical.
Test Facility	Imperial Chemical Industries PLC (1987a)

Vapour Pressure ≤0.013 kPa at 81.7ºC

Method	OECD TG 104 Vapour Pressure. EC Directive 84/449/EEC A4 Vapour Pressure.
Remarks	Weight loss effusion manometer method.
Test Facility	Imperial Chemical Industries PLC (1988)

Water Solubility 0.000025 g/L at (20 ± 0.5)ºC

Method	EC Directive 84/449/EEC A6 Water Solubility.
Remarks	Flask Method/HPLC detection method Due to analytical variability in the first three readings, the fourth and fifth flasks were equilibrated at 20ºC for 24 and 48 hours, respectively.
Test Facility	Imperial Chemical Industries PLC (1987a)

Fat Solubility 16.5 g/100 g standard fat at 37ºC

Method	EC Directive 84/449/EEC A7 Fat Solubility.
Remarks	Analytical Method: concentrations of the notified chemical were determined by HPLC.
Test Facility	Imperial Chemical Industries PLC (1987a)

Hydrolysis as a Function of pH

Remarks	HPLC and GC methods were examined, however, neither possessed sufficient accuracy for use in hydrolysis testing at the required concentration. As the notified chemical contains no functional groups consistent with hydrolysis reactions and the material is virtually insoluble in water, hydrolysis test is not applicable for the notified chemical.
Test Facility	Life Science Research Limited (1992a)

Partition Coefficient (n-octanol/water) $\log P_{ow}$ = 6.3 at (22 ± 1)ºC

Method	EC Directive 84/449/EEC A8 Partition Coefficient.
Remarks	HPLC Method/Flask Method. The range of the determined P_{ow} values were 1.65×10^6 to 2.18×10^6 (i.e. $\log P_{ow}$ = 6.22-6.34).
Test Facility	Imperial Chemical Industries PLC (1987a)

Surface Tension 72.32 mN/m

Method OECD TG 115 Surface Tension of Aqueous Solutions.
EC Directive 84/449/EEC A5 Surface Tension.
Remarks Ring tensiometer method.
Concentration: Saturated solution of notified chemical in HPLC-grade water.
The surface tension indicates the notified chemical is not surface active.
Test Facility Imperial Chemical Industries PLC (1987a)

Adsorption/Desorption log K_{OC} = 5.79

Method Estimated by correlation and computer calculation group contribution method.
Remarks Due to the extremely low water solubility of the notified chemical of 0.00002 g/L, the determination of the K_{OC} was not feasible in a standard test design and was estimated by calculation using 3 methods as 620,000 (i.e. log K_{OC} =5.79). The estimated K_{OC} indicates that the notified chemical would be expected to be tightly bound to organic matter in soil, and hence immobile.
Test Facility Huntingdon Life Sciences (1996b)

Dissociation Constant pKa = 3.2 to 3.6

Method OECD TG 112 Dissociation Constants in Water.
Remarks The extremely low solubility of the notified chemical precludes the determination of dissociation constants experimentally. The value was estimated by calculation.
Test Facility Life Science Research Limited (1992b)

Particle Size Inhalable fraction (<100 µm): ~56.7%
Respirable fraction (<10 µm): 7.5%
Median particle diameter = ~82 µm
Mass Median Aerodynamic Diameter (MMAD) = ~91.5 µm

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

Measured equivalent spherical diameter (µm)	*Volume (%)*
≤2.020	1.12
≤10.08	8.55
≥201.5	1.65
2.020-201.5	97.23

Remarks Coulter counter method. The dispersant used was isoton II or a mixture of isoton II/glycerol (70%/30%), depending on the capillary diameter used. Particles were microscopically observed to be "relatively large irregularly shaped crystals".
The MMAD was calculated from the median particle diameter using the equation:

$$\text{Aerodynamic diameter} = (\text{relative density})^{0.5} \times \text{measured particle diameter}$$

The same equation was used to calculate the respirable and inhalable fractions, by finding proportion of particles with measured diameters <8.97 µm and <89.7 µm, respectively.

Test Facility Huntingdon Life Sciences Limited (1996a)

Flammability Not highly flammable

Method EC Directive 84/449/EEC A10 Flammability (Solids).
Remarks The notified chemical did not ignite in contact with air at ambient temperature, did not propagate combustion in contact with a Bunsen flame, and did not evolve any gases in contact with water.
Test Facility Imperial Chemical Industries PLC (1987b)

Autoignition Temperature >(90 ± 2)ºC

Method	EC Directive 84/449/EEC A16 Relative Self-Ignition Temperature for Solids.
Remarks	The notified chemical did not self-ignite up to its melting temperature.
Test Facility	Imperial Chemical Industries PLC (1987b)

Explosive Properties Not explosive

Method	EC Directive 84/449/EEC A14 Explosive Properties.
Remarks	The notified chemical did not give a positive test result in any of: the BAM Hammer Fall Test, the BAM Friction Test, or the Koenen Steel Tube Test.
Test Facility	Imperial Chemical Industries PLC (1987c)

Oxidising Properties Not oxidising

Method	EC Directive 84/449/EEC A17 Oxidizing Properties (Solids).
Remarks	The notified chemical had a significantly slower burning rate than the reference chemical (Barium nitrate) when mixed with cellulose.
Test Facility	Imperial Chemical Industries PLC (1987b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 401 Acute Oral Toxicity – Limit Test.
Species/Strain	Rat/Wistar Crl:CD(WI)BR
Vehicle	1% w/v aqueous carboxymethycellulose
Remarks - Method	No significant protocol deviations. A screening study was performed using groups of one male and one female, receiving doses of 250, 500, 1000, 2000 and 5000 mg/kg bw. Based on the results of this screening study, a single dose level was used in the subsequent investigation.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	1M, 1F	250	0
1	1M, 1F	500	0
1	1M, 1F	1000	0
1	1M, 1F	2000	0
1	1M, 1F	5000	1M
2	5M, 5F	5000	1M

LD50	>5000 mg/kg bw
Signs of Toxicity	All animals appeared normal on the day of dosing and up to 5 days after treatment. On days 6 and 7, one male appeared emaciated (with reduced body weight), with hunched posture and staining to the body. This animal was found dead on day 8. All other animals appeared normal throughout the study period.
Effects in Organs	Upon necropsy, the male that died showed dark lungs and an enlarged, pale liver with dark and yellow patches. All other animals were unremarkable upon terminal necropsy.
Remarks - Results	It is unknown what effect dosing with 1% carboxymethylcellulose would have on the notified chemical's bioavailability, absorption and toxicity.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	Hazleton UK (1988a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test.
Species/Strain	Rat/Sprague-Dawley
Vehicle	Moistened with distilled water
Type of dressing	Occlusive
Remarks - Method	No significant deviations from the test protocol. The solid test substance was applied to prepared gauze dressing, wetted with distilled water, then applied for 24 hours.

RESULTS

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

LD50	>2000 mg/kg bw
Signs of Toxicity - Local	No local effects were reported.
Signs of Toxicity - Systemic	No clinical signs were noted. Body weight gains were generally lower

	than expected, with the differences most marked in the female animals.
Effects in Organs	Although a gross necropsy was apparently conducted, no results were presented.
Remarks - Results	Given the low water solubility of the notified chemical, it is difficult to estimate the dose that may have actually been received by the rat.
CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	Inveresk Research International (1986a)

B.3. Irritation – skin

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	Moistened with distilled water
Observation Period	72 hours
Type of Dressing	Occlusive
Remarks - Method	No significant protocol deviations. A four-hour application was used.
RESULTS	
Remarks - Results	No adverse skin reactions were noted during the study period (primary irritation index = 0). Given the low water solubility of the notified chemical, it is difficult to estimate if sufficient test substance might have been available to cause irritation.
CONCLUSION	The notified chemical is non-irritating to the skin.
TEST FACILITY	Hazleton UK (1988b)

B.4. Irritation – eye

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White
Number of Animals	2 male, 1 female
Observation Period	72 hours
Remarks - Method	No significant protocol deviations. Solid test substance (100 mg) was applied directly into the conjunctival sac.

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	0.33	0.33	0	2	<48 hours	0
Conjunctiva: chemosis	0	0	0	2	-	-
Conjunctiva: discharge	0	0	0	2	-	-
Corneal opacity	0	0	0	0	-	-
Iridial inflammation	0	0	0	0	-	-

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

Remarks - Results	Moderate discharge and conjunctival erythema and chemosis was noted at 1 hour after instillation. No effects were seen at the 48-hour observation. It is considered that the observed effects may have been due to the instillation of a solid test substance with low water solubility.
CONCLUSION	The notified chemical is slightly irritating to the eye.
TEST FACILITY	Inveresk Research International (1986b)

B.5. Skin sensitisation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 406 Skin Sensitisation – adjuvant test.
Species/Strain	Guinea pig/Dunkin-Hartley
PRELIMINARY STUDY	Maximum Non-irritating Concentration: intradermal: 10% in paraffin oil topical: 25% in paraffin oil
MAIN STUDY	
Number of Animals	Test Group: 20 females Control Group: 20 females
INDUCTION PHASE	Induction Concentration: intradermal: 10% in paraffin oil topical: 10% in paraffin oil
Signs of Irritation	Slight-moderate dermal reactions were noted in the test group (c.f. slight reactions for the control group).
CHALLENGE PHASE	
Concentration	topical: 25% in paraffin oil
Remarks - Method	Because of technical difficulties in formulation, a 10% solution of the test substance was used at induction, rather than 25%. Then, because of an error, a 25% concentration was used for challenge, rather than the 10% determined in the challenge dose ranging study. Prior to topical induction, the same region received a topical application of sodium lauryl sulfate in water (10%, w/w) in order to induce local irritation.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after challenge*	
		24 h	*48 h*
Test Group	25%	0	0
Control Group	25%	0	0

Remarks - Results	Body weight gains were within the historically acceptable range, and no clinical signs were observed.
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	Inveresk Research International (1986c)

B.6. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	EC Directive 84/449/EEC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain	Rat/Sprague-Dawley
Route of Administration	Oral – diet

Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: None
Vehicle	Mixed directly into feed
Remarks - Method	No functional observations were conducted. The following haematology and clinical chemistry parameters were not measured: - Blood clotting time/potential - Cholesterol Heart, brain and epididymides weight were not measured and only the liver spleen, kidneys, adrenals, heart and macroscopically abnormal tissues underwent histopathological investigation. The dose levels for the main study were selected on the basis of a 14-day dose range-finding study. Doses of 0, 1000, 3000 and 10000 ppm were administered to groups of three males and three females.

RESULTS

14-day dose-range finding study

Dose (ppm)	*Number and Sex of Animals*	*Approximate dietary intake (mg/kg bw/day)*	*Mortality*
0	3M, 3F	0	0
1000	3M, 3F	100	0
3000	3M, 3F	300	0
10000	3M, 3F	1000	1F (day 6), 1F(day 12), 1M (day 15)

In the 14-day dose-range finding study, no significant clinical signs of toxicity were observed in animals treated with 1000 and 3000 ppm. Reduced body weight gain was observed in most animals of both of these dose groups (except low dose males). In animals treated with 10000 ppm, emaciation with marked body weight loss (7.4 and 4.8 g/day for males and females, respectively), lethargy and piloerection were observed.

Upon necropsy, gross pathology of animals treated with 10000 ppm found haemorrhages of the stomach, bloody content of the urinary bladder and dilation of the urethra and/or renal pelvis (the last is a congenital abnormality in the strain of animals used).

A dose-dependent increase in absolute and body-weight relative liver weights was observed in all treated animals (25-30%, 60-65% and 130-215% for relative weights in the 1000, 3000 and 10000 ppm groups, respectively). An increased adrenal weight was also observed in all treated males (36%, 39% and 5% for the 1000, 3000 and 10000 ppm groups, respectively).

Main study

Dose (ppm)	*Number and Sex of Animals*	*Mean dietary intake (mg/kg bw/day)*	*Mortality*
0	6M	0	0
	6F	0	0
100	6M	12.2	0
	6F	13.4	0
300	6M	36.2	0
	6F	40.3	0
1000	6M	131.0	0
	6F	146.1	0

Mortality and Time to Death

No mortality was observed during the main study.

Clinical Observations

No evident signs of toxicity were observed during the study. No effects of treatment on body weight gain or on food consumption were observed.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

An increased serum calcium concentration was found in 1000 ppm females (+6%). No other statistically significant effects were observed in the clinical chemistry parameters examined.

A statistically significant increase in platelet count was observed in the 1000 ppm group (+18% and +20% for males and females, respectively). No other statistically significant effects were observed in the haematology parameters examined.

No urinalysis was reported.

Effects in Organs
Statistically significant increases in absolute and body-weight relative liver weights were observed in all animals treated with 1000 ppm (+35% for group mean data). Microscopic examination of the livers of these animals showed centrilobular hypertrophy.

Gross necropsy showed enlarged adrenals (two instances in the 100 ppm group and twice in the 1000 ppm group). An increase in absolute and body-weight relative adrenals weight was observed in animals of both sexes in the 1000 ppm group (+20-24%), however, this effect only reached statistical significance for body-weight relative values in male animals. Microscopic examination of the adrenals showed no histopathologic abnormalities.

A statistically significant increase in body-weight relative kidney weights in the 100 ppm group was considered to be of no toxicological significance in the absence of a dose response and any histopathological correlates.

Upon gross necropsy, petechiae of the thymus (one instance in the 1000 ppm group) and stomach (one instance in the control group) were also observed, but these were considered incidental.

Remarks – Results
The centrilobular hypertrophy observed in the livers of 1000 ppm treated animals suggest that the liver weight increases may be an adaptive metabolic response to xenobiotic treatment. However, as no evidence of reversibility was presented in this study, these effects must be regarded as an adverse effect.

CONCLUSION
The No Observed Effect Level (NOEL) was established as 36 mg/kg bw/day (males) and 40 mg/kg bw/day (females) in this study, based on the absence of significant effects at the 300 ppm dose level.

TEST FACILITY NOTOX CV (1987a)

B.7. *E. coli*, bacterial reverse mutation

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
EC Directive 92/69/EEC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria.
Plate incorporation procedure

Species/Strain	*E. coli* WP2*uvr*A *trp*
Metabolic Activation System	Aroclor 1254-induced rat liver S9 fraction
Concentration Range in Main Test	a) With S9: 0, 312.5, 625, 1250, 2500 and 5000 µg/plate b) Without S9: 0, 312.5, 625, 1250, 2500 and 5000 µg/plate
Vehicle	DMSO
Remarks - Method	No significant deviation from protocol observed. No toxicity was observed in a pre-test at concentrations up to 5000 µg/ml. some precipitation was observed at this highest concentration.

RESULTS

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

Present	>5000			
Test 1		>5000	>5000	Negative
Test 2		>5000	>5000	Negative

Remarks - Results: The positive control chemicals (2-aminoanthracene and *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine) both elicited a positive response in a parallel study.

CONCLUSION: The notified chemical was not mutagenic to bacteria (*E. coli*) under the conditions of the test.

TEST FACILITY: Huntingdon Life Sciences Limited (1996c)

B.8. *S. typhimurium* bacterial reverse mutation

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 471 Bacterial Reverse Mutation Test.
Plate incorporation procedure

Species/Strain	*S. typhimurium*: TA1538, TA1535, TA1537, TA98, and TA100
Metabolic Activation System	Aroclor 1254-induced rat liver S9 fraction
Concentration Range in Main Test	a) With metabolic activation: 0, 8, 40, 200, 1000, 5000 µg/plate b) Without metabolic activation: 0, 8, 40, 200, 1000, 5000 µg/plate
Vehicle	DMSO
Remarks - Method	No significant protocol deviations were noted. A preliminary toxicity study was performed with strain TA98 only.

RESULTS

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

Remarks - Results: The positive control chemicals (benzo[a]pyrene and 2-nitrofluorene) both elicited a positive response in a parallel study.

CONCLUSION: The notified chemical was not mutagenic to bacteria (*S. typhimurium*) under the conditions of the test.

TEST FACILITY: Life Science Research Limited (1985)

B.9. *In vitro* chromosome aberration study (CHL cells)

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 473 In vitro Mammalian Chromosome Aberration Test.

Cell Type/Cell Line	Chinese Hamster Lung (CHL) cell line
Metabolic Activation System	Aroclor 1254-induced rat liver S9 fraction
Vehicle	DMSO
Remarks - Method	No significant protocol deviations. Test 1 (see below) was discarded due to excessive cytotoxicity in the majority of concentrations examined.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period (hrs)*	*Harvest Time (hrs)*
Absent			
Test 1	39.1, 78.1, 156.3, 312.5, 625, 1250, 2500, 5000	6	24
Test 2A	2.4, 4.9, 9.8, 19.5, 39.1, 58.6*, 78.1*, 156.3*	6	24
Test 2B	0.15, 0.3, 0.6, 1.2, 2.4, 4.9*, 9.8*, 19.5*, 39.1, 78.1	24	24
Test 2C	0.08, 0.15, 0.3, 0.6, 1.2, 2.4*, 4.9*, 9.8*, 19.5, 39.1	48	48
Present			
Test 1	39.1, 78.1, 156.3, 312.5, 625, 1250, 2500, 5000	6	24
Test 2	2.4, 4.9, 9.8, 19.5, 39.1*, 58.6,* 78.1*, 156.3**	6	24

* Cultures selected for metaphase analysis.
** Only one of three replicates at 156.3 µg/mL was analysed, due to excessive cytotoxicity in the others.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*		
	Cytotoxicity	*Precipitation*	*Genotoxic Effect*
Absent			
Test 1	>78.1	≥156.3	Not determined
Test 2A	≥156.3	≥156.3	Negative*
Test 2B	≥19.5	>78.1	Negative**
Test 2C	≥9.8	>39.1	Negative
Present			
Test 1	>78.1	≥156.3	Not determined
Test 2	>78.1	≥156.3	Negative

* An increase in aberrant cells (+3.5%) was observed in one culture at 156.3 µg/mL (this value was not significant when compared to the solvent control).
** An increase in aberrant cells (+5.5%) was observed (for gap damage only) at 19.5 µg/mL.

Remarks - Results
All of the increases were within the relevant ranges of normal historical control values, and were not considered to be of biological significance.

The positive control chemicals (mitomycin C and carbendazim) both elicited the appropriate positive responses in a parallel study.

CONCLUSION
The notified chemical was not clastogenic to CHL cells treated *in vitro* under the conditions of the test.

TEST FACILITY
Huntingdon Life Sciences Limited (1996d)

B.10. *In vitro* chromosome aberration study (human lymphocytes)

TEST SUBSTANCE
Notified chemical

METHOD
EC Directive 84/449/EEC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.

Species/Strain: *Homo sapiens* (human male volunteer)
Cell Type/Cell Line: Peripheral lymphocytes
Metabolic Activation System: Aroclor 1254-induced rat liver S9 fraction
Vehicle: DMSO
Remarks - Method: In the presence of metabolic activation the cells were exposed to the test substance for 2 hours rather than 3-6 hours as recommended in the protocol.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period (hrs)*	*Harvest Time (hrs)*
Absent	10*, 33*, 100*, 333, 1000, 3330, 5000	23	23
Present	10, 33*, 100*, 333*, 1000, 3330, 5000	2	23

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (μg/mL) Resulting in:*		
	Cytotoxicity	*Precipitation*	*Genotoxic Effect*
Absent	≥100	≥1000	Negative
Present	≥333	≥1000	Negative

Remarks - Results: The positive control chemicals (mitomycin C and cyclophosphamide) both elicited the appropriate positive responses in a parallel study.

CONCLUSION: The notified chemical was not clastogenic to human peripheral lymphocytes treated *in vitro* under the conditions of the test.

TEST FACILITY: NOTOX CV (1987b)

B.11. *In vivo*/*in vitro* rat primary hepatocyte unscheduled DNA synthesis (UDS) assay

TEST SUBSTANCE: Notified chemical

METHOD: No standard test method specified. The method used was corresponded to a published protocol (Butterworth *et al*, 1987).

Species/Strain: Rat/F344 NH4/hsd

Route of Administration: Oral – gavage

Vehicle: 1.5% aqueous carboxymethylcellulose

Remarks - Method: No significant deviations from OECD TG 486: Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo

A preliminary study of four rats dosed with 1000 mg/kg bw was performed. Primary hepatocyte cultures were isolated from two animals ate 4 hours after dosing, and from the other two animals at 15-16 hours after dosing. As the distribution of ^{3}H-labelling was similar between the time-points, the longer period was chosen.

Three animals per dose level were then administered the specified doses (below). Hepatocyte cultures were prepared 15-16 hours after dosing, and cells were incubated with 5 mCi/mL ^{3}H-thymidine for about 4 hours. Cultures were prepared for nuclear labelling analysis ~16-19 hours after removal of the radioactivity and addition of 0.25 mM thymidine.

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Sacrifice Time (hours)*
vehicle control	3M	0	15-16
I	3M	100	15-16
II	3M	250	15-16
III	3M	500	15-16
IV	3M	1000	15-16
positive control (dimethylnitrosamine)	3M	10 (intraperitoneal)	15-16

RESULTS

Doses Producing Toxicity: No examination of toxicity was reported.

Genotoxic Effects: No statistically significant increases in nuclear ^{3}H-labelling were observed in hepatocytes from treated animals over those from animals treated with vehicle. In addition, no dose-response was observed. In contrast, cells from animals treated with the positive control exhibited significant increases in nuclear labelling that exceeded the criteria that indicate UDS (Butterworth *et al*, 1987).

Remarks - Results: Viabilities of the cultured hepatocytes obtained ranged from 86.5% to 95.9%.

Given its physicochemical properties and the findings from other studies, the notified chemical would be expected to have been sufficiently absorbed from the gastrointestinal tract to expose the hepatocytes to some level of the notified chemical. Indeed, hepatocyte enlargement was observed in animals in the repeat dose toxicity study at lower daily doses than those applied in this study.

The positive control was administered by intraperitoneal injection, and thus absorption would not play as large a role in its induction of UDS.

CONCLUSION The notified chemical was not mutagenic under the conditions of this *in vivo/in vitro* UDS assay.

TEST FACILITY Hazleton Laboratories America (1989)

B.12. *In vivo* mouse micronucleus test

TEST SUBSTANCE Notified chemical

METHOD EC Directive 2000/32/EEC B.12 Mutagenicity - Mammalian Erythrocyte Micronucleus Test.

Species/Strain Mouse/Swiss CD-1

Route of Administration Intraperitoneal injection

Vehicle 1% aqueous methylcellulose

Remarks - Method No significant protocol deviations.

Animals were treated with the test substance once.

A preliminary toxicity study was carried out using 2 male and 2 female mice dosed with the test substance at 2000 mg/kg bw. Only very limited toxicity (minor clinical signs) and no mortalities were observed.

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Sacrifice Time (hours)*
I (vehicle control)	5M/5F	0	24
II (vehicle control)	5M/5F	0	48
III (low dose)	5M/5F	500	24
IV (mid dose)	5M/5F	1000	24
V (high dose)	5M/5F	2000	24
VI (high dose)	5M/5F	2000	48
positive control (mitomycin C)	5M/5F	12 (by oral gavage)	24

RESULTS

Doses Producing Toxicity No mortalities were observed amongst the test substance-treated animals.

Animals treated with 1000 mg/kg bw showed slight hunched posture, piloerection and lethargy at 3 and 4 hours after dosing, no signs were evident by 21 hours.

Animals treated with 2000 mg/kg bw showed slight piloerection and lethargy at all times after dosing until the 48-hour sacrifice time. In addition, these animals showed hunched posture and ptosis until the 4 hours after dosing; these affects had disappeared by 21 hours.

Genotoxic Effects The test substance induced no statistically significant increases in micronucleated, polychromatic erythrocytes (PCEs) at either sampling time.

Remarks - Results No reductions in the PCE/NCE ratio were observed with test substance treatment, so it is therefore unclear if it was able to distribute to the bone marrow (as no cytotoxicity was observed).

It should be noted, however, that the positive control also caused no

	decrease in the PCE/NCE ratio, while still inducing a large, statistically significant increase in the numbers of PCEs.
CONCLUSION	The notified chemical was not clastogenic under the conditions of this *in vivo* mouse micronucleus test.
TEST FACILITY	Huntingdon Life Sciences Limited (1996e)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	EC Directive 84/449/EEC C.5 "Biotic Degradation: Modified Sturm Test"
Inoculum	Activated sludge from municipal sewage treatment plant, De Dommel, 's-Hertogenbosch, The Netherlands.
Exposure Period	28 days
Auxiliary Solvent	$Ba(OH)_2$
Analytical Monitoring	The amount of CO_2 produced was determined by titration of the remaining amount of $Ba(OH)_2$ with standardized HCl solution.
Remarks - Method	The CO_2 evolution test method is employed. No significant deviations from the test method.

RESULTS

Test substance- P 5367 (19.5 mg/ L)		*Reference Substance-Sodium acetate (19.9 mg/L)*	
Day	*% Degradation*	*Day*	*% Degradation*
2	0.5	2	9.3
7	2.0	7	35.0
14	2.1	14	49.3
28	2.3	28	61.4

Remarks - Results

The ready biodegradability of the notified chemical was determined at two different concentrations: 19.5 mg/L (as shown in the above table) and 10.5 mg/L, with the lower concentration showing a final degradation degree of 4.7%.

The reference substance, sodium acetate, showed a degradation of 61.4% within 28-day period and is acceptable to be classified as readily degradation according to the test method.

The degradation of the notified chemical was incomplete after 28 days and did not reach the 10% lag phase threshold over the period of the test. It is therefore not readily biodegradable according to the test guidelines.

CONCLUSION The notified chemical cannot be classified as readily biodegradable.

TEST FACILITY NOTOX CV (1987c)

C.1.2. Bioaccumulation

Remarks - Results

The bioaccumulation properties of the notified chemical were not tested. The notified chemical is expected to have a high octanol/water partition coefficient and high fat solubility, and was shown to be not readily biodegradable. Based on these data, it can be reasonably assumed that it would have potential to accumulate in biological tissues in case of environmental exposure. However, due to the use pattern, exposure of the notified chemical to environment is unlikely to occur, and bioaccumulation in aquatic organisms is not expected.

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	EC Directive 84/449/EEC C.1 "Acute toxicity for fish"
Species	Guppy *(Poecilia reticulata)*
Exposure Period	96 hours
Auxiliary Solvent	DMSO
Water Hardness	11.7 ˚ D H
Analytical Monitoring	HPLC was used for the determination of the solubility of notified chemical in water.
Remarks – Method	Two separate pilot tests were realized at the notified chemical concentration 0.1 mg/L. Dutch Standard Water (DSW) was used as the carrier of the notified chemical in the test. Due to the low solubility of the notified chemical in water, a suspension of the notified chemical was formed and used for the acute toxicity test to fish at concentrations between 0.1 mg/L and 5 mg/L. Toxicity with concentration higher than 5 mg/L was not tested for the reason that macroscopic particles were observed in the suspension.

Actual Concentration (mg/L)	*Number of Fish*	*Mortality*				
		1 h	*24 h*	*48 h*	*72 h*	*96 h*
0	10	0	0	0	0	0
0	10	0	0	0	0	0
0.1	10	0	0	0	0	0
0.1	10	0	0	0	0	0
1.0	10	0	0	0	2	2
5.0	10	0	0	0	0	0
1.0*	10	0	0	1**	1**	1**
1.0*	10	0	0	0	0	0

* Tests for confirmation the result from the previous tests with the same concentration.
** One fish was damaged during renewal of the test medium and removed from the test.

LC50	>5 mg/L at 24 hours. >5 mg/L at 48 hours. >1.0 mg/L at 72 hours. >1.0 mg/L at 96 hours.
NOEC	1.0 mg/L at 96 hours.
Remarks – Results	No effects were observed at suspensions of 0.1 mg/L. Exposure of fish to a suspension of 1 mg/L resulted in significant mortality. The same concentration was tested again in the final test and the result did not appear to be reproducible, with one dead fish out of 10 at 48 h during the renewal of the test medium in one test and none in another test. High rate of mortality at suspension of 5 mg/L was reported in the text Result part, even not reported at the raw data table, which was considered as the physical trauma due to the formation of needle-like particles rather than the chemical toxicity of the notified chemical. The acceptable value of OECD should be 1.0mg/L based on the above test results.
CONCLUSION	The notified chemical shows some toxicity to fish when suspended in water.
TEST FACILITY	NOTOX CV (1987d)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	EC Directive 84/449/EEC C.2 "Acute toxicity for *Daphnia*"
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	Methanol
Water Hardness	11.7 ˚ D H
Analytical Monitoring	HPLC was used for the determination of the solubility of notified chemical in water.
Remarks - Method	The acute toxicity test for *Daphnia magna* was performed in duplicate in suspensions of the notified chemical in DSW at concentrations of 0.01 mg/L and 0.1 mg/L, respectively. As particles suspended in the test medium could have physical effects on the very small *Daphnia magna*, methanol was used as auxiliary solvent in the preparation of the suspensions and evaporated before use for test. A solvent-control test was synchronously performed. One week before the test $K_2Cr_2O_7$ was tested as a reference substance, and the results indicate the test conditions were optimal and the test results with the notified chemical are valid.

RESULTS

Actual Concentration (mg/L)	*Number of D. magna*	*Number Immobilised*	
		24 h	*48 h*
0	10	0	0
0	10	0	0
0*	10	0	0
0*	10	0	0
0.01	10	0	0
0.01	10	0	1
0.1	10	0	0
0.1	10	0	0

* Solvent control: methanol (evaporated before addition of medium)

LC50	>0.1 mg/L at 24 hours >0.1 mg/L at 48 hours
NOEC (or LOEC)	Not determined
Remarks - Results	No significant effects on mobility of *Daphnia* were observed at suspension of up to 1.0 mg/L. It was unclear whether suspensions or clear solutions were tested. On the basis of these results, and the envisaged use of the substance (will be used only in production site, reacts completely to form polymers and no residual substance will remain in the finished products), a chronic test is not considered necessary.
CONCLUSION	The notified chemical has no effect on the mobility of *Daphnia magna* at the levels tested, which were over the saturated concentration of the notified chemical. Hence, it would be classified as non-toxic up to the limit of the water solubility.
TEST FACILITY	NOTOX CV (1987e)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	EC Directive 92/69/EEC C.3 "Algal Inhibition Test"
Species	*Selenastrum capricornutum*
Exposure Period	72 hours

Concentration Range	Nominal: 10 mg/L Actual: 7.4 mg/L
Auxiliary Solvent	10% Tween 80-dimethylformamide
Water Hardness	Not given. The test medium mainly contained 15 mg/L NH_4Cl, 12 mg/L $MgCl_2.6H_2O$, 18 mg/L $CaCl_2.2H_2O$, 15 mg/L$MgSO_4.7H_2O$ and 1.6 mg/L KH_2PO_4.
Analytical Monitoring	HPLC-UV detection
Remarks - Method	None.

RESULTS

An auxiliary solvent, 10% Tween-80 dimethylformamide was employed in making test medium. The nominal and actual test concentrations were 10 mg/L and 7.4 mg/L, respectively.

Biomass		*Growth*	
E_bC_{50} (mg/L at 72 h)	*NOE_bC (mg/L)*	*ErC_{50} (mg/L at 0-72 h)*	*NOErC (mg/L)*
>7.4	<7.4	>7.4	<7.4

Remarks - Results	The maximum concentration employed in the test was nominally 10 mg/L, the highest concentration at which it was possible to obtain a visually stable test dispersion. The NOEC is <7.4 mg/L as 21% inhibition of growth was observed.

CONCLUSION

The notified chemical can be classified as at worst toxic to the growth of *Selenastrum capricornutum*, based on its low water solubility.

TEST FACILITY

Huntingdon Life Sciences Limited (1996f)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 209 Activated Sludge, Respiration Inhibition Test.
EC Directive 87/302/EEC: Activated Sludge, Respiration Inhibition Test.

Inoculum	Activated sewage sludge micro-organisms from the aeration stage of the Anglican Water sewage treatment (Godmanchester) plant
Exposure Period	3 hours
Concentration Range	Nominal: 10 mg/L Actual: 10 mg/L
Remarks – Method	No significant deviations from the test method. An auxiliary solvent, 10% Tween-80 dimethylformamide was employed and a dispersion of 10 mg/L was prepared for the test.

RESULTS

EC_{50}	> 10 mg/L
NOEC	Not given
Remarks – Results	The highest test concentration was 10 mg/L, as this was the highest concentration in the test medium at which a stable dispersion could be formed in the solvent.

CONCLUSION

EC_{50} of the notified chemical with activated sewage sludge has been determined as >10 mg/L for the three hour contact time.

TEST FACILITY

Huntingdon Life Sciences Limited (1996g)

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