File No: EX/165 (STD/1259)

September 2011

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

1,2-Cyclohexanedicarboxylic acid, 1,2-diisononyl ester ('Hexamoll DINCH')

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

For the purposes of subsection 78(1) of the Act, this Full Public Report may be inspected at our NICNAS office by appointment only at Level 7, 260 Elizabeth Street, Surry Hills NSW 2010.

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

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Director
NICNAS

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FULL PUBLIC REPORT

1,2-Cyclohexanedicarboxylic acid, 1,2-diisononyl ester ('Hexamoll DINCH')

This assessment report is for an extension of the original assessment certificate for 1,2-Cyclohexanedicarboxylic acid, 1,2-diisononyl ester ('Hexamoll DINCH'). Based on the submission of new information by the extension notifier, some sections of the original assessment report for BASF Australia Ltd have been modified. These modifications have been made under the heading 'Extension Application' in the respective sections.

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
Holders of the Original Assessment Certificate (STD/1259):
BASF Australia Ltd (ABN 62 008 437 867) of 500 Princes Highway, Noble Park VIC 3174

Applicant for an Extension of the Original Assessment Certificate:
Flint Group Pty Ltd Australia Pty Ltd (ABN 79 006 659 178) of 25-51 Berends Drive, Dandenong South VIC 3175

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: Non-hazardous impurities, Import volume, Name and details of customers, and concentration of notified chemical in formulations.

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
European Union (Identifier 99-04-1211), Canada (NDSL), in USA (TSCA), Japan, Korea and China.

2. IDENTITY OF CHEMICAL

CHEMICAL NAME
1,2-Cyclohexanedicarboxylic acid, 1,2-diisononyl ester

OTHER NAME(S)
1,2-Cyclohexanedicarboxylic acid, diisononyl ester (9CI)
1,2-Cyclohexanedicarboxylic acid, diisononyl ester, branched and linear
Diisononyl cyclohexane-1,2-dicarboxylate
DINCH

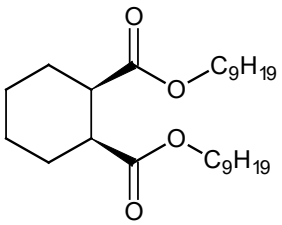
MARKETING NAME(S)
Hexamoll DINCH

Extension Application
Chemical in Flint Group Novasens Colour Range

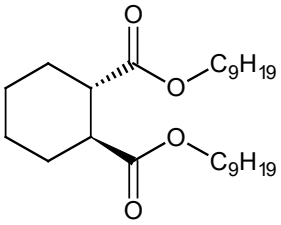
CAS NUMBER
166412-78-8

MOLECULAR FORMULA
$C_{26}H_{48}O_4$

STRUCTURAL FORMULA



90±10% *cis*- isomer



10±10% *trans*- isomer

MOLECULAR WEIGHT
424.6 g/mol

ANALYTICAL DATA
Reference ^{1}H-NMR, ^{13}C-NMR, IR, GC, and UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
99.5%

HAZARDOUS IMPURITIES
None

NON-HAZARDOUS IMPURITIES
Several impurities (isomers and reaction by-products), each present at <0.5%.

ADDITIVES/ADJUVANTS
Formulations of the notified chemical may contain the following:

Chemical Name	Phenol, 4,4'-(1-methylethylidene)bis- (‘Bisphenol A’)		
CAS No.	80-05-7	*Weight %*	≤0.5%
Hazardous Properties	Conc ≥20%:	Xi; R36/37/38; R43	
	1% ≤ Conc <20%:	Xi; R43	

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20ºC AND 101.3 kPa
Clear, colourless liquid. Appears homogeneous by visual inspection.

PROPERTY	VALUE	DATA SOURCE/JUSTIFICATION
Melting Point/Freezing Point	No freezing point. Glass transition <-90ºC Pour point = -54ºC	Did not crystallise Measured MSDS
Boiling Point	>351°C at 101.3 kPa 394°C	Decomposed before boiling at ~351°C Calculated
Density	947.2 kg/m^3 at 20ºC	Measured
Viscosity	44-60 mPa.s at 20ºC	Calculated
Vapour Pressure	2.2×10^{-8} kPa at 25ºC 8.9×10^{-7} kPa at 50ºC	Measured
Water Solubility*	<0.00002 g/L at 25ºC	Measured
Hydrolysis as a Function of pH	Not determined	Insoluble in water
Partition Coefficient (n-octanol/water)	logP_{ow} = >6.2 at 25ºClogP_{ow} = 10.0	MeasuredCalculated

Surface tension	30.7 mN/m at 20ºC	Measured
Adsorption/Desorption	$logK_{oc}$ >5.6 at 23ºC $logK_{oc}$ = 5.82	Measured Calculated
Dissociation Constant	Not determined	No modes of dissociation are expected
Particle Size	Not determined	The notified chemical is a liquid
Flash Point	224ºC	MSDS
Flammability	Not highly flammable	Estimated
Autoignition Temperature	330ºC	MSDS
Explosive Properties	Not explosive	Estimated

* *Note:* Additional solubility data is presented in Appendix A.

DISCUSSION OF PROPERTIES

The notified chemical is considered to be lipophilic, water-insoluble and surface-active. It is not expected to present a physical hazard; while combustible, it is not expected to present a flammable or explosive hazard. For full details of tests on physical and chemical properties, please refer to Appendix A.

Reactivity

The notified chemical is stable under the expected storage and use conditions, but is reported to react with strong oxidising agents. It is not expected to be oxidising of itself.

5. INTRODUCTION AND USE INFORMATION

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

The notified chemical will be imported in 200 L steel drums (majority), 1000 kg intermediate bulk containers (IBCs) or 20 tonne bulk isotainers.

Extension Application

The notified chemical will be imported as a component in a range of ink formulations at a maximum concentration of 40%.

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

Original Application

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	100-1000	100-1000	100-2000	100-2000	100-2000

Extension Application

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	120	180	200	240	300

PORT OF ENTRY

Victoria and NSW

IDENTITY OF MANUFACTURER/RECIPIENTS

BASF Australia Ltd. The notified chemical is expected to be used in plastics and other products by customers around Australia (primarily in Victoria and NSW).

Extension Application

Flint Group Australia Pty Ltd. The notified chemical as imported will be reformulated and/or repackaged at Flint Group Australia Pty Ltd before being transported to various customers throughout Australia.

TRANSPORTATION AND PACKAGING

The notified chemical will be distributed by road, by Orica Australia Limited.

Formulated products:

- Solid products, such as compound for formulation into flexible PVC, will be produced in the form of pellets and packaged in 25 kg bags or 500 kg bulky bins.
- Liquid products will be packaged in 200 L steel drums.

Extension Application
The notified chemical will be transported by road to customers across Australia as a component of ink formulations in 2.5 kg vacuum packed cans, 2.5 and 10 kg plastic pails and 2 kg cartridges in cardboard boxes.

USE
The major applications for the notified chemical will use it as a plasticiser and impact modifier in food packaging, but also in general applications such as wire and cable, automotive, plastisols and other similar applications. The food contact applications can be grouped by the functions of the notified chemical, which are as a PVC plasticiser and as an impact modifier in polystyrene. The plasticiser is used in PVC cling films for fresh meat packaging, for aqueous food and fruits and vegetables, artificial corks, sealing gaskets for beverage containers, flexible tubes for beverages, alcoholic and non-alcoholic, conveyor belts for fatty and other foods, and as a polystyrene food packaging impact modifier.

Extension Application
Offset ink formulations containing the notified chemical at <40% will be used in non-direct food contact applications.

OPERATION DESCRIPTION
The imported notified chemical will be formulated into PVC compound or plastisols, at up to 60% notified chemical content. In both cases, the notified chemical will be transferred to a weighing vessel and then pumped into a closed mixing vessel for blending with PVC and other additives such as stabilisers. Mixing will occur at elevated temperatures for dry blending (100-220°C) or at room temperature for plastisols. Dry blends will be compounded by extrusion and pelletised for packing into bags or bins. Plastisols, which vary from thin liquid dispersions to thick pastes, will be drummed off. The mixing vessels will be cleaned only when required. There may be clean downs required during routine or breakdown maintenance periods, where lines and vessels will be purged and cleaned with inert materials.

Compounded PVC will then be converted into end-use products by processes such as extrusion, calendering or injection moulding. For example, extrusion would be used for the production of flexible tubes for beverages, calendering for food cling film, sheeting and automotive upholstery, and injection moulding would be used for artificial wine corks.

Plastisols will be used for underbody coating, sealing, rotational coating, dipping, slush moulding, and spread coating (such as during the manufacture of tarpaulins). The plastisol liquid or paste may be poured into a mould, which will then be placed in an air-heated tunnel oven (130-160°C). Handling of the plastisol is typically automated, using vacuum pumps to transfer the plastisol directly into the mould.

Extension Application
Ink formulations containing the notified chemical will be warehoused at the notifier's site before being transported to customers or blended with other components into the finished ink product. Blending onsite will involve colour matching, manual decanting of ink formulations containing the notified chemical into industrial mixers, quality testing by laboratory staff and packaging into plastic or metal containers ranging from 1 kg to 200 kg.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

NUMBER AND CATEGORY OF WORKERS

Category of Worker	*Number*	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and storage			
Polymer stage	15	2	50
Product stage	15	1-2	40-50
Compounding and Manufacturing			
Reactor operation	50	12	20
Maintenance	20	1-2	240

QC testing	10	2	240
Transport & storage	10	2/4	240
End use	1000s	1-12	240

EXPOSURE DETAILS

Transport and storage

The notified chemical will be transported by road to a warehouse and then to the compounding facility. Exposure of receivers and transport personnel should only occur in the event of an accidental spillage.

Compounding

Incidental skin contact with the notified chemical may occur when the storemen insert the drum lance into the 200 L drum, or during connection of an IBC or isotank to the weighing vessel. Inhalation exposure to vapours may also occur during the transfer process. After mixing, intermittent skin contact may occur during the packaging process, from powdered blend or liquid plastisol. Quality control samples may also be taken at this stage, as technical personnel will make up small-scale compounds by hand in the laboratory.

During the subsequent compounding of dry blend into pellets, closed systems are used, and any exposure will be incidental. However, manual operations during this process may include opening of packages, connection/insertion of lines/hoses, pumping liquid products, and eventual removal of connections and closing the containers. In addition, maintenance workers may experience skin contact with the notified chemical.

For the specific formulation sites in Australia, approximately one third of the production time for the operators will be dedicated to running compound. During production runs (which can be up to 5 days long), the operators will work two 12-hour shifts, 5 days per week, and 48 weeks per year. Workers will prepare approximately 8 batches per day. Given the time that it takes to connect up and transfer product, the estimated period of direct contact with the notified chemical is less than 30 minutes per day for one person per shift.

Local exhaust ventilation will be employed at all workplace areas where natural ventilation is considered inadequate. Workers, particularly for those operators involved in any open transfer operations, wear personal protective equipment (PPE) including overalls, safety glasses/goggles and face splash shields, protective gloves, and are assumed to operate using appropriate industrial hygiene practices.

Product manufacture

Exposure to the notified chemical may occur during the processing of PVC compound or plastisol to manufacture the end-use product. Once compounded with PVC, the notified chemical is bound within the PVC matrix and exposure is unlikely. However, during product manufacture by processes such as extrusion, calendering and injection moulding, the elevated temperatures required may result in inhalation exposure to the notified chemical, whether from vapours or aerosols.

The methods for product manufacture from plastisols include spread coating, under-body coating, sealing, rotational coating, dipping and slush moulding. Although the processes are largely automated and enclosed, incidental skin contact with the notified chemical may occur during transfer of plastisol from drums to the moulding equipment. Workers are expected to wear PPE including overalls, gloves and eye protection.

End-use of products

Under normal circumstances, dermal exposure to the notified chemical is not expected during handling of PVC products, as it is expected to be physically bound within the PVC matrix. Exudation may occur during any heating of plastics, leading to possible skin and inhalation exposure to low levels of the notified chemical.

Extension Application

Transport and storage of ink formulations containing the notified chemical at <40% is not anticipated to lead to exposure except in case of an accident.

Colour matching will initially be carried out in small amounts in a laboratory environment. Manual decanting and mixing of ink formulations containing the notified chemical at <40% may lead to dermal, ocular and inhalation exposure. However, this is expected to be minimised by the use of exhaust ventilation and personal protective equipment (PPE) including gloves, safety glasses, protective clothing and safety boots.

Colour mixing on a larger scale will involve manual decanting ink formulations containing the notified chemical at <40% into industrial mixers where they will be mixed before being packaged in plastic or metal containers ranging in size from 1 kg to 200 kg. Dermal, ocular and inhalation exposure may occur during blending. However, exposure is expected to be minimised by the use of natural or exhaust ventilation in the area where blending takes place. In addition, workers are expected to wear PPE including gloves, safety glasses, protective clothing and safety boots.

At customer sites, workers manually dispensing inks containing the notified chemical at <40% into offset printing machines using a spatula or via mechanical means may be exposed via the dermal and ocular routes. Workers may also experience exposure during cleaning and maintenance of offset printing equipment. However, exposure is expected to be minimised by the use of gloves, safety glasses and protective clothing. Inhalation exposure is not expected given the high viscosity of the finished inks.

Once the ink containing the notified chemical has been printed onto the surface of packaging, it will be bound in an ink matrix and therefore exposure is not expected.

Occupational exposure estimation

For dermal exposure of workers involved in handling of the notified chemical during compounding and/or product manufacture, assuming non-dispersive use with some intermittent direct contact, EASE exposure modelling estimates the dermal exposure to the notified chemical to be 0-0.1 mg/cm^2/day (EC, 2003). However, the use of EASE for accurately predicting dermal exposures is thought to be limited in accuracy (EC, 2003). The RISKOFDERM project, based on measurements of industrial exposures, describes exposure levels to the hands for the addition of liquids into "large containers (or mixers) with large amounts (many litres) of liquids" (Marquart *et al*, 2006). In this study, a typical case exposure was described as 0.5 mg/cm^2/scenario, though a reasonable worst-case exposure was described as 14 mg/cm^2/scenario. Therefore, based on a reasonable exposure frequency of once daily and a whole-hand exposure (420 cm^2) to a 60 kg adult, a typical dermal exposure of 3.5 mg/kg bw/day is assumed. Worst-case, infrequent (whole-hand) exposures may be as high as 98 mg/kg bw/scenario.

Assuming a closed system with LEV, a highest-probable process temperature of 220ºC (and excluding the possibility of aerosol formation), EASE estimates that the gas/vapour exposure to the notified chemical is likely to be 0-1.8 mg/m^3 (0-0.1 ppm) (EC, 2003). The same value is estimated for an identical system at 25ºC. Therefore as a worst-case estimate, a 60 kg adult male worker exposed to vapours with an inhalation rate of 25.5 m^3/12-hour shift during medium activity (EC, 2003), might experience inhalation exposure to the notified chemical of 0-0.77 mg/kg bw/day.

Therefore, excluding oral exposure and assuming 10% dermal and 100% inhalation absorption (EC, 2003), the typical exposure during handling of the notified chemical is estimated to be 0.35-1.12 mg/kg bw/day.

6.1.2. Public exposure

The notified chemical in its imported form will only be available to industrial customers, and not to the general public. The public may be exposed to the notified chemical from its applications in products such as wire, cable and automotive parts, but the most significant public exposure is likely to occur through ingestion of the notified chemical following its migration from food packaging into food.

Migration into foods

The notified chemical has undergone assessment by the European Food Safety Authority in September 2006 (EFSA, 2006). For this assessment, the specific migration of the notified chemical was measured using various food simulants and representative foodstuffs, under different storage conditions. The specific migration of 10-17.8% notified chemical in plasticised PVC cling film into food simulants and foodstuffs was determined using a validated Gas Chromatography/Mass Spectrometry (GC/MS) method (Otter, 2007):

Test Sample	*Food*	*Extractable fat in food (%)*	*Migration conditions*	*Specific migration (mg/dm²)*
Cling film *(thickness 14 µm, 17.8% notified chemical)*	Sunflower oil	100	6-144 hours/ 10°C & 20°C	29 ± 2
	10% ethanol	0	24 hours/40°C	0.016 ± 0.002
	Turkey (escalope/Schnitzel)	1.0 ± 0.5	5 days/5°C	0.3 ± 0.1
	Pork (neck)	11.3 ± 2.5	5 days/5°C	1.2 ± 0.2
	Pork (escalope/Schnitzel)	0.7 ± 0.3	5 days/5°C	0.14 ± 0.01
		1.8 ± 0.3	5 days/5°C	0.30 ± 0.01
	Pork (liver)	5.0 ± 0.1	5 days/5°C	0.11 ± 0.02
	High fat cheese (nom. 60% fat)	44.3 ± 2.6	10 days/5°C	27.5 ± 2.2
	Low fat cheese (nom. 20% fat)	2.9 ± 1.0	10 days/5°C	2.4 ± 0.7
Cling Film *(thickness 14 µm, 12.2% not. chem.)*	Pork (neck)	14.7 ± 2.9	5 days/5°C	1.0 ± 0.3
	Pork (bacon)	22.1 ± 2.7	5 days/5°C	1.4 ± 0.1
Cling Film *(thickness 14 µm, 10% not. chem.)*	Pork (neck)	17.9 ± 0.5	5 days/5°C	0.5 ± 0.1
	Pork (bacon)	25.81 ± 2.4	5 days/5°C	0.8 ± 0.3

The notified chemical was found to migrate into foods with high fat content (e.g. ≤29 mg/dm^2 into sunflower oil, and ≤27.5 mg/dm^2 into high fat cheese). The migration of the notified chemical into food like fresh meat and low fat cheese was lower than that of foods containing higher fat levels (<2.4 mg/dm^2). The level of notified chemical in fresh meat at equilibrium was found to be proportional to the starting concentration in the cling film and relative to the fat content of the foods. In fatty foods, migration to equilibrium was achieved after 6 hours of contact. Likewise, extraction studies from bottle closures using isooctane (in which the notified chemical is very soluble) show that it is able to extract an equilibrium concentration of the notified chemical after 5.3 hours.

For the use of the notified chemical in bottle sealing gaskets, artificial wine corks and beverage tubes, migration of the notified chemical into mineral water, grapefruit juice, soft drink or 15% ethanol was found to be very low (generally less than 0.11 mg/L, its solubility in 15% ethanol). This level of migration of the notified chemical is expected to apply for all aqueous foods (except alcoholic drinks with high ethanol content) as the low aqueous solubility of the notified chemical would limit its migration. Migration of the notified chemical from polystyrene (at the proposed use concentration) is expected to be lower than that from PVC. A test study using notified chemical-containing polystyrene sticks showed no migration of the notified chemical into olive oil or aqueous 10% ethanol (after 10 days at 40ºC) above the detection limit of the analytical method (unpublished study provided by the notifier). Very low levels of migration of the notified chemical from polystyrene into aqueous 50% ethanol were observed.

For conveyor belts, migration into solid or semi-solid foods is expected to be limited by contact area and short contact times. Computer modelling of fatty food with ≤30 minutes contact time on a conveyor belt containing 12% notified chemical estimates specific migration rates of 12.4 mg/dm^2 at 20ºC and 6.6 mg/dm^2 at 10ºC (Otter, 2007). Therefore, assuming that migration into most foods will be considerably less than migration into oil, and that only the bottom of food is in contact with the conveyor belt (1 dm^2/kg), the migration of the notified chemical is expected to be <5 mg/kg food for ≤30 minutes contact time.

Dietary exposure estimation

On the request of NICNAS, Food Standards Australia New Zealand (FSANZ) has estimated the probable exposure of members of the Australian public to the notified chemical. This estimation was based on Australian food consumption data from the 1995 Australian National Nutrition Survey (NNS), which sampled the 24-hour food intake of 13,858 respondents aged 2 years and older (ABS, 1999). This is considered to be a representative sample of the Australian population and, as such, a diversity of food consumption patterns was reported. Mean consumption figures for all respondents were used to allow addition of potential dietary exposures across different foods.

Food consumption values for various food groups were combined with migration data to provide an estimate of potential dietary exposure to the notified chemical. The total exposure value thus derived provides a worst-case scenario of potential dietary exposure to the notified chemical because it is based on: (1) maximum migration rates, (2) mean food consumption values for broader groups of foods than there were migration data (in some cases) and (3) the highest migration rate where there were several for one food. Estimated dietary exposures were expressed per kilogram of body weight, based on the mean body weight for all respondents in the NNS survey aged 2 years and above, which was 67 kg for the Australian population.

Food	*% Fat content (fresh product)*	*Specific migration (mg/dm^2)*	*Migration (mg/kg)**	*Food Consumption (kg/day)*	*Public Exposure (mg/kg bw/day)*	*% Contri-bution*
All Oils[1]	100	29 ± 2	174	0.026 [1]	0.068	84
Ethanol 10%	0	0.016 ± 0.002	0.096	0	0.000	0
Turkey (escalope)	1 ± 0.5	0.3 ± 0.1	1.8	0.037 [2]	0.001	1
Pork	22.1 ± 2.7	1.4 ± 0.1	8.4	0.029 [3]	0.004	< 1
Pork (liver)	5 ± 0.1	0.1 ± 0.02	0.6	0.001[4]	0.000	0
High Fat Cheese	44.3	27.5 ± 2.2	165	0.002	0.005	6
Low Fat Cheese	11.4	2.4 ± 0.7	14.4	0.016	0.003	4
Total Exposure					0.081	

* A conversion factor of 6 is applied, based on a 1 L cube with 6×1 dm^2 surfaces (i.e. 6 dm^2/L) (Svensson, 2002).

[1] Migration data based on sunflower oil, assumed true for all oils. Consumption value for all oils.

[2] Consumption value for all poultry.

[3] Consumption value for all pork meat and was assigned to the pork commodity with the highest migration data (bacon) to assume a worst-case scenario.

[4] Consumption value for all mammalian offal.

The assumptions made in this dietary exposure estimation included:

- where a specific food was analysed, the notified chemical was assumed migrate equally into all products in that food group (e.g. sunflower oil to all oils), where no other data was available for other foods in the same food group.
- where migration data were assigned to a food classification, all foods in that group were considered to contain the notified chemical;
- all the foods within the group were considered to contain the notified chemical at the levels specified;
- unless otherwise specified, the maximum migration level in each food category has been used;
- food consumption from the 1995 NNS survey represents current Australian food consumption patterns;
- consumers always select products containing the notified chemical;
- 1 L of a food is equal to 1 kg;
- where there were no Australian or New Zealand data on the notified chemical's migration levels into a particular food group, it was assumed that overseas data were representative of these food groups; and
- where a food was not included in the dietary exposure assessment, it was assumed to contain a zero migration of the notified chemical.

Therefore, the worst-case dietary exposure to the notified chemical, based on Australian consumption levels, is estimated to be 0.081 mg/kg bw/day. The primary sources of dietary exposure to the notified chemical were oils (84%) and high fat cheese (6%). It should be noted that while the notified chemical might not be used to package oils directly, this worst-case exposure estimate should include migration of the notified chemical into other foods containing free oils (e.g. continental goods or dressed salads).

One weakness of this exposure estimation is the absence of dietary exposure to the notified chemical from meats other than pork. Consumption of beef by the Australian public is slightly higher than that of pork on a population basis, but that of lamb is less. Overall, this oversight is not expected to result in significantly inaccurate dietary exposure estimate, as pork consumption only accounts for <1% of total exposure to the notified chemical.

Dermal exposure
Members of the public are likely to make limited dermal contact with food packaging, wires, cables and/or automotive parts containing the notified chemical. Significant exposure to the notified chemical in plastic products as a result of casual contact during handling is not expected, as it is expected to be sufficiently bound within the plastic matrix. However, as the notified chemical will not be chemically bound, it may be released from products in low levels over time (e.g. volatilisation from car upholstery). The expected dermal exposure from prolonged contact with plastics containing the notified chemical cannot be accurately estimated, but may be significant as the notified chemical may partition from the plastic into the skin over time.

Extension Application
Members of the public may make contact with packaging printed with ink containing the notified chemical. However, the notified chemical will be bound to the surface of the packaging in an ink matrix and therefore exposure is not expected.

6.2. Human health effects assessment

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

Endpoint and Result	*Assessment Conclusion*
Rat, acute oral	Low toxicity, LD_{50} >5000 mg/kg bw
Rat, acute dermal	Low toxicity, LD_{50} >2000 mg/kg bw
Rabbit, skin irritation	Slightly irritating
Rabbit, eye irritation	Not irritating
Guinea pig, skin sensitisation	No evidence of skin sensitisation
Rat, 28-day oral repeat dose toxicity	NOAEL 318 mg/kg bw/day (M), 342 mg/kg bw/day (F)
Rat, 90-day oral repeat dose toxicity	NOAEL 107.1 mg/kg bw/day (M), 389.4 mg/kg bw/day (F)
Rat, 2-year chronic toxicity/carcinogenicity	NOAEL 40 mg/kg bw/day (M), 200 mg/kg bw/day (F)
Rat, toxicokinetics and metabolism	Distribution to all organs and tissues was observed after rapid absorption. The oral bioavailability was calculated to be ~5-6% of a high dose and ~40-49% of a low dose, indicating saturation of gastrointestinal absorption. Accumulation was not observed in rats, and excretion was rapid, mainly via the faeces. Metabolism to several major metabolites: cyclohexanedicarboxylic acid (urine), monoisononyl cyclohexanedicarboxylate (faeces) & the

Endpoint and Result	*Assessment Conclusion*
	glucuronide of monoisononyl cyclohexanedicarboxylate (bile).
Rat, liver enzyme induction	The notified chemical is an enzyme inductor of phase I and phase II liver enzymes in both male and female rats.
Rat, cell proliferation study	Increased cell proliferation was observed in the liver and the thyroid glands after 1 and 4 weeks of treatment, but after 13 weeks no increases in cell proliferation were observed.
Rat, thyroid function study	Indirectly toxic to the rat thyroid.
Bacterial reverse mutation assay	Non-mutagenic
In vitro chromosome aberration assay	Non-clastogenic
In vitro mammalian cell gene mutation test	Non-mutagenic
In vivo mouse bone marrow micronucleus assay	Non-genotoxic
Rat, developmental toxicity	NOAEL 1200 mg/kg bw/day
Rabbit, developmental toxicity	NOAEL 1000 mg/kg bw/day
Rat, prenatal developmental toxicity	NOAEL 1000 mg/kg bw/day (for parental and F1 toxicity)
Rat, two-generation reproductive toxicity	NOAELs 1000 mg/kg bw/day (for parental and F2 toxicity), & 100 mg/kg bw/day (for F1 toxicity)

Toxicokinetics, metabolism and distribution

From the toxicology studies, the observed differences between oral administration (no systemic effects at 5000 mg/kg bw) and intraperitoneal injection (apathy, reduced spontaneity at 2000 mg/kg bw) immediately suggests that the notified chemical is either not readily absorbed following an oral dose, or that it undergoes extensive first-pass metabolism.

Poor oral absorption was supported in both the toxicokinetics and the metabolism studies, where oral absorption of the notified chemical saturated at higher doses, resulting in the majority of the notified chemical being recovered in faeces (84-100% for animals treated with 1000 mg/kg bw). The oral absorption that occurred was rapid (maximum after 1-2 hrs), but little indication of significant first pass metabolism was observed. The majority of faecal notified chemical was found to be excreted unchanged, and the level of metabolites in urine or bile represented only a small fraction of the administered dose.

None of the available data suggests that systemic absorption of the notified chemical can occur across the skin. Given its lipophilicity, the notified chemical may be taken up by the stratum corneum, but significant absorption it is not expected (EC, 2003).

Following gastrointestinal absorption, the notified chemical distributed to most bodily tissues within 1-8 hrs (highest levels were found in the gastrointestinal tract, adrenal glands, and liver; the lowest levels in brain, muscle, and bone), but it was not found to bioaccumulate. The plasma half-life of the notified chemical was found to be 4.4-11.9 hrs (depending on the administered dose), and its elimination exhibited biphasic kinetics. Excretion of the metabolites of any absorbed notified chemical was approximately equal into urine and bile.

The main metabolites of the notified chemical observed in metabolic studies in rats were the monoisononyl ester of cyclohexanedicarboxylic acid (eliminated through the bile) and cyclohexanedicarboxylic acid (eliminated in the urine). Glucuronide conjugates of the monoester of the notified chemical were observed in bile, and some evidence suggests that minor oxidative metabolites may undergo sulfate conjugation (from urine). While the notified chemical was found to be an inducer of metabolic liver enzymes (see below), its own excretion was unaffected after repeated doses.

Qualitatively, some similarities and some differences are observed between the metabolism, distribution and elimination patterns of the notified chemical and diisononyl phthalate (DINP) (NICNAS, 2007). The two chemicals are similar in structure, except that the notified chemical lacks the aromatic ring structure of DINP. Aromatisation of various derivatives of cyclohexanecarboxylic acid has been observed in liver from rat, guinea pig, rabbits and mice (Svardal and Scheline, 1985). This aromatisation activity was dependent on the presence of the carboxylic acid group, but 1,2-cyclohexanedicarboxylic acid (similar to the notified chemical) was unable to be aromatised. While this study used *trans*-cyclohexanedicarboxylic acid (c.f. predominantly *cis*- in the notified chemical), extrapolation from the results of the metabolism and toxicity studies provided by the notifier support similar conclusions for the notified chemical—i.e. no phthalates were observed in the metabolism study. The notified chemical is not expected to be able to aromatise to form DINP (or its metabolites) *in vivo*.

Acute toxicity

The notified chemical is not expected to be acutely toxic by any route of administration. No significant effects were observed after single large doses in the acute oral and dermal toxicity studies, and only minor systemic effects were observed after intraperitoneal injection in the *in vivo* micronucleus study.

Irritation and Sensitisation

The notified chemical is expected to be at most a weak skin irritant. It does not contain any known structural alerts for skin irritation potential, other than its surface-activity (Hulzebos *et al*, 2005). Moderate erythema was observed up to 72 hours in the acute dermal irritation study, and mild erythema was also observed in the acute dermal toxicity study. These results were not, however, of sufficient severity for the classification of the notified chemical. Only minimal irritation was observed in the eye irritation study.

The notified chemical contains no structural alerts for sensitisation (Barratt *et al*, 1994), was negative in a guinea pig maximisation study, and is therefore not considered to be a skin sensitiser.

Repeated Dose Toxicity

Liver effects and enzyme induction

Treatment of test animals with the notified chemical was found to result in increases in liver weights in the 90-day and 2-year repeated dose studies, in the cell proliferation study, and in the 2-generation study. Other signs of liver effects in these studies included elevations of serum γ-glutamyltransferase activity and decreased serum bilirubin concentrations. No histopathological evidence of liver toxicity was observed.

This spectrum of treatment-related effects is known to result from hepatic enzyme induction. To show that the notified chemical was able to induce liver enzymes, two special studies were carried out. These studies showed that (1) the notified chemical is an inducer of both phase I and phase II enzymes in the liver, and (2) that treatment of rats with the notified chemical induces cell proliferation in the liver that accounts for the increased organ weights observed.

Therefore, any observed effects that are thought to result from liver enzyme induction are interpreted to be adaptive metabolic changes, and not pathological changes.

Thyroid effects

The 2-year combined chronic toxicity/carcinogenicity study with the notified chemical revealed effects on the thyroid as the most significant adverse effect. The key findings were increased absolute and relative thyroid weight, altered thyroid colloid, and an increase incidence of thyroid follicular cell adenomas at 24 months. Thyroid follicular cell proliferation and changes in TSH levels were also observed at comparable dose levels in the 90-day rat study, in a 13 week cell proliferation study, and also in female rats in the 2-generation reproduction toxicity study.

The notifier has argued that these thyroid effects are not significant for human health risk assessment because of differences between rats and humans in thyroid hormone handling and sensitivity to thyroid-disturbing mechanisms. This conclusion is reasonable, and consistent with EFSA and IARC opinion on the significance of thyroid follicular cell tumours induced by chemicals which alter thyroid hormone metabolism and which demonstrate a lack of genotoxic potential (IARC, 1999; Rice *et al*, 1999; EFSA, 2006). It is reasonably well established that in the rat, thyroid follicular-cell tumours are commonly associated with imbalances in TSH levels resulting in sustained stimulation of the thyroid gland by TSH feed-back stimulation of the hypothalamic-pituitary-thyroid axis, leading to secondary hyperplastic or neoplastic changes with thyroid adenoma or carcinoma formation. The human thyroid gland is much less susceptible to this pathological phenomenon than rodents (Capen, 1997). Even in patients with markedly altered changes in thyroid function and elevated TSH levels, there is little if any increase in the incidence of thyroid cancer (Curran and DeGroot, 1991; Capen, 1997). The increased sensitivity of the rodent thyroid gland to perturbations by drugs and chemicals is related to the shorter plasma half-life of thyroxine (T4) in rodents (12-24 h) when compared to humans (5-9 days), due to the considerable differences in the transport proteins for thyroid hormones between species (Capen, 1997). In humans, serum T4 is bound primarily to thyroxine-binding globulin, a protein that is not present in rodents.

The proposal that thyroid effects of the notified chemical in rats are associated with an indirect mechanism was supported by the performance of special mechanistic studies. These demonstrated that, at relevant dose rates in rats, hepatic metabolic pathways involved in T4 conjugation are strongly induced, and that T3, T4 and FSH levels are perturbed in a manner consistent with an indirectly acting enzyme inducer (phenobarbital). The effects observed were not comparable to those associated with a direct inhibitor of iodine incorporation into thyroid hormones (propylthiouracil). The effects of the notified chemical on thyroid hormone metabolism are also not unlike those of polyhalogenated biphenyls, which increase the glucuronidation of T4 and increase TSH, and thyroid uptake of iodine in rats, but less so in mice (Capen, 1997; Craft *et al*, 2002). What is not known (although it appears to be unlikely for the notified chemical), is whether hydroxylated metabolites can have a direct effect on thyroid hormone receptor-activated gene expression, as has been suggested for PCB metabolites (Kimura-Kuroda *et al*, 2007).

A dose-dependent increase in the incidence of altered thyroid follicular colloid (described as “flaky”) was observed in female animals in the 2-year study (at 12 months), in male rats after 13 weeks in the cell

proliferation study, and in female F1 rats in the 2-generation study (<1 year at terminal sacrifice). In another rat strain, Sprague-Dawley, changes in follicular colloid have been reported as a normal effect of ageing, beginning at 56 weeks of age (Rao-Rupanagudi *et al*, 1992). The increased incidence of flaky colloid observed in the 2-year study in both control and notified chemical-treated rats would be consistent with this mechanism after 24 months. However, an increased incidence of this effect in notified chemical-treated animals of ≤1 year in age (absent in control and low-dose animals) was reported in several studies. This effect is not known to be caused by liver enzyme induction. Therefore, as the nature and/or pathogenesis of the effect are not known, it cannot be considered as non-adverse. Altered colloid was observed at 300 mg/kg bw/day (F1 females) in the 2-generation study (NOAEL of 100 mg/kg bw/day).

Kidney effects
The kidney is a probable target organ for the toxicity of the notified chemical. Treatment-related increases in kidney weights were predominantly observed in male rats in the 90-day and 2-year repeated dose studies, in the cell proliferation study, and in the 2-generation study. While these increases correlate with the observation of male-only kidney cortical cell proliferation in the S-phase response study (although this finding was of questionable significance), increased kidney weights were also observed in female rats in the 90-day and 2-generation studies. No data was available regarding the reversibility of kidney weight changes.

Microscopically, deposition of α2μ-microglobulin was observed in the proximal tubules of the renal cortex in all treated males in the 90-day study, but not in the 2-year study. However, α2μ-microglobulin deposition is a rat-specific effect without relevance to the determination of human hazard. The treatment-related vacuolisation of the tubular epithelia of male F1 animals in the two-generation study is considered a relevant effect of treatment with the notified chemical, although what it may indicate is uncertain.

Degenerated epithelial cells were found in the urine of male rats in the 28- and 90-day studies. The notifier's toxicology laboratory reports that these effects are a transient effect in younger animals of the strain used, and has been observed following treatment with several structurally unrelated chemicals. This claim is supported by the fact that these effects were not observed in the 2-year study, or in animals over 20 weeks of age in the 2-generation study. However, in both the 28-day and the 90-day studies, these findings were reported as treatment-related adverse effects, and no data has subsequently made available to support this claim. In the 90-day study, mid- and high-dose animals also showed increased blood cells in urine at one measurement interval.

Increases in kidney weight may be a result of the notified chemical's ability to induce phase I and phase II metabolic enzymes in the kidney. However, such enzyme induction has only been demonstrated for the notified chemical in the liver, and xenobiotic treatment that induces liver metabolism may not induce induction of enzymes in the kidney (e.g. phenobarbital (Khan and Alden, 2002)). Similarly, cell proliferation resulting from treatment with the notified chemical has only been observed in males, yet increases in kidney weight were also observed in females. Therefore, in the absence of data on the notified chemical's ability to induce kidney metabolism, increases in kidney weight as a response to the notified chemical cannot be considered to be of no toxicological relevance.

Kidney effects were observed with an NOAEL of 40 mg/kg bw/day in the 2-year study and 100 mg/kg bw/day in the 2-generation study.

Lack of proliferative effects on peroxisomes
No peroxisome proliferative effects related to activation of the PPARα receptor were observed for the notified chemical (c.f. phthalate esters like DINP). No effects were observed on cyanide-insensitive palmitoyl CoA oxidase in the 90-day study, and no peroxisome accumulation was observed in any of the repeat dose oral toxicity studies. Also unlike DINP, the notified chemical caused no increase in the incidence of hepatic or pancreatic acinar cell tumours, nor did it appear to cause testicular degeneration (NICNAS, 2007).

Toxicity for Reproduction and Development
In four separate studies on the toxicity of the notified chemical on reproduction and development, no effects were observed on mating, male or female fertility, fecundity or gestational parameters, and it was found to be not teratogenic. All observed effects were restricted to general toxicity. The kidney effects observed only in the F1 generation (described above) were noteworthy.

No significant treatment-related effects on anogenital distance were observed in any of the reproductive toxicity studies, suggesting that the notified chemical does not possess endocrine disrupting effects of the kind seen with phthalate esters, e.g. the antiandrogenic effects observed for dibutyl phthalate (Mylchreest *et al*, 1998).

Genotoxicity
The notified chemical was found to be non-genotoxic in three different *in vitro* studies. In addition, an *in vivo* mouse bone marrow micronucleus study gave a non-genotoxic result (although no cytotoxicity to the target

tissue was observed, the notified chemical's distribution to the bone marrow was demonstrated in the toxicokinetics study). Overall, there were no genotoxic effects observed in any test using the notified chemical.

Carcinogenicity
An increased number of thyroid follicular adenomas were observed in the 2-year rat study, and this was proposed by the notifier to occur as a result of an increase in thyroid hormone (T3 and T4) metabolism due to liver enzyme induction by the notified chemical, (as described above). Based on the recommendations of the IARC, the absence of any genotoxic activity indicates that the thyroid follicular adenomas observed are a secondary effect to liver enzyme induction in the rat (IARC, 1999). Thus, these tumours are not considered relevant to human health risk assessment.

After 2 years of treatment, an increase in the number of fibroadenomas was observed in the mammary glands of high dose females. However, the significance of this increase may be discounted because the incidence was only marginally higher than historical data in this strain of rats, and there was no increased incidence of malignancies (adenocarcinomas). The incidence of this tumour in the control group was low in relation to these historical data.

No Observed Adverse effect Level (NOAEL)
The NOAEL of the notified chemical (after oral administration) is considered to be 40 mg/kg bw/day, based on the lowest tested dose (from the 2-year study) where an absence of significant kidney effects was observed.

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

6.3. Human health risk characterisation

6.3.1. Occupational health and safety
Transport and storage workers should only be exposed to the notified chemical in the event of an accidental spillage, and so are unlikely to experience any risk from the notified chemical.

Predominantly incidental dermal exposure can be expected during compounding of plastisols or during the manufacture of plastic products (given the types of duties carried out). The main compounding processes (e.g. dry blending) are expected to be largely automated and enclosed, so incidental inhalation exposures to vapours resulting from high process temperatures are probable.

The estimated typical exposure to the notified chemical during compounding and/or product manufacture is estimated to be 0.35-1.12 mg/kg bw/day (for all routes of exposure). Considering the lowest NOAEL of the notified chemical, 40 mg/kg bw/day, a Margin of Exposure (MOE) range of 35.9-114 is calculated. These MOE values indicate that additional exposure control measures may be necessary during long-term, repeated handling of the notified chemical or during activities around high temperature areas where it will be processed.

Single-event dermal exposures of up to 98 mg/kg bw/scenario are possible. However, the notified chemical is of low acute dermal toxicity (LD_{50} >2000 mg/kg bw) and so such exposure is unlikely to present an acute health risk. However, given that the notified chemical has slight skin irritant characteristics, the wearing of protective equipment would be recommended to prevent against such exposures.

The notifier has described the exposure control measures that are proposed at sites where the notified chemical is handled. These include:

- Adequate exhaust ventilation is expected to be applied during high temperature processes, as vapours of varying hazard are expected in these applications. Thorough ventilation is recommended at all sites where the notified chemical is handled.
- Worker PPE (including overalls, safety glasses, and protective gloves) is expected to be adequate to minimise most foreseeable dermal and ocular exposure.

Exposure to finished plastic articles containing the notified chemical is not expected to result in significant exposure to the notified chemical, and therefore any risk to workers handling these articles is expected to be commensurately negligible.

Given the notified chemical's non-hazardous nature, and the reported handling conditions, it is not considered to pose an unacceptable risk to occupational health and safety.

Extension Application
Given workers will be exposed to lower concentrations (< 40%) of the notified chemical in the new use than was previously assessed (> 99%), the circumstances in the extension application are not expected to impact on the original risk assessment.

6.3.2. Public health

Significant migration of the notified chemical is expected into packaged high-fat foods (≥20% fat content) with PVC cling films and other food packaging. These foods form part of the normal Australian diet, and therefore daily exposure to the notified chemical would be expected to a significant proportion of the population. Based on the available data, a significant proportion of any ingested notified chemical is likely to be systemically absorbed.

In order to estimate the risk associated with this ingestion, a comparison of the toxicological data with the worst-case exposure estimate gives:

NOAEL for kidney effects in rats	=	40 mg/kg bw/day
Safety factor (for extrapolation from animal data)	=	100
Tolerable Daily Intake (TDI)	=	0.40 mg/kg bw/day
Estimated worst-case, long-term dietary exposure	=	0.081 mg/kg bw/day
Estimated exposure as a percentage of TDI	=	20.3%

Therefore, despite numerous conservative assumptions (both in the exposure estimation and in the use of a safety factor from a NOAEL), the expected worst-case public exposure is considered to be acceptable – much lower than a level that might be expected to produce adverse effects. Even accounting for any apparent weaknesses in the exposure estimation, the TDI is unlikely to be exceeded. In addition, as the exposure estimation was well below the TDI, the 'worst-case' dietary exposure estimation was not considered to require further refinement.

Dermal exposure to plastics containing the notified chemical is expected to be low, due to it being incorporated within the plastic matrix. Combined with the low dermal toxicity and low irritant and sensitisation potential of the notified chemical, dermal exposure of the public is unlikely to pose any significant risk to public health, even after prolonged exposure.

In conclusion, the notified chemical is not considered to pose a significant risk to public health at the levels of exposure that are estimated to result from its proposed use.

Extension Applicant

The public are not expected to be exposed to the notified chemical in a bioavailable form from the new use, hence the circumstances in the extension application will not impact on the original risk assessment.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE

Import and Transport

Release of the notified chemical to the environment during importation and transport of the notified chemical is expected to be minimal, unless exposure occurs as a result of accidental spillage.

The notifier estimates that at the maximum import volume, less than 1000 kg of the notified chemical would remain in the import containers. This will be either disposed of to landfill, with the bladders from flexitainers or drums, or as rinsings from isotainers and drums by waste disposal contractors.

Addition of Stabiliser

In some instances a stabiliser may need to be added to the imported product. This will be achieved by pumping the contents of an imported isotainer into a mixing vessel, adding the stabiliser, mixing and returning the product to the isotainer. The mixing vessel will be rinsed and the rinsate disposed of to the sewer. Assuming 30% of the import volume is stabilised, ~210 kg of notified chemical will be disposed of to the sewer with the rinsate.

PVC Compounding

There are two main methods of compounding for processing of PVC, dryblending and plastisol blending. Losses for these methods are described below. In addition to these sources of release, residues in containers may be released. Empty drums will be triple rinsed with washings processed to EPA regulations. IBCs and isotainers are expected to be reused.

- Dryblending will be conducted in lidded vessels. The method will be based on suspension or mass grade PVC and typically consists of mixing all ingredients with a high-speed rotating agitator that heats the

material by friction. Temperatures of 100-200ºC are reached and the liquid plasticiser will be completely adsorbed by the fine PVC powder grain. Residence times in the lidded blender will be of the order of 15 minutes, after which the hot blend will be dropped into a cooling blender for rapid cooling to avoid lumping. During this process the exposure of the hot material to open air will be small. Assuming one air exchange per run, the amount of emitted plasticiser is claimed to be 0.0037%. It is anticipated that these emissions would largely be trapped by local exhaust ventilations systems.

- Plastisol blending will take place in stirred vessels at ambient temperature. To avoid the development of high viscosities by swelling of the PVC particles due to plasticiser uptake, the vessels have to be cooled to remove the heat of friction. Any significant emission of plasticisers at ambient temperature is excluded (emission = 0%).

PVC Product Manufacture
An estimated 0.035% per annum of the import volume of notified chemical would be released into the environment due to the manufacture of PVC products. This release would primarily result from volatilisation during processing into finished articles.

Periodically, extrusion equipment will be cleared of off-grade polymer by a purging process. This purging process will account for approximately 0.4% of the waste notified chemical. The purged material would be recycled or collected and buried in an approved landfill as general waste.

Extension Application
Blending to customer colour specification involving decanting of the inks from containers, mixing of the inks with other ingredients and packaging of the blended products into purpose suited containers may result in releases to the environment. However, any release of the notified chemical in the ink products to the sewage, which is for the worst case scenario, is expected to result in partitioning of the notified chemical to sediment, based on the high log K_{OC} and the low water solubility of the notified chemical. Hence, no significant amount of the notified chemical is expected to remain in the effluent water.

RELEASE OF CHEMICAL FROM USE
Some recycling of PVC products will occur at specialised PVC recyclers (e.g. Cryogrind, Nylex SRM). Ultimately, however, the majority of the objects containing the notified chemical will be disposed of to landfill at the end of their useful life. As the notified chemical is not bound within the PVC matrix, it will be lost from PVC articles containing it. This release may occur through blooming followed by volatilisation or leaching.

Extension Application
Most of the notified chemical is expected to share the fate of the printed matrix of the container. Assuming that paper is the printing matrix (the most likely and conservative case scenario), it is commonly accepted that 50% of the waste paper will end up in landfill and the rest will undergo paper recycling processes. During recycling processes, waste paper will be repulped using a variety of chemical agents which, amongst other things, enhance detachment of ink from the fibres. Due to the high log K_{OC} (> 5.6) and the low water solubility (<0.00002 g/L at 25ºC) of the notified chemical, no significant amount of the notified chemical is expected to remain in the effluent water and be released to the water environment.

RELEASE OF CHEMICAL FROM DISPOSAL
The recommended method of disposal of liquid wastes containing materials such as the notified chemical is by burning in an approved incinerator.

7.1.2 Environmental fate

Endpoint	*Result*	*Assessment Conclusion*
Ready biodegradability	41% in 28 days	Not readily biodegradable
Bioaccumulation	30-day BCF = 189.3 (14-day exposure + 16 day depuration)	Not likely to bioaccumulate

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

7.1.3 Predicted Environmental Concentration (PEC)

The majority of the notified chemical will be incorporated into impact modified food packaging (85%) and in general applications such as wire and cable, automotive, plastisols and other similar applications. During the

lifetime of the articles, the notified chemical may be released from the article either through blooming (movement to the surface of the plastic) followed by evaporation or through leaching.

Wastes generated during compounding with PVC or manufacture of plastic articles will enter either landfill or the sewage system. Simple treat modelling of the notified chemical indicates that 26% will be released to air, 3% to water, 68% to sludge and 1% degraded resulting in 69% removal during passage through a sewage treatment plant (EU, 2001).

The half-life in air through reaction with hydroxide radicals is determined using the AOP program produced by Syracuse Corporation. The following values were generated using the EPIWIN modelling on the notified chemical:

Compartment	Half-life
Air	8.35 hours
Surface water	360 days
Soil/aerobic sediment	720 days
Anaerobic sediment	3240 days

It is anticipated that the notified chemical would display similar half-lives in each of the environmental compartments, and potentially be persistent in some soils and sediments due to it being not readily biodegradable.

7.2. Environmental effects assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish toxicity*	96-hour LC_{50} >100 mg/L	Not toxic to fish
Daphnia Toxicity (acute)	48 h LC_{50} >100 mg/L WAF**	Not toxic to *Daphnia*
Daphnia Toxicity (chronic)	21-day NOEC = 0.021 mg/L	Not toxic to *Daphnia*
Algal Toxicity	72-hour E_rC_{50} >100 mg/L WAF**	Not toxic to algae
	72-hour E_bC_{50} >100 mg/L WAF**	
Inhibition of Bacterial Respiration	180 min EC_{50} >1000 mg/L	Low toxicity to bacteria
Earthworm Toxicity	14-day LC_{50} >1000 mg/kg	Not toxic to earthworms
Emergence and growth of higher plants	20/21-day EC_{50} >1000 mg/kg	Not toxic to higher plants

* Nominal concentration contained considerable undissolved substance.
** The amount of notified chemical present in solution was not determined.

7.2.1 Predicted No-Effect Concentration

Based on the ecotoxicity data provided, the notified chemical is not toxic up to the limit of water solubility. Therefore, a PNEC could not be calculated.

7.3. Environmental risk assessment

The major applications for the notified chemical will be as a plasticiser and impact modifier in food packaging (85%) and in general applications such as wire and cable, automotive, plastisols and other similar applications (15%). The maximum process temperature during the manufacture of food-contact materials containing the notified chemical is ~220°C, well below the thermal decomposition temperature of ~351°C. Therefore, no thermal decomposition is expected under usual processing conditions. The notified chemical may also be used in traditional use functions. These include as a plasticiser for polyvinyl chloride (PVC) and vinyl chloride copolymers. The end use products containing the notified chemical include automobile undercoating, building materials, wires, cables, shoes, carpet backing, pool liners and gloves.

Once the chemical has been incorporated in plastic articles the majority of the notified chemical is expected to remain within the plastic matrices. Hence, the majority of the notified chemical will share the fate of the articles into which it is incorporated. It is anticipated that these will be disposed of to landfill at the end of their useful lifetime. The notified chemical is not expected to leach from landfill. There may also be some recycling.

The recommended method of disposal of wastes containing the notified chemical is incineration. Any incineration of the notified chemical will result in the formation of water vapour and oxides of carbon.

Some blooming and subsequent evaporation or leaching of the notified chemical may be anticipated during the

useful lifetime of the articles into which it has been incorporated. These releases are expected to be dispersed in nature and at low levels. Any material partitioning to the air through evaporation would also rapidly degrade through reaction with hydroxyl radicals.

The above considerations indicate acceptable risk to the environment when the notified chemical is used in the manner and levels indicated by the notifier.

Extension Application
The new use of the notified chemical as a component of finished inks for printing of food and non food cartons is not likely to result in significant additional environmental exposure for this chemical. Therefore, the notified chemical is not considered to pose an unreasonable risk to the environment.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available data the notified chemical is not classified as hazardous under the NOHSC *Approved Criteria for Classifying Hazardous Substances*.

and

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

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

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

Environmental risk assessment
The chemical is not considered to pose a risk to the environment based on its reported use pattern.

Risk assessment relating to extension applicant
The new use and environmental fate described in the extension application are not expected to impact on the original human health and environment risk assessment and recommendations.

Recommendations
CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following engineering controls to minimise occupational exposure to the notified chemical where the liquid imported product and/or formulated products containing it are handled during mixing and blending operations:
  - *Ensure adequate local ventilation*

- Employers should implement the following safe work practices to minimise occupational exposure during the handling of the notified chemical as introduced and in liquid formulations:
  - *Avoid direct skin contact*

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

  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 NOHSC *Approved Criteria for Classifying Hazardous Substances*, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Environment

- Do not empty the notified chemical into drains.

Disposal

- The notified chemical should be disposed of by incineration or landfill in accordance with the local regulations.

- Packaging that is contaminated with the notified chemical should be emptied, thoroughly cleaned and recycled.

Emergency procedures

- Pick up spilled material with suitable absorbent material. Dispose of absorbed material in accordance with local regulations.

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

(1) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from a plasticiser or impact modifier for PVC or polystyrene, a component of industrial printing inks or is likely to change significantly;
  - the amount of chemical being introduced has increased from 2300 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.

No additional secondary notification conditions are stipulated.

Material Safety Data Sheet
The MSDS of the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the MSDS remains the responsibility of the applicant.

Extension Application
The extension applicant has provided an MSDS of a product containing the notified chemical which was reviewed by NICNAS. The accuracy of the information on the MSDS remains the responsibility of the extension applicant.

APPENDIX A: PHYSICO-CHEMICAL PROPERTIES

Melting Point/Freezing Point No melting point.
Glass transition temperature = <-90ºC
Pour point = -54ºC

METHOD OECD TG 102 Melting Point/Melting Range.
Remarks Test was not conducted, as the test substance could not be prompted to crystallise. A glass transition occurred at slightly below -90ºC.
TEST FACILITY BASF (1999a)

Boiling Point >351°C at 101.3 kPa
394°C (calculated)

METHOD EC Directive 92/69/EEC A.2 Boiling Temperature.
Remarks The notified chemical decomposed before boiling at ~351°C (determined by differential scanning calorimetry). The boiling point was obtained via extrapolation.
TEST FACILITY BASF (1999a)

Density 947.2 kg/m^3 at 20ºC

METHOD OECD TG 109 Density of Liquids and Solids.
Remarks Determined using the pycnometer method.
TEST FACILITY BASF (1999a)

Viscosity 44-60 mPa.s at 20ºC

METHOD German Standard method DIN 51562/D 445
Remarks Determined by calculation from the measured kinematic viscosity. Further details of experiment are not known.
DATA SOURCE BASF Technical data sheet "Hexamoll DINCH"

Vapour Pressure 2.2×10^{-8} kPa at 25ºC
8.9×10^{-7} kPa at 50ºC

METHOD EC Directive 92/69/EEC A.4 Vapour Pressure.
Remarks The vapour pressure was determined by extrapolation from measurements at 43.6ºC to 118.2ºC. No details of how these were derived are available. The substance is very slightly volatile (Mensink *et al*, 1995).
TEST FACILITY BASF (1999a)

Water Solubility <0.00002 g/L at 25ºC

METHOD EC Directive 92/69/EEC A.6 Water Solubility.
Remarks Column Elution Method. Quantitative analysis was by gas chromatography.
TEST FACILITY BASF (1999a)

Solubility in other systems ≤0.0001 g/L (in 3% aqueous acetic acid)
0.00011 ± 0.00004 g/L (in 15% ethanol in water)
Soluble in tetrahydrofuran (THF), ethyl acetate, methyl ethyl ketone, toluene, acetone, and dimethylsulfoxide (DMSO)

Remarks Methods unspecified. Measurements carried out at room temperature.
REFERENCE Otter (2007)

Partition Coefficient (*n*-octanol/water) $logP_{ow}$ = >6.2 at 25ºC$logP_{ow}$ = 10.0 (calculated)

METHOD EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks HPLC Method. The retention of the notified chemical on the reverse-phase column was greater than that of DDT ($logP_{ow}$ = 6.2). Calculated using KVVWIN v1.51.
TEST FACILITY BASF (1999a)

Surface Tension 30.7 mN/m at 20ºC

METHOD German standard method DIN EN 14370
Remarks Details of experiment are not known. The notified chemical is considered to be surface active based on this result.
DATA SOURCE BASF Technical data sheet "Hexamoll DINCH"

Adsorption/Desorption Log K_{oc} > 5.6 at 23°C (measured)
$logK_{oc}$ = 5.82 (calculated)

METHOD OECD TG 121 Estimation of the Adsorption Coefficient (K) on soil and sewage sludge using High Performance Liquid Chromatography (HPLC).
Remarks As the retention time of the test substance was higher than that of reference substance (DDT) the value of log K_{oc} of the test substance was estimated as > 5.6.
TEST FACILITY BASF (2002a)

Flash Point 224ºC

METHOD German standard method DIN ISO 3016
Remarks Details of experiment are not known.
DATA SOURCE BASF MSDS "Hexamoll DINCH"

Flammability Not highly flammable

Remarks Not expected to be highly flammable, based on its physicochemical properties and experience in use.

Autoignition Temperature 330ºC

METHOD German standard method DIN 51794
Remarks Details of experiment are not known.
DATA SOURCE BASF MSDS "Hexamoll DINCH"

Explosive Properties Not explosive

Remarks The notified chemical is predicted to be not explosive based on its physicochemical properties and structural considerations.

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Notified chemical (99.7% pure)

METHOD: OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
EC Directive 96/54/EC B.l.tris: Acute Toxicity (Oral)
EPA / OPPTS Guideline 870.1100 Acute Oral Toxicity

- Species/Strain: Rat/Wistar chbb:thorn
- Vehicle: Olive Oil
- Remarks – Method: Initially, 5000 mg/kg bw was administered to three males. Due to the absence of mortality, the same dose was then given to three females .

RESULTS

Dose (mg/kg bw)	*Number and Sex of Animals*	*Mortality*
5000	3 per sex	0/6

- LD_{50}: >5000 mg/kg bw
- Signs of Toxicity: No signs of systemic toxicity were observed, and the expected weight gain was observed during the observation period.
- Effects in Organs: No abnormalities were found at necropsy of animals sacrificed at the end of the study.
- Remarks – Results: None.

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

TEST FACILITY: BASF (1999c)

B.2. Acute toxicity – dermal

TEST SUBSTANCE: Notified chemical (99.7% pure).

METHOD: OECD TG 402 Acute Dermal Toxicity.
EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal)
EPA / OPPTS Guideline 870.1200

- Species/Strain: Rat/Wistar chbb:thorn
- Vehicle: None (administered undiluted)
- Type of dressing: Semi-occlusive
- Remarks – Method: No significant protocol deviations.

RESULTS

Dose (mg/kg bw)	*Number and Sex of Animals*	*Mortality*
2000	5 per sex	0/10

- LD_{50}: >2000 mg/kg bw
- Signs of Toxicity - Local: Very slight erythema or well-defined erythema was observed in all female and male animals, after removal of the dressing.
- Signs of Toxicity - Systemic: No signs of toxicity were observed, and the expected weight gain was observed during the observation period.
- Effects in Organs: No abnormalities were noted at necropsy of animals sacrificed at the end of the study.
- Remarks – Results: None

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

TEST FACILITY: BASF (1999d)

B.3. Irritation – skin

TEST SUBSTANCE: Notified chemical (99.94% pure)

METHOD: OECD TG 404 Acute Dermal Irritation/Corrosion.
EC Directive 92/69/EEC B.4 Acute Toxicity (Skin Irritation)
US EPA OPPTS 870.2500 Acute Dermal Irritation

Species/Strain	Rabbit/New Zealand White A1077 INRA
Number of Animals	1 male, 2 female
Vehicle	None (0.5 mL liquid test substance applied undiluted)
Observation Period	14 days
Type of Dressing	Semi-occlusive.
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			
Erythema/Eschar	1.7	1.7	2.0	2	7 days	0
Oedema	0	0	0	0	0	0

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

Remarks – Results: Moderate erythema was observed in all animals immediately after removal of the patch and this persisted in two animals up to the 48-hour observation. Slight to mild erythema were observed in all animals at the 72-hour observation. These effects were reversible in two animals within 7 days of removal of the patch, and in the third animal within 14 days.

CONCLUSION: The notified chemical is slightly irritating to skin.

TEST FACILITY: BASF (2004a)

B.4. Irritation – eye

TEST SUBSTANCE: Notified chemical (99.7% pure)

METHOD: OECD TG 405 Acute Eye Irritation/Corrosion.
EC Directive 92/69/EEC B.5 Acute Toxicity (Eye Irritation).
US EPA OPPTS Guideline 870.2400 Acute Eye Irritation

Species/Strain	Rabbit/Himalayan Chbb:HM
Number of Animals	3 male
Observation Period	72 hours
Remarks – Method	No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	0.3	0.3	0.3	2	24 hours	0
Conjunctiva: chemosis	0	0	0	0	0	0
Conjunctiva: discharge	0	0	0	1	1 hour	0
Corneal opacity	0	0	0	0	0	0
Iridial inflammation	0	0	0	0	0	0

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

Remarks – Results: Conjunctival discharge was observed in one animal at 1 hour after application, but this effect was reversible within 24 hours. Conjunctival

redness was observed in all animals 24 hours after application. All observed effects were fully reversible at the 48-hour observation.

CONCLUSION The notified chemical is not irritating to the eye.

TEST FACILITY BASF (1999e)

B.5. Skin sensitisation

TEST SUBSTANCE Notified chemical (99.7% pure)

METHOD OECD TG 406 Skin Sensitisation
EC Directive 96/54/EC B.6 Skin Sensitisation

Species/Strain Guinea pig/Hsd Poc:DH

PRELIMINARY STUDY Maximum Non-irritating Concentration:
intradermal 5% (in olive oil)
topical 50% (in olive oil)

MAIN STUDY

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

INDUCTION PHASE Induction Concentration:
intradermal 5% (in olive oil or in a 1:1 mix of Freund's Complete Adjuvant/0.9% NaCl solution)
topical 100% (undiluted)

Signs of Irritation The intradermal induction caused moderate and confluent (grade 2) to intense erythema (grade 3) and swelling in all test animals. Partially open incrustation, in addition to moderate and confluent erythema (grade 2) and swelling were observed in all test animals after percutaneous induction.

CHALLENGE PHASE Test topical application: 50% (in olive oil)
Control topical application: olive oil

Remarks – Method Olive oil was applied as an additional vehicle control challenge to both test and control animals. The test substance was applied to both test and non-induced control animals.
The second control group (intended for a potential second challenge test) only received olive oil, as a second challenge using the test substance was deemed unnecessary due to the lack of ambiguity after the first challenge.

RESULTS

Group	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after:*			
		1st challenge		*2nd challenge*	
		24 h	*48 h*	*24 h*	*48 h*
Test	50% in olive oil	0/10	0/10	-	-
	Olive oil	0/10	0/10	-	-
Control	50% in olive oil	0/5	0/5	-	-
	Olive oil	0/5	0/5	0/5	0/5

Remarks – Results No skin reactions could be observed after the challenge for either the control group or the test group after 24 and 48 hours. As no borderline results were observed after the first challenge, a second challenge was not conducted.
Data provided for a historical positive control (α-hexylcinnamaldehyde) showed the appropriate positive result under equivalent test conditions.

CONCLUSION There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.

TEST FACILITY BASF (1999f)

B.6. Repeat dose toxicity (28 days)

TEST SUBSTANCE	Notified chemical (99.7% pure)
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents. EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral). Japan/MHW: 28-day repeated dose toxicity in mammalian species.
Species/Strain	Rat/Wistar CrI: WI (Glx/BRL/HAN)BR
Route of Administration	Oral – diet
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 14 days
Vehicle	None. The test substance was weighed and added directly to food.
Remarks – Method	The dose level was chosen based on the absence of toxicity observed in a dietary 14-day pre-test study.

RESULTS

Group	*Number and Sex of Animals*	*Dose in feed (ppm)*	*Equivalent dose (mg/kg bw/day)**		*Mortality*
			Males	*Females*	
Control	5 per sex	0	0	0	0/10
Low dose	5 per sex	600	64	66	0/10
Mid dose	5 per sex	3000	318	342	0/10
High dose	5 per sex	15000	1585	1674	0/10
Control recovery	5 per sex	0	0	0	0/10
High dose recovery	5 per sex	15000	1585	1674	0/10

* The equivalent dose was calculated as the mean daily test substance intake in mg/kg body weight over the entire study period.

Mortality and Time to Death

No mortality was observed during the treatment or recovery phases.

Clinical Observations

There were no effects, in any dose group, that were deemed to be treatment-related during the clinical observations, functional observational battery and motor activity measurements. The rearing reflex was found to be significantly decreased in males treated with 1585 mg/kg bw/day, but significantly increased in males treated with 318 mg/kg bw/day. Due to the lack of a dose-response relationship, this effect was considered to be incidental.

There were no treatment-related effects observed on food or water intake, or on body weight gain.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

At the end of the administration period, females treated with 1674 mg/kg bw/day showed a 50% increase in serum γ-glutamyltransferase activities. This effect was reversible upon cessation of treatment.

At the end of the administration period, males treated with 318 or 1585 mg/kg bw/day showed significantly increased serum sodium concentrations and all treated males showed increased potassium levels. These effects were reversible during the recovery period and therefore were not deemed to be toxicologically significant.

At the end of treatment, females treated with 1674 mg/kg bw/day showed reductions in total bilirubin serum concentration. All blood chemistry effects had recovered after the recovery period. No increases in serum levels of cyanide-insensitive palmitoyl-CoA oxidation were observed.

There were no treatment-related changes in the haematology parameters measured.

An increased number of degenerated epithelial cells were detected in the urine of male rats treated with 1585 mg/kg bw/day, which was reversible after the recovery period. No other treatment-related changes were observed in urine parameters.

Effects in Organs

A statistically significant decrease in body-weight relative heart weight (~8%) was observed in all treatment groups, although no effect was observed in absolute weight. These effects were considered to

be of no toxicological significance in the absence of any histopathological correlates.

Other effects (one unilateral ovarian cyst and two gastric ulcers) were considered to have arisen spontaneously.

Microscopic examination of liver did not show any signs of cell hypertrophy or any accumulation of liver peroxisomes.

Remarks – Results

The low (600 ppm) and mid (3000 ppm) dose treatment groups showed no substance related effects in either sex.

Doses of 15000 ppm caused changes in clinical chemistry parameters in animals of both sexes. Indications of mild renal function impairment (urinary epithelial cells, elevated serum Na^+/K^+) were observed in male rats. Female rats showed signs that may be associated with hepatic microsomal enzyme induction, characterised by stimulation of γ-glutamyltransferase synthesis and by increased excretion of bilirubin due to stimulation of phase II reactions.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) was established as 3000 ppm (318 mg/kg bw/day (males) and 342 mg/kg bw/day (females)) in this study, based on the absence of effects on clinical chemistry parameters at this intake level.

TEST FACILITY BASF (2000a)

B.7. Repeat dose toxicity (90 days)

TEST SUBSTANCE Notified chemical (99.6% pure)

METHOD OECD TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents.
EC Directive 87/302/EEC B.26 90-day repeated oral dose using rodent species.
FDA: Redbook II - Subchronic Toxicity Tests with Rodents and Non Rodents.

Species/Strain	Rats/Wistar CrlGlxBrlHan:WI
Route of Administration	Oral – diet.
Exposure Information	Total exposure days: 90 days Dose regimen: 7 days per week Post-exposure observation period: None
Vehicle	None. The test substance was weighed and added directly to food.
Remarks – Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose in feed (ppm)*	*Equivalent dose (mg/kg bw/day)**		*Mortality*
			Males	*Females*	
Control	20 per sex	0	0	0	0/20
Low dose	20 per sex	1500	107.1	128.2	0/20
Mid dose	20 per sex	4500	325.7	389.4	0/20
High dose	20 per sex	15000	1102.9	1311.8	0/20

* The equivalent dose was calculated as the mean daily test substance intake in mg/kg bw over the study period.

Mortality and Time to Death

No mortality was observed during the study.

Clinical Observations

Clinical examinations revealed no treatment-related findings. Several incidental findings were observed (alopecia, mydriasis, aggressiveness, piloerection), but these occurred in single animals only in both control and treatment groups and were thus not considered to be toxicologically significant.

No treatment-related effects were observed on food or water consumption, or on body weight gain.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Significant increases in γ-glutamyltransferase activities (~60%) were found in the serum of the high dose female rats, and this was considered to be treatment-related.

Significant, dose-dependent increases in serum thyroid stimulating hormone (TSH) concentrations were observed in both males and females, but these increases were only statistically significant in high-dose females. This effect is considered to be treatment-related.

An increased number of degenerated transitional epithelial cells were found in the urine of mid and high dose males. Blood was also found in the urine of these animals at day 29.

Effects in Organs

The mean body weight-relative kidney weights were significantly increased in both male and female high dose animals (~8-10%), and in male mid (~7%) and low (~8%) dose animals. In almost all male animals, α2μ-microglobulin was detected in the epithelia and tubular lumen of the promixal tubules of the renal cortex. This staining was dose-dependent and severe in mid and high dose males. While histopathological correlates could not be found with increased kidney weights in females of the high dose group, a relationship to treatment cannot be excluded.

Relative testis weights were increased in all dose groups (~4-7%), but no histopathological correlates were found. The investigators cite the lack of dose response (equivalent response at all three doses) as evidence that these changes were not treatment related.

Relative liver weight weights were significantly increased in all high dose animals (~6% in males and ~13% in females), and in females of the mid dose group (~6%). Relative spleen weights were found to be significantly increased in male rats of the mid dose (~7%) and high dose (~9%) groups. No histopathological changes were found to account for these effects.

Significant increases in mean absolute and relative thyroid gland weights were observed in both males and females of all dose groups (~21% for the high dose groups). The decreases in thyroid weights were considered to be incidental. Minimal to slight hypertrophy/hyperplasia of thyroid gland follicular epithelia was observed for male and female rats in all dose groups including the control group. The incidence of this effect was clearly dose-dependent in males: control (2/20), low dose group (14/20), mid dose group (11/20), high dose group (16/20). A similar dose-dependency were observed in female rats: control (1/20), low dose (1/20), mid dose (3/20), and high dose (15/20) groups.

Remarks – Results

No treatment related adverse effects were noted for the low dose group.

Increased γ-glutamyltransferase and TSH values, increased thyroid gland weights as well as hypertrophy/hyperplasia of the follicular epithelia of the thyroid gland all suggest a common pathogenesis of an enzyme induction process (Curran and deGroot, 1991). Hepatic enzyme induction is characterized by enlargement of the liver, which is followed by increases in liver enzyme activities like γ-glutamyltransferase in the serum. Liver enzyme induction results in increased catabolism of thyroxine, which leads to increased TSH levels through a physiological feedback mechanism. Increased TSH levels result in thyroid follicular hypertrophy. Supporting this hypothesis is the lack of treatment-related effects on serum thyroid hormone (T3 or T4) levels, despite the elevation of TSH. Thus, effects on the thyroid gland were considered to not be adverse, and rather a consequence of liver enzyme induction.

The treatment-related kidney effects, observed in both male and female animals, are considered to be adverse. The accumulation of α2μ-microglobulin observed in male animals might suggest a rat-specific effect, and this effect was not observed in female animals. On the basis of kidney weight changes in both sexes and the appearance of degenerated epithelial cells in the urine of males, the kidney effects are considered to occur at doses of 325.7 mg/kg/day (males) and at 1311.8 mg/kg bw/day (females).

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) was established as 107.1 mg/kg bw/day (males) and 389.4 mg/kg bw/day (females) in this study, based on kidney effects.

TEST FACILITY BASF (2002b)

B.8. Chronic toxicity/carcinogenicity

TEST SUBSTANCE	Notified chemical (99.6%)
METHOD	OECD 453 Combined Chronic Toxicity/Carcinogenicity Studies EC Directive 87/302/EEC B.33 Combined Chronic Toxicity/Carcinogenicity Test EPA OPPTS 870.4300; Combined Chronic Toxicity/Carcinogenicity Japan/MAFF: Combined Chronic Toxicity/Oncogenicity Study
Species/Strain	Rats/Wistar CrlGlxBrlHan:WI
Route of Administration	Oral – diet
Exposure Information	Total exposure: 24 months Dose regimen: 7 days per week Post-exposure observation period: None
Vehicle	None (administered in food)
Remarks – Method	10 animals per dose group of 60 animals made up a satellite group, which was sacrificed after 12 months. Clinical examinations (parameters include body weight, body weight change, food consumption and food efficiency) were conducted prior to the start of the administration period and weekly thereafter. Food consumption and body weight were determined weekly during the first 13 weeks, and at 4-week intervals thereafter. Signs of toxicity or mortality were examined at least once a day. Urinalysis, clinicochemical and hematological examinations were determined for satellite animals after 3, 6 and 12 months of the administration period.

RESULTS

Group	*Number and Sex of Animals*	*Nominal dose (mg/kg bw/day)**	*Mortality (% at 24 months)*	
			Males	*Females*
I (control)	60 per sex	0	20	32
II (low dose)	60 per sex	40	18	28
III (mid dose)	60 per sex	200	26	34
IV (high dose)	60 per sex	1000	14	24

*Dietary concentrations calculated to deliver the nominal dose rates were adjusted weekly during the first 13 weeks, and at 4-week intervals thereafter.

Mortality and Time to Death
Mortality was not adversely affected during the 24-month administration period.

Clinical Observations
No signs of toxicity were observed during clinical examinations.
The body weight and the mortality rate of the animals were not influenced by the administration of the notified chemical after 12 and 24 months. Food and water consumption were not affected by administration of the notified chemical. No treatment-related ophthalmoscopic findings were observed.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis
In all treated males, mean corpuscular volume (MCV) was significantly reduced (low dose, 3.2%; mid dose, 3%; high dose, 4.9%) and mean corpuscular haemoglobin (MCH) was slightly but statistically significantly decreased in the low dose (4.4%) and high dose (4.4%) groups on day 182 (6 months). Low and high dose male rats showed decreased MCV (3.2% and 4.4%, respectively) and MCH values (5.4% and 5.4%, respectively) at 12 months. Slightly but statistically significant increased red blood cell counts was found in mid dose (4.8%) and high dose males (7.2%) after 12 months. After 12 months, high dose females exhibited higher platelet counts (28% increase).

Increased alkaline phosphatase activity in the serum of high dose male rats after 12 months was detected (33%) after notified chemical administration. This was possibly indicative of mild and adaptive impairment of liver function. γ-glutamyltransferase activity was increased substantially in high dose females by 154% on day 181, and by 1450% on day 357. Decreased total bilirubin concentrations were detected in the high dose males on days 182 (28.4%) and 359 (22%). In the high dose females, decreased bilirubin concentrations were detected on days 96 (28.3%), 181 (25.6%), and 357 (40.4%). Hepatic enzyme induction in the rat is

characterised by stimulation of γ-glutamyltransferase activity in the liver and by increased excretion of bilirubin as a result of enhanced activation of "phase II" enzymes. As a consequence, treatment with the notified chemical induced an increase in γ-glutamyltransferase activities and a decrease in total serum bilirubin concentrations.

After 3 months of administration of the notified chemical, urinalysis revealed a significantly increased amount of degenerated transitional epithelial cells in the sediments of high dose males and granular and/or epithelial cell casts in the urine specimens of mid and high dose males. Since these effects were not apparent after 6 and 12 months of treatment, this finding was regarded as an adaptive change only and was temporary in nature. No relevant histopathological findings were seen in the kidneys after 12 or 24 months treatment.

Effects in Organs – General

Dose groups	*Males*			*Females*		
	Low	*Mid*	*High*	*Low*	*Mid*	*High*
Satellite groups (changes in absolute organ weights):						
Kidneys	+3.1%	+20.3%**	+14.2%**			
Liver	+5.2%	+15.9%*	+11.1%	+6.0%	+6.0%	+14.0%**
Thyroid glands				-18.5%*	-2.2%	+2.7%
Satellite groups (changes in relative organ weights):						
Kidneys	-3.8%	+8.0%*	+10.4%			
Liver				+11.5%	+11.7%**	+22.2%**
Final sacrifice groups (changes in absolute organ weights):						
Kidneys	-1.9%	+4.5%*	+3.1%*			
Liver	+0.8%	+6.7%*	+6.8%*	-1.0%	+6.7%	+13.8%**
Thyroid glands	-1.8%	+68.9%**	+52.4%**	+6.3%	+13.9%	+70.4%**
Uterus				-29.2%	-70.1%*	-77.5%**
Final sacrifice groups (changes in relative organ weights):						
Liver	-2.1%	+4.5%*	+1.3%	-2.5%	+4.9%	+14.6%**
Thyroid glands	0.0%	+71.4%*	+42.9%**	0.0%	+11.1%	+55.6%**

*p < 0.05; **p < 0.01

In the satellite groups (12 months sacrifice), the absolute kidney weight was increased in the mid and high dose male groups but not in the female treated groups. The absolute liver weight was also increased in the mid dose males and in the high dose females. The absolute thyroid gland weight was decreased in the low dose female group, but this is not considered treatment-related, as there was no dose-dependent effect.

In the final sacrifice groups (24 months), the mean absolute and relative weights of the thyroid glands were increased for the mid dose male rats and for both genders in the high dose group. The increased absolute kidney weights in the mid dose and high dose male groups were less marked than those seen at 12 months. There were increases in the absolute liver weights in the mid and high dose male groups and in the high dose females. The decreases in absolute uterus weights in the mid and high dose females are considered incidental due to the lesser number of tumours in the treated groups when compared to the control group.

In the satellite groups (12 months sacrifice), there was an increase in the relative kidney weight in the mid and high dose males but not in the female treated groups. Increased relative liver weights were observed in the mid dose and high dose females but not in the males. In the final sacrifice groups (24 months), there was an increase in the relative liver weight in the mid dose males and in the high dose female group. The relative thyroid glands weight was also increased in the mid and high dose males and in the high dose female group.

In the mid and high dose males, an increased number of animals with an enlarged thyroid were observed. In the high dose female group, the number of masses in the thyroid glands was slightly increased. There was an increased incidence of altered colloid in the thyroid gland, but only seen in females in the 12-month satellite groups.

After 24 months, the number of mid and high dose females with masses in the mammary gland was increased while the number of females with masses in the uterus was decreased in all dose groups. The lack of dose-response relationship suggests that the uterine effects were incidental and not treatment-related. In females, the number of foci in the liver was increased relative to controls, but there was no clear dose-response relationship, and this finding may have been unrelated to treatment. In males, there was a slight increase in

numbers of liver foci, but only in the mid- and high-dose satellite groups (12 months).

Dose Group (24 months)	*Males*				*Females*			
	Control	*Low*	*Mid*	*High*	*Control*	*Low*	*Mid*	*High*
Thyroid gland								
enlarged	1	0	8	9	2	1	2	2
mass	3	1	3	2	0	1	1	4
altered colloid (12 mth)					0	5	3	8
Mammary gland mass					5	3	11	11
Uterine mass					11	5	9	3
Liver foci								
12 months	3	5	7	7				
24 months					5	10	20	11

Effects in Organs – Tumours

Dose Group (24 months)	*Males*				*Females*			
	Control	*Low*	*Mid*	*High*	*Control*	*Low*	*Mid*	*High*
Thyroid gland adenoma	3	5	11*	14**	1	3	3	9**
Hyperplasia, follicular cell	8	6	9	15	3	4	5	14
Mammary Gland								
adenocarcinoma					3	1	5	1
fibroadenoma					1	2	5	9**
Pancreas, adenoma islet cell	1	5	4	4	0	0	0	0

*p < 0.05; **p < 0.01

After 24 months of treatment, dose-related follicular cell hyperplasia and increased number of follicular adenomas were observed in the thyroid glands of male rats administered 200 mg/kg bw/day and in both genders administered 1000 mg/kg bw/day. These effects were clearly treatment-related and consistent with the increased thyroid gland weights in mid and high dose male groups and in the high dose female group.

There was a significant increase in the number of fibroadenomas in the mammary gland of high dose females after 24 months treatment. While the number of islet cell adenomas in the pancreas in treated males appeared to be increased, the changes were neither statistically significant nor dose-related. A further analysis (at the request of the US FDA) of the incidence of pancreatic and mammary fibroadenomas, using a different statistical approach in a Supplementary report confirmed the results of the main report. However, it was suggested that neither the pancreatic nor mammary fibroadenoma response should be attributed to treatment with the notified substance. The primary basis for discounting the toxicological significance of these tumours was that the incidence of mammary fibroadenomas in controls (2%) was low compared to historical control data (6-16.1%), the incidence in the high-dose group (18%) was only marginally higher than the historical incidence, and there was no increased incidence of malignancies (adenocarcinomas). The incidence of pancreatic islet cell adenomas was also within the historical control range (5-12%) as well as lacking a clear dose-response relationship in the current study.

Remarks – Results

The thyroid glands are clearly a target organ for the effects of the notified substance in rats. There was a dose-related increased incidence of follicular adenomas in the thyroid gland of mid and high dose male rats and high dose female rats. However, thyroid effects in rats are potentially secondary effects associated with liver enzyme induction and of limited relevance to humans. Such an indirect mechanism is plausible based on the findings of increased GGT activity and lower serum bilirubin levels in this study, and supported by further studies (see special studies below) on enzyme induction and cell proliferation.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) was established as 40 mg/kg bw/day (males) and 200 mg/kg bw/day (females) in this rat study, based on liver weight changes (both sexes) and kidney weight changes (males).

TEST FACILITY BASF (2005b), BASF (2006a)

B.9. Toxicokinetics

TEST SUBSTANCE	^{14}C-radiolabelled notified chemical (99.9%)
METHOD	OECD 417 Toxicokinetics EC Directive 87/302/EEC B.36 Toxicokinetics US EPA OPPTS 870.7485 Metabolism and Pharmacokinetics. Japan/MAFF Tests on In Vivo Fate in Animals, 2001

STUDY DESIGN AND OBJECTIVE

The study is intended to determine the absorption, distribution, elimination and biokinetics of the test substance in male and female Wistar rats after oral (gavage) and intravenous administration.

The following oral dose levels and experimental designs were used (test substance suspended in 0.5% aqueous carboxymethylcellulose and 1% Cremophor EL):

(1) Pre-test:	1000 mg/kg bw (2M and 2F)
(2) Blood/plasma levels:	50, 300 and 1000 mg/kg bw (4M and 4F per dose group)
(3) Balance/excretion:	20 and 1000 mg/kg bw (4M and 4F per dose group; also 4M and 4F for the 14-day repeat dose study)
(4) Tissue distribution:	20 and 1000 mg/kg bw (12M and 12F per dose group)
(5) Excretion via bile:	20 and 1000 mg/kg bw (4M and 4F per dose group)

The 20 mg/kg bw dose level was chosen because it was considered to be similar to the maximum of the expected human exposure range. The notified chemical was also dosed intravenously (by injection into the tail vein of 4M and 4F rats) at 3 mg/kg bw (in untreated rat plasma), for balance/excretion studies.

RESULTS

(1) Pre-test

No clinical signs were observed after a single oral dose, within a 48-hour observation period.

(2) Blood/plasma levels

Kinetic parameters were similar between the three dose levels and in both sexes. Absorption was rapid at all dose levels, with a maximal plasma concentration observed after 1-2 hours. In the mid and high-dose animals, a biphasic absorption was observed, with an initial peak occurring at 1 hour and a second at 4-8 hours after dosing. Thereafter, plasma concentrations declined rapidly and biphasically with half-lives of 4.4-11.9 hours.

An evaluation of AUC values (μg Eq.hour/g) showed that the total of absorption processes were saturated with increasing doses over the entire dose range investigated (e.g. increasing the dose from 300 to 1000 mg/kg bw only resulted in a 1.25-fold increase in AUC values, c.f. a >3-fold increase in dose).

(3) Balance/excretion

After a single oral dose of 20 mg/kg bw, mean total recoveries of radioactivity of 93.1% in males and 92.60% in females. Within 168 hours after administration about 30% (males) and 32% (females) of the administered radioactivity were excreted in urine. After 168 hours the total amount of radioactivity excreted via faeces was approximately 63% for males and 59% for females.

After a single oral dose of 1000 mg/kg bw, mean total recoveries of radioactivity were 92.01% in male rats and 99.42% in female rats. Within 168 hours after administration, 86.41% (males) and 93.55% (females) of the administered radioactivity were excreted via faeces. After 168 hours the total amount of radioactivity excreted in urine was found to be 5.37% for males and 5.30% for females.

No radioactivity was detected in exhaled air following any dose.

After repeated oral administration of the high dose (fourteen doses of 1000 mg/kg bw/day non-radiolabelled test substance followed by one dose of 1000 mg/kg bw radiolabelled test substance), the excretion pattern in both sexes was virtually identical to those after a single oral administration of 1000 mg/kg bw. This indicates that pre-treatment neither changed the excretion pattern nor time-course of excretion, and gives no indication that enzyme induction occurred.

After a single intravenous dosing of the radiolabelled test substance (3 mg/kg bw), mean total recoveries were 95.25% (males) and 93.12% (females). Within 168 hours, 43.45% (males) and 44.80% (females) of the total dose was found in urine, and 48.06% (males) and 42.95% (females) was found in faeces. These findings indicated that the test substance was excreted approximately equally into urine and bile.

The results of the balance and excretion experiments show that there was no gender difference with respect to

the excretion pattern at both dose levels. The time course of radioactivity found in urine and faeces indicates rapid excretion and confirms the results from plasma kinetics studies. Saturation of gastrointestinal absorption is indicated by the lower proportion of radioactivity excreted in the urine at the high dose (~5%) compared to the low dose (~30%) level.

(4) Tissue distribution
Tissue distribution radioactivity measurements were performed at 1, 8, 21 and 28 hours after oral administration of 1000 mg/kg bw, and at 1, 4, 9 and 16 hours after oral administration of 20 mg/kg bw. Generally, tissue radioactivity levels for both sexes were comparable at the respective time points and dose levels, and tissue concentrations declined similarly at both dose levels. The highest radioactivity was found in the gastrointestinal tract, adrenal glands, and liver; the lowest was found in brain, muscle, and bone.

For both dose groups and both sexes, the tissue concentrations of radioactive substance declined rapidly after reaching the peak serum concentration (1-8 hours post-dosing). Initial half-lives of radioactivity concentrations in plasma, kidney and liver were calculated to be 3-10 hours (in both male and female rats) with terminal half-lives of 32-74 hours. Initial half-lives of 7-20 hours were calculated for adipose tissue. Overall, the half-lives do not indicate a potential for accumulation of the test substance.

(5) Excretion via bile
Excretion via bile was determined by bile duct cannulation. Within 48 hours of administration, excretion of the test substance in bile was ~0.5% of the high dose (both sexes) and 5.93% (males) and 12.60% (females) of the low dose. There is indication that the saturation of biliary excretion occurs with increasing dose levels.

CONCLUSION
After rapid but incomplete absorption, the notified chemical distributes to all organs and tissues, with rapid excretion mainly via the faeces. The oral bioavailability of the notified chemical was calculated to be ~5-6% (high dose) and ~40-49% (low dose), based on the total excreted radioactivity. Saturation of gastrointestinal absorption was observed with increasing doses. The notified chemical showed little potential to accumulate in rats.

TEST FACILITY BASF (2003c), BASF (2005c)

B.10. Metabolism

TEST SUBSTANCE ^{14}C-radiolabelled notified chemical (>99% pure)

METHOD OECD 417 Toxicokinetics
EC Directive 87/302/EEC B. Toxicokinetics
US EPA OPPTS 870.7485: Metabolism and Pharmacokinetics
Japan/MAFF: Metabolism Animals

STUDY DESIGN AND OBJECTIVE
The objective of the study was to investigate the nature of the biotransformation products of a ring-radiolabelled test substance in excreta and bile of Wistar rats. The test substance was administered orally (suspended in 0.5% aqueous carboxymethylcellulose with 1% Cremophor EL), or intravenously (suspended in plasma of untreated rats).

The following doses and experimental design was used:

(1) *Metabolite pattern in urine and faeces extracts:* single oral dose of 20 or 1000 mg/kg bw (4M and 4F per dose group); repeated 1000 mg/kg bw/day for 14 days (4M, 4F); intravenous 3 mg/kg bw (4M, 4F).

(2) *Metabolite pattern in bile:* single oral dose of 20 or1000 mg/kg bw (4M and 4F per dose group).

Metabolite patterns were determined by radio-HPLC after oral dosing at 12-24 hours (urine and faeces) and at 0-12 hours (bile), and after intravenous administration at 0-48 hours (urine) and of 12-48 hours (faeces).

Metabolites were isolated from bile and purified for LC-MS/MS and NMR analysis. In the case of urine and faeces, the unfractionated sample (urine) or sample extract (faeces) was analysed by LC-MS/MS instead of isolating individual metabolites.

RESULTS

Metabolite pattern in urine

The radioactivity detected in urine represented between 0.6% and 4.0% of an orally administered dose. One predominant metabolite, cyclohexanedicarboxylic acid (51-75% of radioactivity present in urine) was identified in the urine of animals after single or repeated oral treatment. Two to five minor metabolites were also detected, although none exceeded 1% of the dose in the investigated fractions. These were tentatively identified as sulfate-conjugated oxidative metabolites of the cyclohexanedicarboxylic acid monoester.

After intravenous administration, the urinary metabolite patterns were very similar to those following an oral dose, with the cyclohexane dicarboxylic acid accounting for 16-17% of the dose (between 0-24 hours). The detected minor metabolites were qualitatively similar to those detected after oral administration.

Metabolite pattern in faeces

The unabsorbed, unchanged test substance accounted for 84-100% of the radioactivity in faeces extracts after oral administration (24-76% of the administered radioactive dose at the investigated time points), reflecting its low oral bioavailability. A small amount of cyclohexane dicarboxylic acid monoisononyl ester was detected in the faeces extracts of low dose animals.

Metabolite patterns in faeces after intravenous administration were different compared to those after oral administration. No unmetabolised test substance was detected, although numerous metabolites were detected. These included cyclohexanedicarboxylic acid monoisononyl ester, which accounted for approximately 3% of the administered dose over 12-48 hours. The residual metabolites were characterised as comprising oxidation/hydroxylation products of the monoester.

Metabolite pattern in bile

The LC-MS/MS data of bile identified two to four groups of peaks. The most prominent metabolite was identified as the glucuronic acid conjugate of the monoisononyl ester, which represented 54-65% of the radioactivity in the bile (3.75/7.60% of the dose for females/males). Small amounts of the monoisononyl ester were also detected. The third metabolic fraction was characterised to contain degradation products of the monoisononyl ester (with or without further conjugation) and other derivatives/conjugates that may lack both isononyl groups.

CONCLUSION

A metabolic pathway of the notified chemical was independent of the dose level or of the sex of test animals:

(1) Partial hydrolysis of the diisononyl ester of cyclohexane dicarboxylic acid to yield the monoisononyl ester;
(2) Two further metabolic transformations of the monoisononyl ester were observed:
    i. its direct glucuronidation along with oxidation/hydroxylation and subsequent conjugation;
    ii. the hydrolysis of the remaining ester bond to yield free cyclohexanedicarboxylic acid (which accounts for the acid being the predominant metabolite in urine).

After intravenous administration of the notified chemical, the same metabolic transformations were observed, although its metabolism to polar metabolites was efficient and complete – no unmetabolised diisononyl ester of cyclohexane dicarboxylic acid was detected in urine or faeces.

TEST FACILITY BASF (2005d)

B.11. Liver enzyme induction study

TEST SUBSTANCE	Notified chemical (99.7% pure)
METHOD	2-week Liver enzyme induction study (no official test guideline available) OECD GLP Principles used
Species/strain	Rat/Wistar CrlGlxBrlHan:WI
Route of administration	Oral - diet
Exposure information	Total exposure days: 2 weeks Dose regimen: 7 days per week
Vehicle	None. The test substance was weighed and added directly to food.
Remarks - Method	The notified chemical was administered to Wistar rats (5/sex) at a dietary concentration of 15000 ppm over 2 weeks to determine the potential of the test substance to induce hepatic liver enzymes. This dose level was ~50% higher

than the high dose administered in the 2-year chronic toxicity/carcinogenicity rat study.

Equivalent doses: males (1,418 mg/kg bw/day), females (1,568 mg/kg bw/day).

Upon completion of the study, the following parameters indicative of liver enzyme induction were examined:

- Cytochrome P450 (Cyt.P450)
- Ethoxyresorufin O-deethylase (EROD)
- Pentoxyresorufin O-dependentylase (PROD)
- Benzoxyresorufin O-debenzylase (BROD)
- 4-Methylumbelliferone glucuronyltransferase (MUF-GT)
- 4-Hydroxybiphenyl glucuronyltransferase (HOBI-GT)

RESULTS

The treated rats showed a significant increase in liver Cyt.P450 activity in both male and female rats (2.2 fold for both sexes). Male and female rats also showed significant increases in the activities of liver EROD (2.7 and 1.6 fold, respectively), PROD (30 and 43 fold, respectively), BROD (11 and 24 fold, respectively), MUF-GT (3.3 and 2.4 fold, respectively), and HOBI-GT (7.2 and 2.7 fold, respectively).

CONCLUSION

The notified chemical is an inducer of both phase I (oxidative) and phase II (glucuronyl transferase) metabolic pathways in livers of both male and female rats.

TEST FACILITY BASF (2005e)

B.12. Cell proliferation study

TEST SUBSTANCE	Notified chemical (99.6% pure)
METHOD	S-phase cell proliferation study (no official test guidelines available) OECD GLP Principles used
Species/strain	Rat/Wistar CrlGlxBrlHan:WI
Route of administration	Oral - diet
Exposure information	Total exposure duration: 1, 4 and 13 weeks Dose regimen: 7 days per week
Vehicle	None. The test substance was weighed and added directly to food.
Remarks - Method	This study was conducted to determine the effects of the notified chemical on S-phase cell proliferation in critical toxicity target organs in Wistar rats after administration in the diet for 1, 4 and 13 weeks. Cell proliferation (S-phase response) was assessed in liver, kidneys and thyroid glands using BrdU (5'-bromo-2-deoxyuridine), administered one week prior to necropsy using subcutaneously implanted osmotic minipumps.

Group	*Dose level (mg/kg bw/day)**		*Treatment duration (weeks)*	*Number and Sex of Animals*
	Males	*Females*		
Control	0	0	1	10 per sex
			13	10 per sex
Low dose	40	40	1	10 per sex
			4	10 per sex
			13	10 per sex
Mid dose	200	200	1	10 per sex
			4	10 per sex
			13	10 per sex
High dose	1000	1000	1	10 per sex
			4	10 per sex
			13	10 per sex

*The concentration (ppm) of the notified chemical in food was adjusted weekly by body weight and food intake.

RESULTS

Induction of cell proliferation was found in all three organs examined (liver, thyroid glands and kidneys).

Highest levels of proliferation were found after 1 week of treatment, but were less pronounced after 4 weeks, and approached control levels after 13 weeks of treatment. While increased cell proliferation was observed at all dose levels, the pattern of response was both organ- and sex-dependent.

Liver weight increases were observed in high dose males at 4 weeks, and at all dose levels in females at week 4 but only in the high dose group at week 1. There were no changes in absolute or relative weights for kidneys or thyroid glands, although data for thyroid glands for the mid dose and high dose males and high dose females was missing due to technical issues. An increase in liver cell proliferation was observed in male rats after 4 weeks for the low and high dose groups and after 1 week for the high dose group.

There was no cell proliferation observed in the kidneys of females at any dose or time period. In males, significant proliferation was observed in the mid and high dose groups at 1 and 4 weeks but not at week 13. These effects were primarily seen in the cortex. At 13 weeks, the only significant increased labelling was seen in the outer stripe of the medulla but only in the low dose group. The lack of a clear dose-response relationship for the 13-week kidney findings suggests that their toxicological significance is questionable.

A significant increase in cell proliferation in the thyroid glands, as measured by BrdU staining, was observed at all dose levels in both male and female rats at 1 and 4 weeks but not at week 13. The incidence of follicular cell hypertrophy was both dose- and time-dependent, as shown in the table below:

Observation	*Week*	*Dose groups (mg/kg bw/day)*							
		Males				*Females*			
		Control	*40*	*200*	*1000*	*Control*	*40*	*200*	*1000*
Follicular hypertrophy	1	2	2	2	7	0	1	3	5
	4	2	2	2	8	0	0	2	3
	13	1	3	10	9	0	0	0	10
Altered colloid	13	0	1	4	6	0	0	0	2

*10 rats examined per group (only 8 for 4-week male controls).

CONCLUSION
Cell proliferation induced by the notified chemical was observed in the liver and thyroid glands, and to a lesser extent in kidneys (males only). Liver and thyroid cell proliferative effects were apparent in all dose groups of both sexes, but mainly after 1 and 4 weeks of treatment. By week 13, the cell proliferation response had subsided. However, there was evidence of follicular cell hypertrophy, mainly in the mid and high dose groups of both sexes, which progressively increased towards 13 weeks of treatment.

TEST FACILITY BASF (2005f)

B.13. Thyroid function study

TEST SUBSTANCE Notified chemical (99.7%)

METHOD Thyroid function study in male Wistar rats using perchlorate discharge as a diagnostic test (no official test guideline available). OECD GLP principles used.

Species/strain	Rat/Wistar CrlHan:WI	
Number/sex of animals	6 males per group	
Route of administration	Oral – diet	
Exposure information	Dose:	15000 ppm (equivalent to 1301 mg/kg bw/day)
	Total exposure days:	4 weeks
	Dose regimen:	7 days per week
Vehicle	None. The test substance was weighed and added directly to food.	
Remarks - Method	The aim of the present study was to use the perchlorate discharge assay (PDA) to investigate if the effects of the test substance on the thyroid gland in male Wistar rats occur via a direct effect inhibiting the iodination in the thyroid gland or by indirect mechanisms (i.e. the liver).	
	The effects of the notified chemical were compared to a direct-acting chemical, propylthiouracil (PTU; 2000 ppm in the diet), which directly effects thyroid function by blocking iodide incorporation, and an indirectly-acting chemical, phenobarbital (PB; 1000 ppm in the diet), which increases TSH levels via	

enhanced T4 clearance from induction of liver glucuronyltranferase activity.

T3, T4 and TSH concentrations were determined from blood samples on day 27. After 4 weeks (day 29), the animals received 0.5 mL of radiolabelled NaI (^{125}iodide) by intraperitoneal (i.p.) injection, followed six hours later with an i.p. injection of either 0.9% saline solution or 10 mg/kg bw potassium perchlorate (3 rats per group). Rats were sacrificed 2.5 minutes after saline or perchlorate administration. Radioactivity was counted in the blood and thyroid to determine the ratio of ^{125}iodide between thyroid and blood.

RESULTS

Remarks - Results

Both phenobarbital (1000 ppm) and the notified chemical (15000 ppm) caused statistically insignificant changes in T3, T4 and TSH, a significant increase in thyroid weight and uptake of radiolabelled iodide uptake into the thyroid, and a significant increase in the ratio of ^{125}iodide measured in the thyroid versus the blood (phenobarbital: 57% and 77% with and without perchlorate treatment respectively; versus 134% and 28% for the notified chemical).

In contrast, PTU administration (2000 ppm) caused a significant decrease in T4 and T3 concentrations, a significant increase in TSH concentrations, a marked increase in thyroid weights, a significant reduction in ^{125}iodide uptake in thyroid after perchlorate administration, a discharge of ^{125}iodide after perchlorate administration, and a significant reduction (95% and 85%, with and without perchlorate) in the ratio of ^{125}iodide measured in the thyroid versus the blood.

CONCLUSION

The significant increase of ^{125}iodide uptake in the thyroid after administration of the notified chemical and the absence of radiolabelled iodide discharged after co-administration with perchlorate supports the finding that the notified chemical acts like phenobarbital, and indirectly promotes thyroid toxicity in the rat by inducing hepatic metabolic enzyme activities.

TEST FACILITY

BASF (2005g)

B.14. Mutagenicity – bacteria

TEST SUBSTANCE

Notified chemical (>99% pure)

METHOD

OECD TG 471 Bacterial Reverse Mutation Test.
EC Directive 92/69/EEC B.13/14
Pre-incubation test (test 1) and plate incorporation method (test 2).

Species/Strain

S. typhimurium: TA1535, TA1537, TA98, TA100.
E. coli: WP2 uvrA.

Metabolic Activation System

Aroclor 1254-induced rat liver S9 mix.

Concentration Range in Main Test

a) With metabolic activation: 20-5000 µg/plate (test 1)
4-2,500 µg/plate (test 2)
b) Without metabolic activation: 20-5000 µg/plate (test 1)
4-2,500 µg/plate (test 2)

Vehicle

Acetone

Remarks – Method

No significant protocol deviations. No preliminary test was reported.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Present				
Test 1	-	≥2,500	≥2,500	Negative
Test 2	-	≥2,500	≥2,500	Negative
Absent				
Test 1	-	≥2,500	≥2,500	Negative
Test 2	-	≥2,500	≥2,500	Negative

Remarks – Results	A weak bacteriotoxic effect was occasionally observed under all test conditions. The test substance did not lead to an increase in the number of revertant colonies, either with or without S9 mix.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	BASF (2000b)

B.15. Genotoxicity – *in vitro* chromosome aberration test

TEST SUBSTANCE	Notified chemical (>99% pure)
METHOD	OECD TG 473 In vitro Mammalian Cytogenetic Test. EEC Directive 92/69/EC B.10 In Vitro Mammalian Chromosome Aberration Test
Species/Strain	Chinese hamster (source of cultured cell line)
Cell Type/Cell Line	V79 cells
Metabolic Activation System	Phenobarbital/3-naphthoflavone-induced rat liver S9 mix
Vehicle	Acetone
Remarks – Method	The final concentration of cyclophosphamide (positive control) with metabolic activation was changed to 0.7 µg/mL (2.5 µM).

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period (hours)*	*Harvest Time (hours)*
Present			
Test 1	6.3, 12.5, 25*, 50*, 100*, 200*	4	18
Test 2	50, 100*, 200*, 300, 400*	4	18
Test 3	12.5, 25*, 50*, 100*, 200*, 400	4	28
Absent			
Test 1	6.3, 12.5, 25*, 50*, 100*, 200*	4	18
Test 3A	25*, 50*, 100*, 200, 500, 1000*	18	18
Test 3B	25*, 50*, 100*, 200, 500, 1000*	18	28

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Present				
Test 1	-	>200	≥200	Negative
Test 2	-	>400	≥100	Negative
Test 3	-	≥100	≥50	Negative
Absent				
Test 1	-	>200	≥100	Negative
Test 3A	-	>1000	≥100	Negative
Test 3B	-	>1000	≥100	Negative

Remarks – Results

No statistically significant increases in structural chromosomal changes or polyploid metaphase frequencies were associated with treatment, compared with concurrent controls.

A slight increase in chromosomal aberration frequency was observed at 200 in the presence of S9 in Test 1; however, this increase was not statistically significant and was not reproducible under similar conditions in Test 2. In addition, a single statistically significant increase in chromosomal aberration rate was observed at 100 in Test 2 in the presence of S9. This increase was within the range of historical

controls and was not observed at higher doses in the same study.

The positive controls produced the expected significant increases in the frequency of chromosomal aberrations, demonstrating the sensitivity of the experimental conditions employed.

CONCLUSION The notified chemical was not clastogenic to V79 cells treated *in vitro* under the conditions of the test.

TEST FACILITY RCC (2000)

B.16. Mutagenicity – *in vitro* gene mutation test

TEST SUBSTANCE Notified chemical (99.6% pure)

METHOD OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.
EC Directive 2000/32/EC B.17 Mutagenicity - *In vitro* Mammalian Cell Gene Mutation Test.

Species/Strain	Chinese hamster (source of cultured cell line)
Cell Type/Cell Line	CHO cells (substrain K1)
Metabolic Activation System	Aroclor 1254-induced rat liver S9 mix
Vehicle	Acetone
Remarks – Method	The dose range was selected on the basis of a pre-test for cytotoxicity, where no toxic effects were observed up to 5000 µg/mL, despite the presence of distinct test substance precipitation. In Test 1, the S9 to cofactors ratio was 3:7 in the S9 mix, whereas in Test 2, this ratio was 1:9.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Present				
Test 1	0, 312.5, 625, 1250, 2500, 5000	4 hrs	7-9 days	1 wk
Test 2	0, 312.5, 625, 1250, 2500, 5000	4 hrs	7-9 days	1 wk
Absent				
Test 1	0, 312.5, 625, 1250, 2500, 5000	4 hrs	7-9 days	1 wk
Test 2	0, 312.5, 625, 1250, 2500, 5000	4 hrs	7-9 days	1 wk

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Present	>5000			
Test 1		>5000	N.D.*	Negative
Test 2		>5000	N.D.*	Negative
Absent	>5000			
Test 1		≥1250	≥312.5	Negative
Test 2		>5000	≥312.5	Negative

*Not determined. Could not be determined due to precipitation of S9 mix in the culture medium.

Remarks – Results No changes in cell morphology (from completely attached, fibroblast-like cells) were observed during the study. While the test substance did not induce any increases in the frequency of mutant colonies in this study, precipitation of either the notified chemical or S9 mix (or both) were observed at all dose levels. Therefore, the effective concentration of test substance present in the culture medium throughout the experiment is not known, but is expected to be significantly lower than the nominal concentration. Any non-mutagenic conclusions for the notified chemical are dubious on the basis of this study alone.

The positive control substances produced the expected significant increases in the frequency of mutant colonies, demonstrating the sensitivity of the experimental

conditions employed.

CONCLUSION The notified chemical was not observed to induce mutations in CHO cells treated *in vitro* under the conditions of the test.

TEST FACILITY BASF (2001b)

B.17. Genotoxicity – *in vivo*

TEST SUBSTANCE Notified chemical (99.6% pure)

METHOD OECD TG 474 Mammalian Erythrocyte Micronucleus Test.
EC Directive 2000/32/EC Annex 4C

Species/Strain Mouse/NMRI
Route of Administration Intraperitoneal injection (10 mL/kg bw)
Vehicle Olive oil
Remarks – Method This study includes an extra observation time (2-4 hours), which differs from the original protocol.

Dose (mg/kg bw)	*Number and Sex of Animals*	*Sacrifice time (hours)*
0	6 males	24
0	6 males	48
500	6 males	24
1000	6 males	24
2000	6 males	24
2000	6 males	48
40 (CP)	6 males	24

CP=cyclophosphamide (positive control).

RESULTS

Doses Producing Toxicity All animals in the highest dose group (2000 mg/kg bw) showed evidence of toxicity (reduction in spontaneous activity, apathy) up to 24 hours after treatment with the test substance.

Genotoxic Effects No increase in micronucleated PCEs was observed in the bone marrow of treated animals. The positive control showed a substantial increase in the frequency of induced micronuclei, indicating that the test system was able to respond appropriately to this chemical.

Remarks – Results No decrease in the PCE/NCE ratio was observed at the maximum dose recommended by the test guideline, suggesting the absence of cytotoxic effects on the bone marrow. However, the notified chemical is expected to distribute to the bone marrow under the conditions of the test.

CONCLUSION The notified chemical was not found to be clastogenic or aneuploidogenic under the conditions of this *in vivo* mouse micronucleus test.

TEST FACILITY RCC (2001)

B.18. Developmental toxicity (rabbit)

TEST SUBSTANCE Notified chemical (99.6% pure)

METHOD OECD 414 Prenatal Developmental Toxicity Study
EC Directive 87/302/EEC B. Teratogenicity Test – Rodent And Non-Rodent

Species/Strain Rabbit/Himalayan Chbb:HM
Route of Administration Oral – diet.
Exposure Information Exposure period: day 6 – day 29 post insemination (p.i.)
Dose regimen: daily
Vehicle None (administered undiluted).

Remarks – Method	No significant protocol deviations. Implantation sites were observed at necropsy in 19-24 rabbits/group, and therefore a sufficient number of females for the purpose of the study were available.

RESULTS

Group	*Number of Animals*	*Dose (mg/kg bw/day)*		*Mortality*
		Nominal	*Actual (mean)*	
Control	25 females	0	0	0/25
Low dose	25 females	100	102.2	0/25
Mid dose	25 females	300	310.7	0/25
High dose	25 females	1000	1028.5	0/25

Mortality and Time to Death
There were no substance related mortalities in any of the dose groups. One low dose animal died on day 26 p.i. There were no findings at necropsy to explain the sudden death.

Effects on Dams
There were no substance-related effects on the does regarding food consumption, body weight, body weight change, uterine weights, corrected body weight change or clinical and necropsy observations up to and including a dose of 1000 mg/kg body weight/day.

There were no significant toxicological differences between the controls and the substance treated groups on the gestational parameters (i.e. conception rate, mean number of corpora lutea, total implantations, resorptions and live foetuses and foetal sex ratios) or in the values calculated for the pre- and post-implementation losses.

Effects on Foetus
There were no substance-related differences reported for the placental and foetal body weights. The external, soft tissue and skeletal examinations of the foetuses revealed no toxicologically relevant differences between the control and the substance treated groups.

Remarks – Results
Under the conditions of this study, the test substance elicited no signs of maternal toxicity, had no influence on gestational parameters and induced no signs of developmental toxicity up to 1000 mg/kg bw/day administered to pregnant Himalayan rabbits.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established to be 1000 mg/kg bw/day in this study, based on maternal and prenatal developmental toxicity.

TEST FACILITY BASF (2004b)

B.19. Developmental toxicity (rat)

TEST SUBSTANCE	Notified chemical (99.7% pure)
METHOD	OECD 414 Prenatal Developmental Toxicity Study EC Directive 87/302/EEC B. Teratogenicity Study – Rodent And Non-Rodent EPA OPPTS 870.3700: Prenatal Developmental Toxicity Study
Species/Strain	Rat/Wistar CrlGlxBrlHan:WI
Route of Administration	Oral – gavage.
Exposure Information	Exposure period: day 6 – day 19 post coitum Dose regimen: daily
Vehicle	Olive oil
Remarks – Method	No significant protocol deviations. Implantation sites were found at necropsy in 21-24 rabbits/group; therefore, a sufficient number of females for the purpose of the study were available.

RESULTS

Group	*Number of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	25 females	0	0/25
Low dose	25 females	200	0/25
Mid dose	25 females	600	0/25
High dose	25 females	1200	0/25

Mortality and Time to Death
There were no substance-related or spontaneous mortalities in all of the groups at all dose levels.

Effects on Dams
There were no substance-related effects on the dams concerning food consumption, body weight, body weight change, uterine weights, corrected body weight change, clinical and necropsy observations up to and including a dose of 1200 mg/kg body weight/day.

There were no significant toxicological differences between the controls and the substance treated groups on the gestational parameters (i.e. conception rate, mean number of corpora lutea, total implantations, resorptions and live foetuses and foetal sex ratios) or in the values calculated for the pre- and post-implementation losses.

Effects on Foetus
There were no substance-related differences reported for the placental and foetal body weights. The external, soft tissue and skeletal examinations of the foetuses revealed no toxicologically relevant differences between the control and the substance treated groups.

Remarks – Results
Under the conditions of this study, the test substance elicited no signs of maternal toxicity, had no influence on gestational parameters and induced no signs of developmental toxicity up to and including a dose of 1200 mg/kg bw/day administered to pregnant rats.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established to be 1200 mg/kg bw/day in this study based on maternal and prenatal developmental toxicity. This is above the limit dose of 1000 mg/kg bw/day.

TEST FACILITY BASF (2002c)

B.20. Pre-/postnatal developmental toxicity study

TEST SUBSTANCE Notified chemical (99.7% pure)

METHOD The method was based on the publication of Mylchreest *et al* (1998), with elements of the following Guidelines:
OECD 414 Prenatal Developmental Toxicity Study
OECD TG 415 One-Generation Reproduction Toxicity Study
EC Directive 87/302/EEC B. Teratogenicity Study
EC Directive 87/302/EEC B. One-Generation Reproduction Toxicity Test
EPA OPPTS 870.3800: Reproduction and Fertility Effects
EPA OPPTS 870.3700: Prenatal Developmental Toxicity Study

Species/Strain: Rats/Wistar CrlGlxBrlHan:WI
Route of Administration: Oral – gavage.
Exposure Information: Only F0 generation females
Exposure period: day 6 – day 20 post partum
Dose regimen: daily
Vehicle: Olive oil
Remarks – Method: The F0 females were allowed to litter and rear their pups until day 21 after parturition. At this time all male pups and up to 3 female pups per litter were selected and raised until days 100 to 105 post partum (with no additional exposure) and particularly examined for their sexual maturation (testes descending, day of vaginal opening/balanopreputial separation).

Anogenital distance measurements were performed on all live F1 pups on

day 1 after birth and the anogenital index (anogenital distance/pup weight) was calculated for all pups. Further more, all surviving male pups were checked for the presence of signs of areolae/nipples from day 12 until day 15 post partum.

The dose groups (10 females) were smaller than is recommended in the OECD Guidelines (20 females), but the same as those used by Mylchreest *et al* (1998).

RESULTS

Group	*Number of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	10 females	0	0/10
Low dose	10 females	750	0/10
High dose	10 females	1000	0/10

Mortality and Time to Death
There were no substance-related mortalities in any of the parental female rats in all of the doses tested.

Effects on Dams
The parental female rats showed no substance-related changes to food consumption during gestation and lactation. No test substance-related effects in organ weights, gross findings, reproductive performance and clinical examinations were observed for the parental female rats of both the 750 and 1000 mg/kg bw/day dose groups. Gestation, parturition and lactation of the parental female rats were unaffected by the administration of the test substance.

Effects on 1st Filial Generation (F1)
Clinical examinations, sexual maturation, organ weights, gross and histopathological findings and sperm motility all showed no indications of substance-related adverse effects.

There was a marginal (about 7-8% lower than the respective control values), but statistically significant decrease of the anogenital distance (AGD) in the high dose males and of the anogenital index (AGI) in high dose males and females. These were considered to be spurious, with no biological relevance because:

- all other corresponding sexual developmental parameters did not show any substance-related adverse effects;
- the female AGI was lowered to the same extent as the male AGI, which is contradictory for the reduction in AGI being an indicator of an impaired androgen-mediated development of the male reproductive tract; and
- the variability in the open literature (Clark, 1998) were considered to be similar to those seen in the present study.

In addition, any effects on sexual development/reproductive performance were investigated in the follow-up full-scale two-generation study (see below).

Remarks – Results
The findings of this pre-/postnatal developmental toxicity study of the notified chemical shows no indications that the test substance induced any adverse effects in the parental female rats. There were no indications of any developmental toxicity in the F1 pups in terms of data obtained during gestation and lactation. No substance-related clinical and pathological observations were made for the F1 progeny. The administration of the test substance to the parental female rats showed no influence on sexual organ morphology and sexual maturation of the selected F1 rats of both genders, or on sperm motility of the males.

CONCLUSION
Based on the conditions of this study, the No Observed Adverse Effect Level (NOAEL) for reproductive performance and systemic toxicity of the parental female rats is 1000 mg/kg bw/day.

The NOAEL for developmental toxicity (based on the growth and development of the offspring, including sexual organ morphology and sexual maturation) is also 1000 mg/kg bw/day for F1 progeny.

TEST FACILITY BASF (2002d)

B.21. Toxicity to reproduction – two generation study

TEST SUBSTANCE	Notified chemical (99.6% pure)
METHOD	OECD 416 Two-Generation Reproduction Toxicity Study EC Directive 87/302/EEC B35. Two-Generation Reproduction Toxicity Test EPA OPPTS 870.3800: Reproduction and Fertility Effects
Species/Strain	Rats/Wistar CrlGlxBrlHan:WI
Route of Administration	Oral – diet.
Exposure Information	Exposure period - female: Continuous until time of sacrifice Exposure period - male: Continuous until time of sacrifice Dose regimen: 7 days per week.
Vehicle	None (administered undiluted)
Remarks – Method	A technical error was found to have caused false positive vaginal smears for sperm in the F0 generation, which resulted in a high incidence of supposed male infertility of treated animals. This technical error related to contamination with rat sperm of the physiological saline solution used to prepare the vaginal smears. Therefore a second litter (i.e. F1B) was generated from the F0 generation.

Study design:

Weeks of study	*F0*	*F1*	*F2*
1-10	Exposure of F0 animals prior to first mating		
11-12	Mating period for F1A litters		
14-15		F1A born and litters culled on day 4 p.p. to 8 pups each	
17-18		F1A litters weaned day 21 p.p.; 25 of each sex selected for F1 parental generation; remaining pups sacrificed	
19-20	Mating period for F1B litters	Exposure of F1A animals prior to mating	
28-30	Necropsy of F0 adults (after birth and weaning of F1B litters)	Mating period for F2 litters	
31-32			F2 born and litters culled on day 4 p.p. to 8 pups each
34-35			F2 litters weaned day 21 p.p.; Necropsy of litters
36-37		Necropsy of F1 adults	

Dose groups

Generation	*Group*	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	
			Nominal	*Actual**
F0	control	25 per sex	0	0
	low dose	25 per sex	100	99-109
	mid dose	25 per sex	300	283-334
	high dose	25 per sex	1000	968-1101
F1	control	25 per sex	0	0
	low dose	25 per sex	100	94-101
	mid dose	25 per sex	300	271-301
	high dose	25 per sex	1000	942-1037

* Range of mean test substance intakes, which for females varied according to the test period (i.e. pre-mating, gestation, lactation)

RESULTS

Mortality and Time to Death

There were no substance-related mortalities in any of the male and female parental (F0) and F1 animals in any of the groups.

Effects on Parental (F0) animals:

The administration of the test substance at the dosed concentrations did not adversely affect the reproduction and delivery data of the F0 generation female animals.

The 1000 and 300 mg/kg bw/day F0 generation females showed increases in serum γ-glutamyltransferase activity, decreases in total bilirubin level. Increases in the absolute and relative liver and kidney weights of both genders were observed for the high and mid dose levels.

Effects on 1st Filial Generation (F1)

No substance-related differences occurred between the control and dose groups concerning viability and mortality of the F1 pups.

The relative and/or absolute liver and kidney weights of the F1 parental rats were significantly increased for all dose groups. F1 generation females treated with 1000 and 300 mg/kg bw/day showed increases in serum γ-glutamyltransferase activity and decreases in total bilirubin level. Decreased total serum bilirubin concentrations were also observed in 1000 mg/kg bw/day dose F1 males. Vacuolisation of the tubular epithelia was detected in the kidneys of all high dose males and 9/25 males in the mid dose group.

The absolute and relative thyroid weights for females were increased in the high dose group. Minimal to slight hypertrophy/hyperplasia of the follicular epithelia of the thyroid glands was recorded in 21/25 female rats of the high dose group and 10/25 female rats in the mid dose group. Also observed was minimal or slight (multi)focal accumulation of a flaky colloid within the lumen of the follicles of the thyroid glands in 12/25 female rats in the 1000 mg/kg bw/day high dose group and in 10/25 female rats in the 300 mg/kg bw/day mid dose group.

Effects on 2nd Filial Generation (F2)

No substance-related differences occurred between the control and dose groups concerning the viability and mortality of F2 pups for all dose levels.

Remarks – Results

Gross and histopathological findings did not indicate that the test substance adversely affected reproductive performance or fertility in the parental or first filial generation rats for all dose groups. There were no substance-induced signs of developmental toxicity in the progeny of F0 and F1 generation animals.

Clinical examinations for general signs of toxicity of the parental (F0) and first filial generation (F1) rats revealed no substance-induced effects for all doses of the test substance.

Clinical pathology results showing the increase in γ-glutamyltransferase activity and decreased total bilirubin levels are substance-related effects and are thought to occur as a result of the induction of the hepatic microsomal enzyme system. The increased liver weights and decreased bilirubin levels are expected to be at least partly due to this induction of hepatic microsomal enzymes. Effects due to induction of the microsomal enzyme system are interpreted as an adaptive metabolic response and are thus not considered as adverse.

Hypertrophy/hyperplasia of the follicular epithelia of the thyroid glands in the F1 generation females was considered to be a consequence of liver enzyme induction, as described in other studies, and thus is not considered to be an adverse effect of treatment.

The vacuolisation of tubular epithelia of the kidneys in the mid and high dose F1 generation males, and the observation of flaky colloid in lumen of thyroid glands follicles in the mid and high dose F1 generation females, are considered to be treatment-related.

CONCLUSION

Under the conditions of this two-generation reproduction study, the NOAEL for fertility and reproductive performance is 1000 mg/kg bw/day for F0 and F1 generation rats of both genders.

The NOAEL for general toxicity is 1000 mg/kg bw/day (F0 rats of both genders) and 100 mg/kg bw/day for the F1 male and female rats (based on tubular vacuolisation and flaky thyroid follicular colloid).

The NOAEL for developmental toxicity (growth and development of offspring) was 1000 mg/kg bw/day for the F1 and F2 pups.

TEST FACILITY BASF (2003a)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE	Notified chemical (test concentration 27 mg/L)
METHOD	OECD TG 301 B Ready Biodegradability: CO2 Evolution Test. EC Directive 92/69/EEC C.4-C Biodegradation: Determination of the "Ready" Biodegradability: Carbon Dioxide Test International Standard ISO 9439:1999 – Water Quality.
Inoculum	Activated sludge from laboratory wastewater plants treating municipal sewage. Concentration of dry substance was 30 mg/L.
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	CO_2 generation
Remarks - Method	Since the test substance is not sufficiently soluble in water, no DOC degradation was determined. The test was extended to 60 days to show continuing degradation over time.

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
7	4	14	73
14	10		
21	27		
28	41		
38	64		
49	76		
60	93		

Remarks - Results	The test substance is not readily biodegradable as the degradation of the test substance was below 60% at 28 days.
CONCLUSION	The notified chemical cannot be classed as readily biodegradable.
TEST FACILITY	BASF (2000c)

C.1.2. Bioaccumulation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 305 Bioconcentration: Flow-through Fish Test (adapted June 1996). US EPA, OPPTS 850.1730 Fish BCF
Species	Zebra fish (*Brachydanio rerio*)
Exposure Period	Exposure: 14 days Depuration: 16 days
Auxiliary Solvent	Acetone
Concentration Range	Nominal: 0.04 µg/L (low) 0.4 µg/L (high) Actual: 0.041 µg/L (low) 0.41 µg/L (high)
Analytical Monitoring	Liquid scintillation counter
Remarks - Method	The control group was set up as a solvent (acetone) control. ^{14}C-radiolabelled test substance was used. Fish samples were taken together with samples on 6 and 5 occasions during the uptake and depuration phase respectively and were analysed for the content of the test substance by measuring the total radioactivity in fish. No toxicity in fish was expected to the solubility limit of the test substance in water (<0.05 mg/L).

RESULTS

Bioconcentration Factor	189.3 based on the mean BCFss(184.9) and BCF_k (193.7).
DT_{50}	0.5 days (low concentration), 0.6 days (high concentration)
Remarks - Results	The BCF is reported for the whole organism.
	The lipid content was in a range between 2.3% and 3.6% over the entire uptake and elimination period.
	The fish in the concentration groups showed no change in appearance and behaviour in comparison with the control group. No mortality was observed over the total exposure and depuration period for all test groups.
	Steady state was reached within 3 days for both concentrations.
	Approximately 90% of the steady state concentration of the test substance was excreted after 1.5 days for the low concentration and 1.6 days for the high concentration, indicating a very fast depuration from the organism. The elimination could be sufficiently described by first order kinetics. Based on kinetic rate constants the bioconcentration factor was 213.7 in the lower concentration group and 173.7 in the higher concentration group. The mean value was 193.7.
CONCLUSION	The notified chemical has a bioconcentration factor of less than 200, indicating that it is not likely to bioaccumulate.
TEST FACILITY	BASF (2006b)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical .
METHOD	EC Directive 92/69/EEC C.1 Acute Toxicity for Fish – 96 hour, static.
Species	Zebra fish (*Brachydanio rerio*)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	Approx. 250 mg $CaCO_3$/L
Analytical Monitoring	Probit analysis was used to determine the LC_{50}.
Remarks – Method	The stability of the test substance in the test water was not determined.
	To prepare the test solution 1 g test substance was added to 10 L test water and the mixture was homogenised with ultra-turrax stirrer. The test solution was stirred for about 1 day before fish were placed into the aquaria to ensure that the limit of solubility was reached.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
0	-	10	0	0	0	0	0
100	-	10	0	0	0	0	0

LC_{50}	>100 mg/L at 96 hours.
NOEC	100 mg/L at 96 hours.
Remarks – Results	No mortality occurred at a concentration of 100 mg/L. No abnormalities or symptoms were observed in the test fish during the test period.
	Undissolved test substance in the form of droplets at the water surface was visible throughout the exposure period.
CONCLUSION	The notified chemical is not toxic up to the limit of its water solubility to Zebra fish.
TEST FACILITY	BASF (2000d)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical (suspended in water).
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test part 1 - 48 hour, static. EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia - 48 hour, static. EPA OPPTS 850.1010 – Aquatic Invertibrate Acute Toxicity Test, Freshwater Daphnids. International Standard ISO 6341: 1989 – Water quality – Determination of the inhibition of the mobility of *Daphnia magna* STRAUS
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	2.49 mmol/L
Analytical Monitoring	Temperature was measured continuously throughout the test period, pH and oxygen were measured at the start of the test (0 hr) and at 48 hours.
Remarks - Method	A concentration control analysis was not conducted in this test. The stock of the notified chemical was made by stirring the test substance into water, then removing any undissolved test substance by centrifugation. In this way, Water Accommodated Fractions (WAF) were prepared by dilution of this eluate.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	-	20	0	0
100	-	20	0	0
50	-	20	0	0
25	-	20	0	0
12.5	-	20	0	0

LC_{50}	> 100 mg/L WAF at 48 hours (immobilisation)
NOEC	100 mg/L WAF at 48 hours (immobilisation)
Remarks - Results	No organism were immobilised in this test at the nominal concentration studied. There is no indication as to whether solutions remained clear or of the amount of test substance in the WAFs.
CONCLUSION	The notified chemical is not toxic to *Daphnia magna* up to the limit of its water solubility.
TEST FACILITY	BASF (1999g)

C.2.3. Chronic toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 211 *Daphnia magna*. Reproduction Test – 21 day, semi-static. EEC Guideline X1/691/86, Draft 4: Prolonged toxicity study with *Daphnia magna*: Effect on reproduction.
Species	*Daphnia magna*
Exposure Period	21 days
Auxiliary Solvent	Tween 20 (25 µg/L)
Water Hardness	2.2 – 3.2 mmol/L
Analytical Monitoring	Gas Chromatograph after extraction with hexane and evaluation by internal standard dibutylphthalate in hexane (concentration control

analysis).

Remarks - Method: The test was performed with the inclusion of 25 µg/L Tween 20 in the stock solution. Only one concentration (0.03 mg/L) of the notified chemical was tested (limit test).

Tween 20 (25 µg/L) was weighed in a glass beaker. The test substance (30 mg/L) was transferred into the beaker with the solvent. The solution was stirred for 60 minutes at 20 ± 2°C. The glass beaker was subjected to an ultra sonic bath for 10 minutes and later the solution was further stirred for 21 hours. The solution was transferred into a graduated flask, which was filled to 1L with M4 medium. 1 mL of this solution was transferred into 1000 mL M4 medium. The solution was renewed every 2-4 days.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*14 d*	*21*
0	0	10	0	0
0.03	0.021	10	0	0

NOEC: ≥ 0.021 mg/L at 21 days
LOEC: > 0.021 mg/L at 21 days
LCD: ≥ 0.021 mg/L at 21 days

Remarks - Results: As there were no significant differences between survival and reproduction for control and exposed daphnia, the observed adverse effect level is 0.021 mg/L – i.e. no effects are expected at the limit of solubility.

CONCLUSION: The notified chemical did not cause chronic toxicity at the highest concentration tested (limit water solubility).

TEST FACILITY: BASF (2004c)

C.2.4. Algal growth inhibition test

TEST SUBSTANCE: Notified chemical (aqueous extract)

METHOD: OECD TG 201 Alga, Growth Inhibition Test.
EC Directive 92/69/EEC C.3 Algal Inhibition Test.
EPA OPPTS 850.5400 – Algal Toxicity, Tiers I and II

Species: *Scenedesmus subspicatus*
Exposure Period: 72 hours
Concentration Range: Nominal: 6.25, 12.5, 25, 50 and 100 mg/L
Auxiliary Solvent: None
Water Hardness: Not reported
Analytical Monitoring: None

Remarks - Method: A concentration control analysis was not conducted in this test because the detection limit of the analytical method was beyond the water-solubility of the test substance.

To make up the stock solution, the test substance was stirred in demineralised water for 20 h at approximately 20 ± 2°C. Undissolved test substance was removed by centrifugation (c. 60 min at ~17700 *g*). The nominated concentration of 125 mg/L in the eluate was diluted to prepare the test solutions.

The test substance has the potential to adsorb to glass. Therefore the test vessels were filled with an aqueous extract (eluate) of the test substance and stirred for 24 h at 100 rpm at room temperature. The eluate was changed once. The vessels were carefully rinsed with water before the test started.

RESULTS

Biomass		*Growth*	
E_bC_{50} mg/L at 72 h	*NOEC mg/L*	*E_rC_{50} mg/L at 72 h*	*NOEC mg/L*
> 100 mg/L WAF	≥ 100 mg/L WAF	> 100 mg/L WAF	≥ 100 mg/L WAF

Remarks - Results: No inhibition of biomass or growth rate was found. In fact in both cases these were higher than the control; this was significantly so in the case of the former.

CONCLUSION: The notified chemical is not toxic to algae, up to the limit of its water solubility.

TEST FACILITY: BASF (2000e)

C.2.5. Inhibition of microbial activity

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 209 Activated Sludge, Respiration Inhibition Test.
EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test
International Standard ISO 8192-1986 Water Quality – Test for inhibition of oxygen consumption by activated sludge.

Inoculum: Activated sludge from laboratory wastewater plant treating municipal swage. Concentration of dry substance is 1 g/L.

Exposure Period: 180 mins

Concentration Range: 1000 mg/L (nominal)

Remarks – Method: 3,5-dichlorophenol was used as a reference.

RESULTS

EC_{50}: >1000 mg/L (nominal)

Remarks – Results: The oxygen concentration decreased more significantly for the test substance than for the blank controls.

The oxygen consumption rate did not differ between the test substance and the controls.

The oxygen consumption rate of the reference substance is significantly lower than both the rate of the test substance and the blank samples and the reference met the validity criteria (EC_{50} = 6.5 mg/L).

CONCLUSION: The notified chemical has low toxicity to bacteria.

TEST FACILITY: BASF (1999h)

C.2.6. Acute toxicity to the earthworm

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 207: Earthworm, acute toxicity tests.

Species: *Eisenia foetida*

Remarks - Method: The test involves keeping earthworms in samples of a precisely defined artificial soil to which a range of concentration of the test substance has been applied. These were 62.5, 125, 250, 500 and 1000 mg/kg.

RESULTS: LC_0 (14 day) >1000 mg/kg (nominal)
LC_{50} (14 day) >1000 mg/kg (nominal)
LC_{100} (14 day) >1000 mg/kg (nominal)

Remarks - Results: No negative impact on worm biomass was detected.

No other particular behavioural or morphological changes were observed

	at the end of the test. The determined pH value of 7.0 deviated from the aspired value of 6 ± 0.5. There was also deviation in the water constant of the dry test substrate from 2.2 g/100 g dry weight to the aspired value of < 2 g/100 g dry weight, but this had no effect on the result of this study. There was a deviation of the water content of the test substrate at the end if the test (36.9 g/100 g dry weight). The aspired value was 33 ± 2 g/100 g dry weight. This no effect on the outcome of this study.
CONCLUSION	The notified chemical is not toxic to earthworms.
TEST FACILITY	BASF (2001c)

C.2.7. Effect on emergence and growth of higher plants

TEST SUBSTANCE	Notified chemical
METHOD Species	OECD TG 208: Terrestrial plants, Growth Test International Standard ISO 11269-2:1995: Soil Quality Determination of the Effects of Pollutants on Soil Flora – Part 2: Effects of Chemicals on the Emergence and Growth of Higher plants. *Avena sativa* (oilseed rape) *Brassica napus* (oats) *Vicia sativa* (vetch)
Remarks - Method	The test involves incorporation of the notified chemical at various concentrations into soil with seeds subsequently sown. The number of seedlings that emerge is recorded. At least two weeks after 50% of the seedlings have emerged in the control, the plants arte harvested. Weight and shoot lengths recorded. The normal test concentrations used were 62.5, 125, 250, 500 and 1000 mg/kg.
RESULTS	The EC50 test results, relating to dry mass of the soil, for all three species (*Avena sativa, Brassica napus and Vicia sativa)* are as follows: EC50 (emergence rate) >1000 mg/kg (nominal) EC50 (dry matter) >1000 mg/kg (nominal) EC50 (fresh matter) >1000 mg/kg (nominal) EC50 (shoot length) >1000 mg/kg (nominal) The NOEC/LOEC tests results relating to the dry mass of the soil for all the three species (*Avena sativa, Brassica napus and Vicia sativa*) are as follows: NOEC/LOEC (emergence rate) ≥1000 mg/kg (nominal) NOEC/LOEC (dry matter) ≥1000 mg/kg (nominal) NOEC/LOEC (fresh matter) ≥1000 mg/kg (nominal) NOEC/LOEC (shoot length) ≥1000 mg/kg (nominal)
Remarks - Results	The results for *Avena sativa* were obtained after 20 Days and the tests results for *Brassica napus* and *Vicia sativa* were obtained after 21 days.
CONCLUSION	The notified chemical is not toxic to higher plants.
TEST FACILITY	BASF (2005h)

BIBLIOGRAPHY

ABS (1999) National Nutrition Survey: Foods Eaten Australia 1995. Australian Bureau Of Statistics. ABS Catalogue No. 4804.0 (ISBN 0 642 25723 X). © Commonwealth of Australia 1999

Barratt MD, Basketter DA, Chamberlain M, Admans GD and Langowski JJ (1994) An Expert System Rulebase For Identifying Contact Allergens. *Toxic. in vitro*. 8(5):1053-1060.

BASF (1999a) Chemical and Spectroscopic Characterization of "1,2-Cyclohexane dicarboxylic acid, di(isononyl)ester". Study No. 99L00402. Experimental Toxicology and Ecology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (1999b) Physico-chemical Properties of 1,2-Cyclohexane dicarboxylic acid, di(isononyl)ester. Study No 99L00403. GKA Analytik, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (1999c) 1,2-Cyclohexane dicarboxylic acid, di(isononyl)ester: Acute Oral Toxicity in Rats. Department of Toxicology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (1999d) 1,2-Cyclohexane dicarboxylic acid, di(isononyl)ester: Acute Dermal Toxicity in Rats. Department of Toxicology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (1999e) Acute Eye Irritation/Corrosion In Rabbits. Department of Toxicology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (1999f) 1,2-Cyclohexane dicarboxylic acid, di(isononyl)ester – Maximisation Test in Guinea Pigs. Department of Toxicology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (1999g) Determination of the Acute Effect of 1,2 Cyclohexanedicarboxylic acid, bis (isononyl) ester on the Swimming Ability of the Water Flea *Daphnia magna* STRAUS According to OECD 202 and GLP, EN 45001 and ISO 9002. Ecology and Environmental Analytics, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (1999h) Determination of the Inhibition of Oxygen Consumption by Activated Sludge by 1,2 Cyclohexanedicarboxylic acid, bis(isononyl) ester in the Activated Sludge Respiration Inhibition Test BASF Aktiengesellschaft Ecology and Environmental Analytics (unpublished report provided by the notifier).

BASF (2000a) 1,2-Cyclohexane dicarboxylic acid, di(isononyl) ester: Repeated Dose Oral Toxicity Study in Wistar Rats. Administration in the Diet for 4 Weeks and Recovery Period of 2 Weeks. Experimental Toxicology and Ecology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (2000b) Salmonella Typhimurium/Escherichia Coli Reverse Mutation Assay (Standard Plate Test and Preincubation Test) with 1,2-Cyclohexane dicarboxylic acid, di (isononyl)ester. Department of Toxicology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (2000c) OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test. BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (2000d) 1,2-Cyclohexane dicarboxylic acid, di(isononyl)ester: Acute Toxicity Study on the Zebra Fish. Department of Toxicology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (2000e) 1,2-Cyclohexanedicarboxylic acid, bis (isononyl) ester – Determination of the Inhibitory Effect on the Cell Multiplication of Unicellular Green Algae *Scenedesmus subspicatus*. Experimental Toxicology and Ecology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (2001a) Characterization of "1,2-Cyclohexane dicarboxylic acid, di(isononyl)ester" before start of toxicological and ecological studies. Study No. 01 L00125. ZAX Analytik, BASF AG, D-67056 Ludwigshafen (unpublished report provided by the notifier).

BASF (2001b) In Vitro Gene Mutation Test with 1,2 Cyclohexane dicarboxylic acid, di(isononyl)ester in CHO Cells (HPRT Locus Assay). Experimental Toxicology and Ecology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (2001c) 1,2-Cyclohexane dicarboxylic acid, di(isononyl)ester – Determination of the Acute Lethal Effect of Chemicals on the Earthworm *Eisenia foetida* (Michaelsen). Experimental Toxicology and Ecology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (2002a) Physico-chemical Properties of "1,2 Cyclohexane dicarboxylic acid, di(isononyl)ester". Study No. 02L00200. GKA Analytik, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (2002b) 1,2 Cyclohexane dicarboxylic acid, di(isononyl)ester - Subchronic Oral Toxicity Study in Wistar Rats. Administration in the Diet for 3 Months. Experimental Toxicology and Ecology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (2002c) Pre-/Postnatal Developmental Toxicity Study in Wistar Rats Oral Administration (gavage). (Study No. 60R0223/99095, 18 October 2002) Experimental Toxicology and Ecology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (2002d) Prenatal Developmental Toxicity Study in Wistar Rats Oral Administration (gavage). (Study No. 30R0223/99124, 25 January 2002) Experimental Toxicology and Ecology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (2003a) Two-Generation Reproduction Toxicity Study in Wistar Rats Continuous Dietary Administration (Study No. 70R0107/01021, 10 June 2003) Experimental Toxicology and Ecology, BASF Aktiengesellschaft. Vol 1 Report and Summary tables, Vol 2 Table sections individual values, Vol 3 Supplement (unpublished report provided by the notifier).

BASF (2003b) ^{14}C-1,2-Cyclohexanedicarboxylic acid, diisononyl ester – Study of the Biokinetics in Rats. Experimental Toxicology and Ecology BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (2003c) Supplementary Information: Kinetics of 1,2 Cyclohexanedicarboxylic acid, bis(isononyl) ester and di(2-ethylhexyl)adipate in rat tissues after a single oral administration (unpublished report provided by the notifier).

BASF (2004a) Hexamoll DINCH - Acute Dermal Irritation/Corrosion in Rabbits. Experimental Toxicology and Ecology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (2004b) Prenatal Developmental Toxicity Study in Himalayan Rabbits Administration in the diet. (Study No. 42R0107/01135, 18 August 2004) Experimental Toxicology and Ecology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (2004c) 1,2-Cyclohexanedicarboxylic acid, diisononyl ester – Determination of the Chronic Effect on the Reproduction of the Water Flea *Daphnia magna* STRAUS. Experimental Toxicology and Ecology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (2005a). Characterisation of 1,2-Cyclohexane dicarboxylic acid di(isononyl) ester After Termination of Toxicological Studies. Study Number 04L00301. ZAX Analytik, BASF AG, D-67056 Ludwigshafen (unpublished report provided by the notifier).

BASF (2005b) Combined Chronic Toxicity/Carcinogenicity Study in Rats; Administration in the Diet up to 24 Months. Experimental Toxicology and Ecology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (2005c) Amendment No. 1 to "^{14}C-1,2-Cyclohexanedicarboxylic acid, diisononyl ester – Study of the Biokinetics in Rats". Experimental Toxicology and Ecology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (2005d) Investigations on the Metabolism of ^{14}C-1,2-Cyclohexane dicarboxylic acid diisononyl ester (DINCH) in Rats. Ecology and Environmental Analytics, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (2005e) 1,2-Cyclohexanedicarboxylic acid, diisononyl ester: Liver Enzyme Induction Study in Wistar Rats: Administration in the Diet Over 2 Weeks. Experimental Toxicology and Ecology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (2005f) 1,2-Cyclohexanedicarboxylic acid, diisononyl ester: S-Phase Response Study in the Liver of Wistar Rats. Administration in the Diet for 1, 4 and 13 Weeks. Experimental Toxicology and Ecology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (2005g) 1,2-Cyclohexanedicarboxylic acid, diisononyl ester: Thyroid Function Study in Male Wistar Rats Using Perchlorate Discharge as a Diagnostic Test. Administration in the Diet Over 4 Weeks. Experimental Toxicology and Ecology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (2005h) 1,2-Cyclohexanedicarboxylic acid, diisononyl ester: Determination of the effect of chemicals on the emergence and growth of higher plants (*Avena sativa, Brassica napus, Vicia sativa).* Experimental Toxicology and Ecology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (2006a) Amendment to Study Report with Title "1,2-Cyclohexanedicarboxylic acid, diisononylester – Combined Chronic Toxicity/Carcinogenicity Study in Rats: Administration in the Diet up to 24 Months". Experimental Toxicology and Ecology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

BASF (2006b) Bioconcentration: Flow-through fish test. Experimental Toxicology and Ecology, BASF Aktiengesellschaft (unpublished report provided by the notifier).

Capen CC (1997) Mechanistic Data and Risk Assessment of Selected Toxic End Points of the Thyroid Gland. *Toxicol Pathol.* 25(1): 39-48.

Clark RL (1998) Endpoints of Reproductive System Development, In: Daston G and Kimmel C (eds), *An Evaluation and Interpretation of Reproductive Endpoints for Human Risk Assessment*. International Life Sciences Institute, Vol 10-20 (1998).

Craft ES *et al* (2002) Comparative Responsiveness of Hypothyroxinemia and Heaptic Enzyme Induction in Long-Evans Rats Versus C57BL/6J Mice Exposed to TCDD-like and Phenobarbital-like Polychlorinated Biphenyl Congeners. *Tox Sci.* 68:372-380.

Curran PG and DeGroot LJ (1991) The Effect of Hepatic Enzyme-inducing Drugs on Thyroid Hormones and the Thyroid Gland. *Endocr Rev.* 12(2): 135-50.

EC (2003) Technical Guidance Document on Risk Assessment, Part I. Institute for Health and Consumer Protection, European Chemicals Bureau, Joint Research Centre, European Commission.

EFSA (2006). Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request related to a 12th list of substances for food contact materials. Adopted on 26 & 27 September 2006. *The EFSA Journal,* 395 to 401, 1-21.

EU (2001) Risk assessment, 1,2-Benzenedicarboxylic acid, di-C9-11-branched alkyl esters, C10-rich and di-"isodecyl" phthalate, Finalised report. European Union, France.

FORS (Federal Office of Road Safety) (1998) Australian Code for the Transport of Dangerous Goods by Road and Rail (ADG code), 6th Edition, Canberra, Australian Government Publishing Service.

Hulzebos E, Walker JD, Gerner I and Schlegel K (2005) Use of Structural Alerts to Develop Rules for Identifying Chemical Substances with Skin Irritation or Skin Corrosion Potential. *QSAR Comb. Sci.* 24, 332-342.

IARC (1999) International Agency for Research on Cancer. Species Differences in Thyroid, Kidney and Urinary Bladder Carcinogenesis. Capen CC, et al., (eds.). IARC Scientific Publications no.147. IARC, Lyon, 1999 (cited in Rice et al., 1999).

Khan KNM and Alden CL (2002) Kidney. In: Handbook of Toxicological Pathology (2nd edition). WM Haschek, CG Rousseaux and MA Wallig, *Eds*. Volume 2, Chapter 33, p 269. © Academic Press, Electronic Edition.

Kimura-Kuroda J, *et al* (2007) Disrupting Effects of Hydroxy-polychlorinated Biphenyl (PCB) Congeners on Neuronal Development and Cerebellar Pukinje cells: A Possible Causal Factor for the Development of Brain Disorders. *Chemosphere* 67:S412-S420

Mensink BJWG, Montforts M, Wijkhuizen-Maslankiewicz L, Tibosch H and Linders JBHJ (1995) Manual For Summarising and Evaluating the Environmental Aspects of Pesticides. National Institute of Public Health and Environmental Protection, Bilthoven, Netherlands.

Mylchreest E, Cattley RC and Foster PMD (1998) Male Reproductive Tract Malformations in Rats Following Gestational and Lactational Exposure to di(*n*-butyl) phthalate: An antiandrogenic mechanism? *Toxicol Sci*. 43:47-60.

NICNAS (2007) Phthalates hazard compendium: A summary of physicochemical and human health hazard data for 25 phthalates. http://www.nicnas.gov.au

NOHSC (1994) National Code of Practice for the Labelling of Workplace Substances [NOHSC:2012(1994)]. National Occupational Health and Safety Commission, Canberra, Australian Government Publishing Service.

NOHSC (2004) Approved Criteria for Classifying Hazardous Substances, 3rd edition [NOHSC:1008(2004)]. National Occupational Health and Safety Commission, Canberra, AusInfo.

NOHSC (2003) National Code of Practice for the Preparation of Material Safety Data Sheets, 2nd edition [NOHSC:2011(2003)]. National Occupational Health and Safety Commission, Canberra, Australian Government Publishing Service.

Otter R (2007) Data Summary for NICNAS Registration. BASF Germany.

Rice JM, Baan RA, Blettner M, Genevois-Charmeau C, Grosse Y, McGregor DB, Partensky C, Wilbourn JD (1999). Rodent Tumors of Urinary Bladder, Renal Cortex, and Thyroid Gland in IARC Monographs: Evaluations of Carcinogenic Risk to Humans. *Toxicol Sci*. 49(2):166-71.

RCC (2000) *In Vitro* Chromosome Aberration Assay in Chinese Hamster V79 Cells with 1,2-Cyclohexane dicarboxylic acid, di(isononyl)ester. RCC Cytotest Cell Research GmbH (unpublished report provided by the notifier).

RCC (2001) Micronucleus Assay in Bone Marrow Cells of the Mouse. RCC Cytotest Cell Research GmbH (unpublished report provided by the notifier).

Rao-Rupanagudi S, Heywood R and Gopinath C (1992) Age-related changes in thyroid structure and function in Sprague-Dawley rats. *Vet Pathol*. 29(4):278-87.

Svardal AM and Scheline RR (1985) The aromatization of cyclohexanecarboxylic acid to hippuric acid: substrate specificity and species differences. *Mol Cell Biochem*. 67(2):171-9.

Svensson K (2002) Exposure from food contact materials. ILSI Europe Report Series, International Life Sciences Institute (ILSI), with the Joint Research Centre (JRC), European Commission .

United Nations (2003) Globally Harmonised System of Classification and Labelling of Chemicals (GHS). United Nations Economic Commission for Europe (UN/ECE), New York and Geneva.