European Union Risk Assessment Report

DIPHENYLAMINE

CAS-No.: 122-39-4

EINECS-No.: 204-539-4

RISK ASSESSMENT

Final version of 29.05.2008

FINAL APPROVED VERSION

LEGAL NOTICE

Cataloguing data can be found at the end of this publication

Luxembourg: Office for Official Publications of the European Communities, [ECB: year]
ISBN [ECB: insert number here]

Printed in Italy

Information on the rapporteur

Contact Point:

Bundesanstalt für Arbeitsschutz und Arbeitsmedizin (BAuA)
Anmeldestelle Chemikalien / Zulassungsstelle Biozide
(Federal Institute for Occupational Safety and Health
Division for Chemicals and Biocides Regulation)
Friedrich-Henkel-Weg 1-25

44149 Dortmund (Germany)

fax: +49(231)9071-2679
e-mail: chemg@baua.bund.de

The first draft of the Human Health Section of the Comprehensive Risk Assessment Report of **diphenylamine**, a substance chosen from the EU 3rd priority list in 1997 was distributed for preliminary written procedure in April 2005.

The Human Health Section was discussed in-depth in October 2005 (TC NES IV´05) and was distributed for the final written procedure in January 2007.

The Environment Section of the Risk Assessment Report diphenylamine was distributed to MS for preliminary comments in June 2003 (TM II/03), for the first in-depth discussion in November 2003 (TM IV/03) and for the last visit written procedure in May 2008.

Foreword

We are pleased to present this Risk Assessment Report which is the result of in-depth work carried out by experts in one Member State, working in co-operation with their counterparts in the other Member States, the Commission Services, Industry and public interest groups.
The Risk Assessment was carried out in accordance with Council Regulation (EEC) 793/931 on the evaluation and control of the risks of "existing" substances. "Existing" substances are chemical substances in use within the European Community before September 1981 and listed in the European Inventory of Existing Commercial Chemical Substances. Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of these substances if they are produced or imported into the Community in volumes above 10 tonnes per year.
There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each substance on a priority list, a Member State volunteers to act as "Rapporteur", undertaking the in-depth Risk Assessment and recommending a strategy to limit the risks of exposure to the substance, if necessary.
The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/942, which is supported by a technical guidance document3. Normally, the "Rapporteur" and individual companies producing, importing and/or using the chemicals work closely together to develop a draft Risk Assessment Report, which is then presented at a meeting of Member State technical experts for endorsement. The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Health and Environmental Risks (SCHER) which gives its opinion to the European Commission on the quality of the risk assessment.
If a Risk Assessment Report concludes that measures to reduce the risks of exposure to the substances are needed, beyond any measures which may already be in place, the next step in the process is for the "Rapporteur" to develop a proposal for a strategy to limit those risks.
The Risk Assessment Report is also presented to the Organisation for Economic Co-operation and Development as a contribution to the Chapter 19, Agenda 21 goals for evaluating chemicals, agreed at the United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992 and confirmed in the Johannesburg Declaration on Sustainable Development at the World Summit on Sustainable Development, held in Johannesburg, South Africa in 2002.
This Risk Assessment improves our knowledge about the risks to human health and the environment from exposure to chemicals. We hope you will agree that the results of this in-depth study and intensive co-operation will make a worthwhile contribution to the Community objective of reducing the overall risks from exposure to chemicals.

---

1 O.J. No L 084, 05/04/199 p.0001 – 0075

2 O.J. No L 161, 29/06/1994 p. 0003 – 0011

3 Technical Guidance Document, Part I – V, ISBN 92-827-801 [1234]

CONTENTS

0 OVERALL CONCLUSIONS/RESULTS OF THE RISK ASSESSMENT

CAS No. 122-39-4

EINECS No. 204-539-4

IUPAC Name Diphenylamine

Overall results of the risk assessment:

(X) i) There is need for further information and/or testing

(X) ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

( ) iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account

Summary of conclusions:

Environment

Conclusion (i) There is need for further information and/or testing

This conclusion applies for sewage treatment plants (stp) (micro-organisms) for all production and intermediate processing sites, professional use of lubricants and storage aid and

formulation of storage aid. For the aquatic compartment (water and sediment), the conclusion applies for all industrial/professional uses. Further information is needed from formulation and professional use of lubricants and from formulation of storage aid to lower the predicted risk in soil compartment. The conclusion is drawn also for the secondary poisoning via aquatic foodchain for the production and the only known intermediate processing site. Additionally, more information is needed in order to lower the predicted risk for secondary poisoning via terrestrial route in the scenario of professional use of lubricants. The PECs in the above mentioned scenarios may be lowered if new information is provided on the following points:

- Current tonnage of all uses
- For the rest of the European volume of 10 000 t/a which is not covered by the known producer and importer, a confirmation is needed whether the rest is completely imported or produced in additional European sites.
- It is very probable, that more than one intermediate processing sites are located in Europe. Information on their size, emissions, waste water treatment and effluent dilution rate is necessary.
- Information on the size and waste water treatment of lubricant formulation sites is necessary.
- More information on the end-uses of lubricants containing Diphenylamine is needed in order to be able to allocate the tonnage for lubricant use to several generic use scenarios.
- There is no information available whether there actually are any formulation sites of storage aid located in Europe or not. Confirmation for this issue is needed. If any formulation sites are located in Europe, site specific data on their size, emissions and waste water treatment is needed. If not, the scenario can be deleted.
- Measured data are needed in order to be able to compare the model results with the reality.

ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

This conclusion is drawn for the sewage treatment plant (micro-organisms) for the formulation of lubricants. It applies also for soil for the production and intermediate processing sites (based on the assumption that these sites are not connected to a municipal sewer). For secondary poisoning via aquatic food chain, no concern was identified for the only known intermediate processing site, formulation and professional use of lubricants, and formulation of storage aid. For secondary poisoning via terrestrial food chain, no risk was identified for the production and intermediate processing, formulation of lubricants and formulation of storage aid. In addition the predicted regional concentrations in water, sediment and soil do not pose risk to the organisms (risk ratio is around 0.5).

Finally, it should be noted, that two very minor uses of Diphenylamine, use in explosives and as stabilizer and colouring agent, were not considered in this assessment.

Human Health

Workers

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

Consumers

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

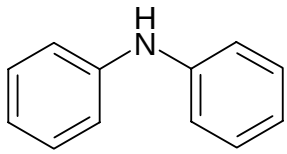
Man exposed indirectly via the environment

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

1 GENERAL SUBSTANCE INFORMATION

Identification of the substance

CAS No.:	122-39-4
EINECS No.:	204-539-4
IUPAC Name:	Diphenylamine
Synonyma:	Benzenamine, N-phenyl-
	Diphenylamin
	Anilinobenzene
	Benzene, (phenylamino)-
	N,N-diphenylamine
	N-Phenylaniline
	N-Phenylbenzenamine
Molecular weight:	169 g/mol
Empirical formula:	C_{12} H_{11} N
Structural formula:	



Purity/impurities, additives

Purity:	> 99.2 %
Impurities:	≤ 0.03% Aniline
	≤ 0.02 % 4-Aminodiphenyl

Physico-chemical properties

Diphenylamine is a colourless solid (at room temperature and normal pressure) with a floral odour. Data on the physical and chemical properties are given in Table 1.1.

Table 1.1: Data on the physical and chemical properties of diphenylamine

Melting point	53 °C [1)]	Auer Technikum, 1988
Boiling point	302 °C	Römpp, 1995
Relative density	1.159	Ullmann, 1974
Vapour pressure	0.033 Pa at 20 °C [2)]	Auer Technikum, 1988
Surface tension	72.3 mN/m at 20 °C (saturated solution) [3)]	Bayer AG, 1999a
Water solubility	40 mg/l at 25 °C	Bayer AG, 1986a
Partition coefficient	LogPow 3.4 [4)]	Lyman et al., 1982
Flash point	not determined	substance is a solid
Auto flammability	no self-ignition up to the melting point (53 °C)	BAM, 1997
Flammability	not flammable [5)]	Bayer AG, 1998
Explosive properties	no explosive properties [6)]	BAM, 1997
Oxidizing properties	no oxidizing properties [6)]	BAM, 1997

[1)] In the IUCLID a melting range of 52.5 – 55.5 °C is given (Deutsches Arzneibuch, cited in BUA report 15, 1988). This value could not be confirmed by other entries in the commun chemical literature. Also information about the purity of the test substance, the test method and the test conditions is missing. Therefore the melting point of 53 °C is recommended for the risk assessment.

2) A further value for the vapour pressure at 20 °C (0,0215 Pa) is cited in the IUCLID. This value was received by internal measurements at Bayer. No information about the purity of the test substance, the test method and the test conditions was available. Therefore the vapour pressure of 0.033 Pa at 20 °C is recommended for the risk assessment.

[3)] OECD-ring method in compliance with guideline 92/69/EWG, A.5

[4)] The partition coefficient n-octanol/water was also calculated according to Leo Hansch and resulted in a logPow of 3.29. For the risk assessment an experimental value is preferred; but the cited logPow of 3.6 in the IUCLID (Tonogai, Y. etal., J.Toxicol.Sci.7, 1982) could not be

confirmed, because the literature was not available. Therefore the reliable logPow of 3.4 from the secondary literature was used for the risk assessment.

5) According to A.10 the substance did not propagate combustion. The tests according to A.12 and A.13 were not conducted. Due to the properties and the handling of the substance it has not to be assumed that flammable gases can be formed in contact with water or the substance has pyrophoric properties

6) No test conducted because of structural reasons

Classification

- (Classification according to Annex I)

Classification and labelling according to the 22nd ATP of Directive 67/548/EEC

T	Toxic
R 23/24/25	Toxic by inhalation, in contact with skin and if swallowed
R 33	Danger of cumulative effects
N	Dangerous to the environment
R 50/53	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

- (Proposal of the rapporteur)

Environment

According to the data presented below and the criteria of Directive 93/21/EEC, the Annex I entry is confirmed with respect to the Environment:

N	Dangerous to the environment
R 50/53	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

Human Health

Xn, Xi	Harmful
R 22	Harmful if swallowed
R 41	Risk of serious damage to eyes

Remarks

Studies confirming the existing classification with T, R23/24/25 and R33 are not available.

Data on eye irritating properties of the substance are conflicting and poorly documented, but it may be assumed that diphenylamine may pose a risk of serious damage to eyes. There exist two guideline-compliant studies which both report severe eye irritation caused by diphenylamine. In one of these studies irreversibility of effects after 21 days is stated. Hence, appropriate labelling with R41 "Risk of serious damage to eyes" is proposed.

2 GENERAL INFORMATION ON EXPOSURE

2.1 Volume

In the EU 15 four companies informed on HPV scale production or import of Diphenylamine during the 1990s but they have announced to have stopped the activity. . Srour (1994) predicted for the year 1998 a total EU production capacity of 18 000 t/a and a production volume of 12 000 t/a. Imports were expected to be 1 500 t/a and exports outside the EU 3 500 t/a.

Based on Srour (1994) and the tonnage indicated in IUCLID for years 1992-1993, a total EU market volume of approx. 10 000 t/a (~ 9 000 t/a production + ~ 1 000 t/a imports) is assumed for the risk assessment. Spin database (2003) indicates a total use volume of ca. 50 t/a in years 2000-2001 and 17 t/a for 2005 for the Nordic countries (FIN, S, NO, DK).

The production and import volume which was reported by the Industry participating in the risk assessment during 1998-2003 was in the range of 5000 t/a.

Industry has indicated that a plant in Slovak Republic is producing Diphenylamine. The production volume is in the range from 10000-20000 t/a. In 2003 no export of Diphenylamine from the Slovak Republic to the EU was reported. (Centre 2004)However, the tonnage of 10 000 t/a will be sustained in the risk assessment due to the following two reasons:

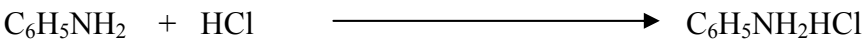
1) Two companies have notified Diphenylamine under the Council Directive concerning placing plant protection products on the market 91/414/EEC ("PPP-Directive"). They have neither been included in IUCLID nor provided information on volumes. Therefore, a significant import (or production) volume might be missing. Diphenylamine is included in the 3rd priority list (Commission Regulation 2002/1490/EEC) of the substances to be assessed under the PPP-Directive. Data for the assessment should be available to the rapporteur Irelandby 30 November 2004. The PPP-risk assessment will not cover releases from production and formulation of the substance, which are taken into account in this risk assessment. In addition, it is not yet clear whether the PPP-assessment will cover the releases from use of the plants (fruits and vegetables).

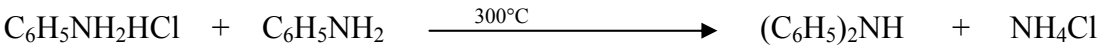
2) Industry has indicated that a plant in Slovak Republic is producing Diphenylamine for the EU market. The Competent Authority of Slovak Republic has confirmed that Diphenylamine is produced in Slovak Republic and practically completely exported. Further information in the possession of the Competent Authority on the production is confidential. Murin et al. (1997) reports a production volume greater than 10 000t/a.

2.2 Production process

The production of Diphenylamine can be performed by self-condensation of aniline in the presence of a small amount of a strong mineral acid, such as anhydrous hydrochloric acid (0.5 wt. % of aniline), ferrous chloride or ammonium bromide at elevated temperature and

pressure (Moore, 1978). The technical substance is gained by vacuum distillation and is additionally purified for the use as fruit storage aid.





2.3 Use pattern

2.3.1 Main uses

According to Industry, most of Diphenylamine is used as a chemical intermediate and only a minor amount as additive in final products. According to a prediction of Srour (1994) for the year 1998 a quantity of 8 450 t/a was used as intermediate and further 1 000 t/a for open uses. In addition, according to the Spin database (2003), the Swedish product register contains consumer products where Diphenylamine is used.

Taking into account these estimations and the more recent confidentially reported use quantities by industry, it is assumed that approximately 97.5 % (9 750 t/a) of the current market volume of 10 000 t/a in the EU 15 is used as an **intermediate** for:

-Antioxidants: Diphenylamine can be alkylated nucleophilic with acetone or alkenes to antioxidants widely used in the rubber industry and for lubricants.

-Antiozonants: Diphenylamine nitrosation followed by reductive alkylation with ketones gives antiozonants from the p-phenylendiamine-type used in the rubber industry

-Phenothiazine: Chemical reaction of Diphenylamine with sulphur gives phenothiazine used as stabiliser for plastics.

-Dyestuffs: After chemical reaction several dyestuffs can be prepared.

Approximately 2 % (~ 200 t/a) are assumed to be used as the active substance in **storage aid** (solution or spray) in the post harvest treatment of e.g., for apples and pears to prevent the formation of storage scald on fruit. Storage scald is an abiotic disorder in the fruit skin cells. Diphenylamine is registered at the present as plant protection product in Ireland, United Kingdom, France, Spain, Portugal, Italy and Greece (see also chapter 2.1). According to the UK Pesticides Safety Directorate, two professional products with names ‘No Scald’ and ‘Shield’ are currently on the UK market. The other of the two registrants under the PPP-Directive 91/414/EEC has indicated that they import Diphenylamine to Europe. No information is available whether the second registrant formulates its product in Europe or not. The European Union has set maximum residue limits (MRLs) for Diphenylamine in its legislation (Commission Directive 2000/57/EWG). The MRL for apples is 5 mg/kg, for pears 10 mg/kg and for all other commodities 0.05 mg/kg. These MRLs replaced the national limits by 1 April 2001. In the Farm Chemicals Handbook (1997) a limit of 10 mg/ kg is mentioned for apples and pears in the U.S.

According to the specific information from industry, alkylated diphenylamine **lubricant oils** contain a small amount of unreacted Diphenylamine. These lubricant oils are petroleum or synthetic oils. Diphenylamine has been reported to be used also in its unreacted for as antioxidant in lubricant oils. However, there is contradicting information from industry whether this use is still an intended use. The Nordic Product Register SPIN and the Finnish Product Register show that altogether 3.3 t/a are presently registered for lubricant use in Sweden, Denmark and Finland. The products in the Finnish Product Register contain < 1 % Diphenylamine. The product registers indicate that lubricants containing Diphenylamine are used in the classes 'Sale, maintenance and repair of motor vehicles and motorcycles; retail sale of automotive fuel' and 'Manufacture of machinery and equipment'. The first use is covered in TGD by IC 0 ('Others'), the latter one by IC 16 ('Engineering industry') or by IC 8 ('Metal extraction, refining and processing industry'). Based on the specific information from industry and the Nordic product registers, a total amount of 50 t/a (0.5 % of the total tonnage) is assumed to be used in lubricant oils within metalworking industry and automotive repair and sale. The concentration of 1 % Diphenylamine in lubricant oils is assumed.

2.3.2 Minor uses

The following minor uses have been reported in the literature.

–Diphenylamine is used as a **stabiliser** in nitrocellulose, celluloid **in the explosive industry**. The function of Diphenylamine in explosives is to fix the decomposition products during storage (e.g. NO, NO_2, HNO_3) and thus prevent further destruction (Drzyzga, 1999). According to information provided by industry, an order of magnitude of approx. 10 t/a is used for this purpose in Europe. The Finnish Product Register, on the other hand, includes four Diphenylamine products where it is a component of gunpowder. The registered amount is ca. 20 t/a for Finland alone, which suggests a much larger European use volume than the figure provided by industry.

No reliable estimation of the quantities released to the environment from these products is available. It can be assumed that a considerable fraction of Diphenylamine used will be destroyed during the use of the explosives and that the remaining releases to the environment predominantly occur into restricted areas. Therefore, for the purpose of this risk assessment possible emissions from this use are not taken into account.

In addition, Diphenylamine is used as a **stabiliser in perfume oils** with a content of 0.1 % (Drzyzga, 1999). According to a data base at the German Federal Institute for Health Protection of Consumers and Veterinary Medicine (Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin/ BgVV, 2001) there is currently only one notification as trace-ingredient (< 0.01 %) in balsamic turpentine oil. This use is considered to be negligible for this risk assessment.

2.3.3 Former uses

Diphenylamine was used until the year 2003 among few other substances in low tax fuels and heating oils as colouring agent to distinguish them from other fuels. This use was set aside by the Commission Decision 2001/574/EC establishing a common fiscal marker for gas oils and kerosene.

In the BUA-report on Diphenylamine (GDCh, 1988) referring to Daniels (1983) it is mentioned that in special cases about 200-300 ppm Diphenylamine is added as stabiliser to commercial vinylacetate. The most recent (July 2001) information by the largest German producer of vinylacetate, Celanese Chemicals Europe GmbH, confirms that Diphenylamine is not used for this purpose anymore.

In veterinary medicine, Diphenylamine was reported to be used as additive in anti-screwworm mixtures and as active ingredient in biocidal products against body lice, house flies and bloodsucking larvae of mites (Drzyzga, 1999). The veterinary use as anthelminticum is also mentioned in a report prepared by the German Federal Environmental Agency (UBA, 1995). However, current information by Liebisch (2001) indicates that Diphenylamine is not used any more as an endo- or ectoparasitical additive in veterinary products. In addition, the UK Veterinary Medicines Directorate has also confirmed that Diphenylamine is not currently used in any veterinary products in the UK. Correspondingly, Diphenylamine is not mentioned for these purposes in the current "Tierarzneimittelkompendium der Schweiz" (veterinary medical product handbook). For these reasons any possible release from this use pattern is not considered in this risk assessment.

The use as a stabiliser for commercial carbon tetrachloride is now no longer of importance as production and use of carbon tetrachloride has been strongly regulated by the EC Regulation 3093/94 since 1994.

-The use of N-nitroso-Diphenylamine (NDPA) as inhibitor in the rubber industry was terminated in 1983 (Srour 1994). NDPA decomposes partly to Diphenylamine, this may be the reason that Diphenylamine was found in rubber articles up to the early 80's (Babish et al. 1983, Danielson et al. 1983).

The approximate quantitative breakdown of the use pattern of Diphenylamine is compiled in table 2.1.

2.3.4 Overview of the uses considered in the exposure assessment

Table 2.1 concludes the main industrial uses and fractions of tonnage within each category. These figures are used in the risk assessment.

Table 2.1: Quantitative breakdown of the use pattern

MC	**IC**	**UC**	**Mass balance (% of use)**
Non dispersive	Chemical industry (3)	Intermediate for antioxidants, antiozonants, phenothiazine, for dyes and other uses (33)	97.5
Wide dispersive	Agricultural/Food industry (1)	Plant protection product used as storage aid, for conservation of fruits, e.g., apples and pears (38)	< 2
Non dispersive	Metal working industry (8), Engineering industry (16), Others (0)	Lubricants	0.5
Wide dispersive	Others (0)	Stabiliser for nitro-based explosives (49)	~ 0.1
Wide dispersive	Others (0)	Stabiliser, colouring agent	Traces

3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 General discussion

Release into the environment

Releases of Diphenylamine to the environment are expected to occur mainly in chemical industry during production, formulation of heating oil, lubricant additive and plant protection product and processing (use as intermediate). In addition, professional use of Diphenylamine in lubricant oils and as storage aid (plant protection product) cause releases. Diphenylamine is also released from private use of heating oil and unintentionally from consumption of fruits, which are treated with storage aid containing Diphenylamine. The generic use scenarios developed are explained in Chapter 3.1.2.

Indirect releases to the soil compartment via sludge application may occur from uses which are connected to the municipal sewer. From Diphenylamine uses these are formulation and professional use of lubricants and consumption of Diphenylamine treated fruits. Because no information on the fate of sludge was provided by industry, sludge application is taken into account in all scenarios except in production and intermediate processing. Some of the uses emit Diphenylamine to air which is deposited into soils.

Presently, only one production site and one HPV-scale importer are known to the rapporteur in the EU 15. They stopped the activity. In addition, there is information on one major intermediate processing site. Site specific assessments for these two sites are presented in the confidential Appendices B1-B2. The two site specific local scenarios incorporate the site specific data provided so far, but the calculations are still to a large extent carried out on a generic basis. Use and emission information from the two known sites have been partly contradictory or is missing. The tonnage covered by the known processing site does not cover the whole European tonnage. Thus, there is reason to assume, that there are other major sites processing or formulating Diphenylamine of which there is no information at all available (see also chapter 2.1).

For the above mentioned reasons, the risk assessment is conducted using generic scenarios and values according to the Technical Guidance Document.

For this version of the assessment, EUSES 1.0 was used to estimate the fate and exposure. Details on the information and defaults used for running EUSES are included in the Appendix A (EUSES –report).

Degradation

Hydrolysis

Based on the molecular structure hydrolysis of Diphenylamine is not expected under environmental conditions.

Photolysis in water

The quantum yield of the direct photodegradation of Diphenylamine in water showed to be 0.093 with polychromatic light. From this value a half-life of 1.9 h in summer and 33.1 h in winter are calculated (50 degree of latitude, clear sky, clean water near the surface, values integrated over the whole day; Bayer AG, 1999b). Assuming a daily sunlight period of 12 h an overall mean half-life of 1.46 d results.

In most natural water bodies, the rate of photoreaction is affected by dissolved and suspended matter. Using the standard parameters of the regional model (water depth, suspended matter concentration), the reduction in the light intensity may be as large as 98 %. Assuming an even distribution of the substance in the water phase according to the Mackay-type fugacity models only a small part of Diphenylamine in the upper layer of the surface water is available for photolysis in water.

With the above mentioned conditions (mean half life winter/summer of 1.46 d and only 2 % of the solved substance in the upper layer of surface water is exposed to photodegradation), an effective mean half-life of 73 d is estimated. The corresponding $kphoto_{water}$ of $3.96 * 10^{-4}$ h^{-1} is used in the risk assessment.

Photodegradation in the atmosphere

The atmospheric degradation by OH radicals is calculated for an OH radical concentration of $5 * 10^5$ OH/cm³ to a half-life of approximately 1.5 h (Bayer AG, 1999b). Another atmospheric half-life of approximately 1 h was estimated (GDCh, 1988). Using the QSAR-model package PropertEst version 1.3 including AOPWIN version 1.87 for a k_{OH} of $2 * 10^{-10}$ $cm^3*molec^{-1}*s^{-1}$ a half-life of 1.93 h results. This value is used for risk assessment.

From the spectroscopic data available for Diphenylamine, direct photolysis in atmosphere is not to be expected.

Biodegradation

Murin et al. (1997) reported a biodegradation of 26 % in a Closed Bottle Test (OECD 301 D, 1992) and 38 % in a Mod. MITI (II) Test after 28 days. An activated sludge from a waste water treatment plant (wwtp) in Usserod, DK that was obviously not adapted to Diphenylamine was used as inoculum. Although the authors report diminished oxygen consumption in the toxicity control of the Closed Bottle Test as compared to the reference control, they reported no quantitative decrease of the consumption or the amount of biodegradation achieved in the toxicity control. Hence, it may be concluded that the degradation was above 25 % and thus the test can be regarded as valid according to

92/69/EEC. This means, on the other hand, that the test result was not significantly influenced by toxic effects. The result of the Mod. MITI (II) Test (biodegradation of 38 %) may have been influenced by toxic effects as the substance concentration in this test was 30 mg/l and a test on inhibition of respiration according to OECD 209 showed an EC_{50} of 18.7 mg/l.

Christodoulatos et al. (1997) showed primary degradation of Diphenylamine in batch shaker flasks at 30 °C and 100 rpm by pure Pseudomonas strains and by adapted mixed activated sludge as well. All tested cultures were capable of degrading Diphenylamine as sole carbon source, as well as together with glucose as co-substrate. The Diphenylamine disappearance rate constant was found to follow first order kinetics. The primary degradation rates after 11 days in the shaker flask experiments were 71 % and 89 % (HPLC).

Transformation of Diphenylamine with aniline acclimated activated sludge has been demonstrated by Malaney (1960) in Warburg respirometric experiments. The oxygen demand in 180 h was about 30 % of the ThOD. An evaluation of the respirometric curve reveals an almost linear increase of the oxygen uptake with the time up to 180 h. This indicates that presumably additional biodegradation potential is available (Christodoulatos et al. 1997).

Dahn (1997) investigated the elimination of Diphenylamine from propellants coming up from dismantling of ammunition. Diphenylamine was eliminated in a first step to 99.6 % (2 000 mg/l to 6.6 mg/l) after alkaline hydrolysis at elevated temperature. In a second step the author reported elimination of Diphenylamine of 100 % (2 mg/l to < 0.003 mg/l) in the biological stage of a pilot plant. Diphenylamine was not detectable, neither in the sludge (detection limit = 0.25 mg/kg) nor in the effluent (d.l. = 3 µg/l). Detailed information on the inoculum was not given.

In a bioreactor system primary degradation of Diphenylamine has been shown by adapted mixed activated sludge. The average biodegradation rate constant from the bioreactor experiments is 0.54 d^{-1}, half-life is 1.3 d (Christodoulatos et al., 1997).

Over 96 % primary degradation of ^{14}C labelled Diphenylamine in 24 h was found in a laboratory model sewage sludge system. Diphenylamine was completely mineralized only to an extent of 15 % in 50 h. Identified metabolites (4-hydroxyDiphenylamine, aniline, indole) are not persistent in the sludge (Gardner et al., 1982).

Sediment

Diphenylamine has been transformed cometabolically by bacteria in sediment under anaerobic conditions mainly to aniline or its derivatives (Drzyzga 1996, 1997). But there is no quantitative information available.

Conclusion

Using non adapted inocula, mineralisation of Diphenylamine was achieved only up to approx. 25 %. According to Technical Guidance Document, the substance has to be regarded as not readily biodegradable. Furthermore, currently Diphenylamine cannot be considered as inherently biodegradable in spite of the fact that extensive primary degradation of Diphenylamine especially by communities of adapted micro-organisms has been

demonstrated several times. The result of the Mod. MITI (II) Test (Murin et al., 1997) resulted in a degradation of only 38 % but may have been influenced by toxic effects as the substance concentration in this test was 30 mg/l. A test on inhibition of respiration according to OECD 209 showed an EC_{50} of 18.7 mg/l.

According to the available degradation data, the rate constants presented in Table 3.1 are applied for the assessment.

Table 3.1: Degradation constants

Compartment	Degradation rate constant	Half-life
Waste water treatment plant	$kbio_{sewage\ treatment\ plant} = 0\ h^{-1}$	∞
Aquatic compartment	$kbio_{surface\ water} = 0\ d^{-1}$	∞
Aquatic compartment	$kphoto_{water} = 3.96 * 10^{-4}\ h^{-1}$	73 d
Soil	$kbio_{soil}$ = negligible	∞
Sediment	$kbio_{sediment}$ negligible	∞

Distribution

The Henry's law constant of 0.139 Pa * m³/mol calculated from the physico-chemical properties indicates that Diphenylamine is moderately volatile from water.

The adsorption and desorption behaviour of Diphenylamine was investigated by Briggs (1981). The measured adsorption coefficient water-organic matter $logK_{OM}$ is 2.54. From this figure the partition coefficient water-organic carbon K_{OC} of 596 l/kg was derived by the author. According to the OECD-Test Guideline 106 "Adsorption-Desorption Using a Batch Equilibrium Method", an equilibrium time of min. 24 h is generally sufficient. In contrast, the chosen equilibrium time in this experiment was only 2 h. It seems, that this short equilibrium time may have led to an underestimation of the sorption potential of Diphenylamine. Therefore this figure is not taken into account in this risk assessment.

In the Technical Guidance Document an equation for the calculation of K_{OC} of anilines using $logK_{OW}$ is provided. On this basis, a K_{OC} of 907.8 l/kg was estimated.

This K_{OC} indicates a moderate sorption potential. Using the value of 907.8 l/kg, the solids-water partition coefficients with the default organic carbon contents as proposed by the Technical Guidance Documents have been determined for each compartment (see Table 3.2).

Table 3.2: Solid-water partition coefficients

Parameter	Organic carbon content [%]	Partition coefficient [l/kg]
Kp (soil-water)	2	18.2
Kp (sediment-water)	5	45.4
Kp (suspended matter-water)	10	90.8
Kp (raw sewage-water)	30	272
Kp (activated sludge-water)	37	336

The environmental compartment-water partition coefficients (Eqs. 22 and 24 in TGD) are presented in Table 3.3.

Table 3.3: Compartment-water partition coefficients

Parameter	Partition coefficient [m^3/m^3]
$K_{soil\text{-}water}$	27.4
$K_{sediment\text{-}water}$	23.5
$K_{susp\text{-}water}$	23.6
$K_{air\text{-}water}$	$5.88 * 10^{-5}$

Elimination in waste water treatment plant

Table 3.4 presents the fate of Diphenylamine in waste water treatment plant as estimated with EUSES using the 9-box model (with $\log K_{ow}$ of 3.4, the Henry's law constant of 0.139 Pa m^3 mol^{-1} and a biodegradation rate of 0 h^{-1}).

Table 3.4: Elimination of Diphenylamine in wwtp.

	Fraction [%]
Directed to air	0.2
Directed to water	89.7
Directed to sludge	10.1
Degraded	0
Removed from water	10.3

Accumulation

Geoaccumulation

The estimated K_{OC}-value indicates potential for geoaccumulation. Diphenylamine is expected to be only slightly mobile. Nevertheless, in the groundwater below a former military airfield in Lower Saxonia Diphenylamine was measured up to 8 ppb (Entenmann & Schäcke, 1994).

Bioaccumulation

Cyprinus carpio was tested with Diphenylamine concentrations of 0.01 mg/l and 0.1 mg/l in a flow through test (temperature 25 ˚C, duration 8 weeks) reaching steady state. BCFs were calculated from measured Diphenylamine concentrations in whole fish (wet weight) and in water. BCF-values were 51 - 253 and 101 – 242, respectively (CITI, 1992). The exact results, flow rate and depuration times were not given, but the results give and idea of the magnitude of the BCF.

A flow-through study with *Pimephales promelas* resulted in a BCF of 30 at a test concentration of 43.7 µg/l after 32 d (Veith et al., 1979). The authors mention gaschromatographic analysis as well as scintillation counting from homogenised whole fish for 30 substances tested. According to the authors, all BCF-values were related to the substances tested.

A BCF based on logKow was calculated according to Technical Guidance Document and resulted in a BCF of 155 l/kg. This is in accordance with the measured data from CITI (1992) and indicates a need for an assessment of secondary poisoning. Since no averaged value was given in the CITI (1992) study, the calculated BCF of 155 l/kg will be used in the assessment.

No measured BCF for earthworm is available. BCFearthworm of 31 was calculated according to the TGD (eq. 82d) based on $\log K_{ow}$ of 3.4 and will be used for the estimation of secondary poisoning via terrestrial food chain.

Natural occurrence

Diphenylamine is a natural occurring substance. It has been detected in several plants e.g., in

-*Calamintha nepeta*, a perennial green shrub in the mediterranean region. Diphenylamine was found as a compound in the essential oil of leaves and stem (Mastelic et al., 1998)

-Soy protein isolates (d.l. – 0.02 ppm) (Boatright et al., 1997)

-*Eucommia ulmoides*, as compound in the leaves (gutta-percha, used in Chinese medicine) (Guo et al., 1995)

-*Narcissus trevithian*: it was found as a compound in the essential oil of the flower (van Dort et al., 1993)

-Important medical and food plants: lemon species, onion, dill, tea and coriander between 1 – 50 mg/kg (Karawya et al., 1986)

-*Allium cepa* (onion): it was found in green leaves, but mainly in the bulbs (Karawya et al., 1984, 1986)

-Green and black tea as aroma stuff (Nose et al., 1971, Karawya et al., 1984)

-The extract obtained by steam distillation of Fenugreek seeds (Trigonella foenum) not treated before with pesticides contained between 0.5 and 5 % Diphenylamine (Girardon et al., 1985).

-Konjak flour: odorous substance (Sun et al., 1997)

Furthermore, Diphenylamine has been found as volatile compound in headspace samples produced by bacteria on spoiled beef (0.3 – 0.8 ppb) (Intarapichet et al., 1992).

3.1.2 Aquatic compartment

The calculation of exposure for the local scenarios developed on the basis of major uses and production was conducted with EUSES using generic values and models presented in the Technical Guidance Document for the only known (closed in 2001) production site and the only known processing site, specific scenarios have been calculated using the data provided. These scenarios are included in the confidential Appendices B1 and B2.

From the emission data for Diphenylamine listed in the US Toxic Release Inventory (TRI) for the year 1997, emission factors in the range from 5 * 10^{-4} % to 3 % have been estimated. These figures are for additional information only and are not taken into account for the risk assessment.

3.1.2.1 Estimation of $Clocal_{water}$ for production

Site specific data

A **$Clocal_{water}$ of 89.8 µg/l** and a **$PEC_{stp,micro-organisms}$ of 3.59 mg/l** for the only known (closed in 2001) European production site has been estimated (for details, see Appendix B1).

Generic scenario

On the basis of information from Industry, it is assumed that there are no production sites with additional processing capacities. A generic $Clocal_{water}$ for production of Diphenylamine was calculated (EUSES use pattern 2). Based on the information from former and present producers and the information presented in Chapter 2.1, it is assumed, that a typical plant has a production volume of 5000 t/a. In the following, the major parameters for the calculation are given:

Emission factor (Table A 1.2; waste water):	0.003
Emission factor (Table A 1.2; surface water):	0

No. of days (Table B 1.6):	300
Fraction directed to surface water from stp:	0.896

The emission scenario document for IC 3 gives a wwtp effluent of 10 000 m^3/d and a dilution factor of 1:40, a **$Clocal_{water}$ of 0.112 mg/l** and **$PEC_{stp,micro-organisms}$ of 4.48 mg/l** result (for calculation details, see Appendix A; production was included in the use pattern 2).

3.1.2.2 Estimation of $Clocal_{water}$ for formulation

Formulation of lubricant oil (IC/UC = 16/35;0/35;08/35)

According to industry, a small amount of Diphenylamine is used in lubricant oils in a concentration of 1 %. There is contradicting information regarding to whether Diphenylamine is merely an impurity in alkylated diphenylamine antioxidants for lube oils, or whether it is as itself used as a primary constituent. Diphenylamine is used in petroleum or synthetic lubricant oils. Based on the specific information available from industry, it is assumed, that 50 t/a is used for this purpose. The amount of 5000 t/a lubricant oil produced results assuming the 1 % fraction for Diphenylamine.

Industry has not provided any specific information on the uses of these lubricant oils. According to the Nordic Product Register and The Finnish Product Register, the substance is used in the classes 'Sale, maintenance and repair of motor vehicles and motorcycles; retail sale of automotive fuel' and 'Manufacture of machinery and equipment'. The first use is covered in TGD by IC 0 ('Others'), the latter one by IC 16 ('Engineering industry') or by IC 8 ('Metal extraction, refining and processing industry'). Based on specific industry information, formulation is carried out in the chemical industry (IC 3). The environmental concentrations are calculated based on the Technical Guidance Document. For the emissions to waste water, the default emission factor of 0.02 from Table A 2.1 is used. A standard wwtp of 2 000 m^3/d and a dilution factor of 1:10 are assumed. It is assumed, that 10 % of the formulation occurs in the region, of which the fraction of mainsource is set to 1 on the basis of specific information from industry. The formulation is assumed to be continuous (No of days = 300). A **$Clocal_{water}$ of 14.9 µg/l and $PEC_{stp,micro-organisms}$ of 0.149 mg/l** result (for details, see Appendix A, use pattern 1).

Formulation of the plant protection product – storage aid for fruits (IC/UC = 01/38)

At this moment it is assumed, that an amount of about 200 t/a Diphenylamine is used as plant protection product for scald prevention of fruits or vegetables, mainly for apples and pears. There is no specific information available on European formulation facilities. For this first approach it is assumed that the formulation of the plant protection product takes place in the agricultural industry (IC=01) and 10 % of the use takes place in the region.

According to information available from the U.S., Diphenylamine concentration in the formulation is approximately 15-30 %. UK has confirmed that one product on the market has Diphenylamine content of 31 %. Assuming the upper Diphenylamine content of 30 % for European preparations, a tonnage of 670 t/a results. For this formulation volume a fraction of

main source of 0.6 and a duration of emission of 201 d/a (Table B 2.1) applies. Emission factor for waste water is 0.02 (Table A 2.1).

Assuming a standard wwtp of 2 000 m^3/d and a dilution factor of 1:10, a **$Clocal_{water}$ of 59.7 µg/l** and **$PEC_{stp,micro-organisms}$ of 0.598 mg/l** are calculated (for details, see Appendix A, use pattern 4).

3.1.2.3 Estimation of $Clocal_{water}$ for processing

An estimated amount of 89.5 % (corresponding to 8950 t/a) of the total EU quantity of 10 000 t/a are used as an intermediate for the production of antioxidants, antiozonants, phenothiazine, dyes and other products.

Intermediate processing by non-producers, site specific data (IC/UC = 03/33)

At the present, one processing site only using Diphenylamine as intermediate is known (see also chapter 2.1). The processing site has delivered measured data on emissions to water, on which basis a specific local scenario has been developed. It is presented in Appendix B2. A **$Clocal_{water}$ of 62.1 µg/l** and a **$PEC_{stp,micro-organisms}$ of 2.49 mg/l** have been estimated (for details, see Appendix B2).

Intermediate processing by non-producers (IC/UC = 03/33) – generic scenario

A generic local scenario was also calculated because the only known intermediate processing site does not cover all the tonnage allocated to the use as intermediate. According to the information on intermediate processing sites in the original IUCLID data set, all of the sites have been of major size. A processing volume of 4000 t/a at one site is used for calculations. In the following, the major parameters for calculation of exposure are presented.

Emission factor (waste water):	0.007 (Table A 3.3)
No. of emission days:	300

Emission during the emission period is 93 kg/d. The emission scenario document for IC 3 gives a wwtp effluent of 10 000 m^3/d and a dilution factor of 1:40 a **$Clocal_{water}$ of 0.208 mg/l** and **$PEC_{stp,micro-organisms}$ of 8.34 mg/l** (for further details, see Appendix A, use pattern 2).

Processing of lubricant oil (IC/UC = 16/35;0/35;8/35)

Lubricant oils containing Diphenylamine are used in the classes ‘Sale, maintenance and repair of motor vehicles and motorcycles; retail sale of automotive fuel’ and ‘Manufacture of machinery and equipment’ according to the Nordic product registers. The first use is covered in TGD by IC 0 (‘Others’), the latter one by IC 16 (‘Engineering industry’) or by IC 8 (‘Metal extraction, refining and processing industry’). No information on the share of each branch of the use volume is available. For the two first industrial use categories, the Technical Guidance Document gives the same A- and B-tables (A 3.16 and B 3.14). For IC 8, the available emission scenario document does not give any further advice on the estimation of the emission from the industrial use for pure oils lubricants. However, Table A 3.7 of the TGD

gives an emission factor of 0.185 for waste water, which is higher than the corresponding emission factor for IC 16 and IC 0 (there: 0.1).

Giving the above reasons, a realistic worst case approach is derived by assuming that the whole use takes place in the IC 16 ('Engineering industry'). The regional tonnage is assumed to be 10 % of the total use. The Technical Guidance Document gives an emission factor of 0.1 for emissions to waste water from Table A 3.16 and a fraction of mainsource of 0.9 and 45 emission days from Table B 3.14. A connection to a municipal standard wwtp of 2 000 m^3/d and a dilution factor of 1:10 can be assumed according to the emission scenario document for lubricants (IC 8). On this basis, a **$Clocal_{water}$ of 437 µg/l and $PEC_{stp,micro-organisms}$ of 4.38 mg/l** have been calculated (for details, see Appendix A, use pattern 1).

Professional use (processing) of storage aid (IC/UC = 01/38)

Technical Guidance Document does not recommend to calculate local scenario for professional use of a plant protection product, because the use belongs to the scope of the Directive 91/414/EEC. However, it should be kept in mind that the use of storage aid may cause significantly elevated concentrations in the surrounding environment or in a connected waste water treatment plant. The total emissions from the professional use of storage aid are taken into account in the estimation of regional and continental concentrations.

3.1.2.4 Estimation of $Clocal_{water}$ for private use of Diphenylamine-treated fruits/ vegetables

Emissions from the private consumption of Diphenylamine -treated fruits and vegetables can be assumed to be very dispersive by nature. The releases from this use are taken into account in the regional and continental scale.

3.1.2.5 Monitoring data

Sporstol et al. (1985) detected Diphenylamine in 21 % of all samples from polluted fjord areas as well as from industrial effluents and municipal wwtp´s in Norway (limit of quantitation: 1 µg/l).

According to an U.S. EPA report (EPA, 1985) the gross analysis data from the U.S. EPA STORET data base contained at that time 152 observations for Diphenylamine with a mean concentration of 5 µg/l and a range of 3.2 – 10 µg/l. Further details on the origin of the water samples were not given.

Pahren and Melton (1979) reported the presence of trace organics in effluents from water treatment plants in the U.S., California. In the effluent of a Lake Tahoe plant, Diphenylamine was detected at 1.54 ppt and in the effluent of a plant in Pomona, at a level of 9.77 ppt.

In Japan, Diphenylamine was analysed from 80 water samples and 29 fish samples. The detection limits were in a range of 0.6–5 µg/l in water and 150-250 µg/kg in fish. In all samples Diphenylamine content was below the respective detection limit (Environment Agency Japan, 1985).

Diphenylamine was detected by Anna et al. (1984) in sewage sludge of a waste water treatment plant of a chemical production site and in sewage sludge of a common municipal and industrial wwtp. The sludge of purely municipal wwtp did not contain detectable amounts of Diphenylamine. Detection limit for Diphenylamine was not stated explicitly. For other substances values as low as 0.5 µg/kg dw could be determined.

Furthermore, plenty of monitoring data on Diphenylamine content in storage-aid treated food exist. Reported concentrations (Frank et al., 1990; Green & Lillemark, 1994; KAN-DO, 1995; Johnson et al., 1997) vary significantly but indicate clearly a broad use of Diphenylamine. The concentrations reported have remained mainly well below the maximum residue limits for fruits.

Yasuhara et al. (1997) determined the Diphenylamine concentration in leachate from waste disposal sites in Japan. Samples from 8 different landfills receiving domestic waste, ash from incinerator, sewage sludge, waste plastics etc. were investigated with Diphenylamine concentrations ranging from 6.4 – 25.5 ng/l with a mean concentration of approx. 0.01 µg/l.

3.1.2.6 Sediment

Due to the sorption potential of Diphenylamine, an assessment of the compartment sediment seems to be necessary. The concentrations in sediments of each local scenario were calculated applying the equilibrium partitioning method of the Technical Guidance Document (eq. 50). For the calculation, the regional PEC_{water} of 0.79 µg/l was added to the $Clocal_{water}$ –values given in chapter 3.1.2. to first obtain the local PEC_{water} –values for input into the equation 50. The $K_{susp\text{-}water}$ of 23.4 m^3/m^3 was applied. The results are listed in the following.

Production (site specific): $PEC_{sediment}$ = 1.84 mg/kg wwt

Production: $PEC_{sediment}$ = 2.31 mg/kg wwt

Formulation of lubricant: $PEC_{sediment}$ = 0.32 mg/kg wwt

Formulation of storage aid: $PEC_{sediment}$ = 1.24 mg/kg wwt

Processing (intermediate, site specific) $PEC_{sediment}$ = 1.28 mg/kg wwt

Processing (intermediate use): $PEC_{sediment}$ = 4.29 mg/kg wwt

Professional use of lubricant (processing): $PEC_{sediment}$ = 8.99 mg/kg wwt

Measured data

In Japan, Diphenylamine was analysed in a number of samples from sediment (n=20). The detection limits were in a range of 200-740 µg/kg. In all samples, Diphenylamine content was below the detection limit (Environment Agency Japan, 1985).

In the Hamburg port mud, Diphenylamine was qualitatively detected in the years 1983 and 1984 (Schenk, 1986).

3.1.3 Atmosphere

Concentrations in air were predicted according to Technical Guidance Document using EUSES for those scenarios where direct emissions to air are assumed to occur as default. Table 3.5 lists the resulting annual average concentrations in air and annual deposition fluxes. Since the regional PEC_{air} = $2*10^{-8}$ mg/m^3, the Clocal –values are approximately equal to PECs.

3.1.3.1 Estimation of $Clocal_{air}$ for production and processing (intermediate use)

According to the Technical Guidance Document, no emissions to air are expected from production of Diphenylamine or its processing as intermediate for generic scenarios. However, the site specific information indicates that emissions to air do occur from these activities. The site specific concentrations in air are calculated in the Appendices B1 and B2.. **$Clocal_{air}$ of $1.23*10^{-4}$ mg/m^3** for production and **$Clocal_{air}$ of $2.22 *10^{-5}$ mg/m^3** for intermediate processing were estimated.

3.1.3.2 Estimation of $Clocal_{air}$ for formulation

Formulation of lubricant oil (IC/UC = 16/35)

No branch specific information on emissions to air are available, except on the size of the formulation site (see Chapter 3.1.2.2). The concentration in air is calculated based on Technical Guidance Document with EUSES. For air, the default emission factor of 0.0025 from Table A 2.1 is applied. It is assumed, that 10 % of the formulation occurs in the region, of which the fraction of mainsource is set to 1 on the basis of the information from industry. The formulation is assumed to be continuous (No of days = 300). A **$Clocal_{air}$ of 0.012 µg/m^3** results.

Formulation of the plant protection product – storage aid for fruits (IC/UC = 01/38)

There is no specific information available on European formulation facilities and products. For this first approach it is assumed that the formulation of the plant protection product takes place in the agricultural industry (IC=01) and 10 % of the use takes place in the region. When the use information from the assessment under the Directive 91/414/EEC becomes available, this scenario will be updated, if necessary (see Chapter 2.1 and Chapter 3.1.2.2). A fraction of main source of 0.6 and a duration of emission of 201 d/a (Table B 2.1) are applied. Emission factor for air is 0.0025 (Table A 2.1). The concentration in air has been calculated based on Technical Guidance Document with EUSES. A **$Clocal_{air}$ of 0.0463 µg/m^3** results.

3.1.3.3 Overview of the annual average concentrations and deposition rates

Table 3.5 shows the annual average concentrations in air and annual deposition rates from those scenarios where release to air is assumed as default. EUSES uses these data as input for calculation of the concentrations on soil.

Table 3.5: Local annual average concentrations in air and total annual deposition rates

Scenario	**$Clocal_{air\_ann}$ [mg/m³]**	**$DEPtotal_{ann}$ [mg/m² * d]**
Production (site specific)	$1.0 * 10^{-4}$	$1.8 * 10^{-4}$
Formulation of lubricant	$9.5 * 10^{-6}$	$1.4 * 10^{-5}$
Formulation of storage aid	$3.8 * 10^{-5}$	$5.7 * 10^{-5}$
Intermediate processing (site specific)	$1.8 * 10^{-5}$	$2.8 * 10^{-5}$

Measured data

In Norway Diphenylamine was qualitatively determined in rain water and snow in the years 1973 and 1974 by Lunde et al. (1977).

3.1.4 Terrestrial compartment

Direct pollution of soils with Diphenylamine potentially takes place in restricted areas (e.g. arms refuse dumps) due to its use as stabiliser in explosives. For example, at an ammunition depot in West Germany a maximum concentration of 1.7 g/kg soil and a maximum ground water concentration of 3.9 µg/l in the vicinity of this arms refuse dump was measured (Orth et al, 1994). Furthermore, a contamination of a former military air field with Diphenylamine and derivatives of the compound in concentrations up to 730 ppb in soil and up to 8 ppb in ground water was reported (Entenmann & Schäcke, 1994). Overall, the reported pollution of soil seems to occur in restricted military areas and are not considered to be of general relevance for the risk assessment of soil.

The assessments for the known production site and the known intermediate processing site are presented in Appendices B1 and B2. The results are listed below.

Atmospheric deposition to soil according to site specific local releases are of an order of magnitude of 10^{-5} to 10^{-4} mg/m²*d. The deposition around the sites is of minor importance for local Diphenylamine concentrations in soil. Application of sludge onto soils around the largest sites does not occur, but sludge is either deposited into an industrial landfill or incinerated. However, many of the industrial sites considered in this risk assessment at generic level (e.g., lubricant processing) may be connected to municipal sewage treatment plants. No information on how sludge is handled was received from industry. Therefore, soil concentrations around all generic sites except production and intermediate processing (which are assumed to have their own biological treatment plants) are calculated taking into account both the application of sludge and deposition. For the generic and site specific production and intermediate processing sites deposition is assumed to be the only input path.

Details of each generic scenario are explained in chapter 3.1.2. Concentrations in agricultural soil around sites were calculated according to the Technical Guidance Document with EUSES and are presented below. Csoil refers to the situation, where the Diphenylamine load from sludge application is averaged over 30 days, the mixing depth is 5 cm and rate of sludge application 0.5 kg dwt * m^2 /year. $C_{agr,soil}$ refers to the situation when sludge application is averaged over 180 days, mixing depth is 20 cm and the application rate given above. Cgrassland in turn refers to the situation where the averaging time for application it the same as for Cagr,soil but mixing depth is 10 cm and the rate of application is 0.1 kg dwt * m^2/year. To obtain local PECs, the regional PEC for natural soil is added to the Clocal –values. Due to the low regional PEC for natural soil (1.04 *10^{-5} mg/kg wwt), the following Clocal –values are approximately equal to local PEC-values.

Production (site specific)	C_{soil} =	1.7 * 10^{-3} mg/kg wwt
	$C_{agr,soil}$ =	1.7 * 10^{-3} mg/kg wwt
	$C_{grassland}$ =	2.8 * 10^{-3} mg/kg wwt
Production:	C_{soil} =	3.8 * 10^{-4} mg/kg wwt
	$C_{agr,soil}$ =	3.8 * 10^{-4} mg/kg wwt
	$C_{grassland}$ =	6.4 *10^{-4} mg/kg wwt
Formulation of lubricant:	C_{soil} =	0.53 mg/kg wwt
	$C_{agr,soil}$ =	0.53 mg/kg wwt
	$C_{grassland}$ =	0.18 mg/kg wwt
Formulation of storage aid:	C_{soil} =	2.13 mg/kg wwt
	$C_{agr,soil}$ =	2.12 mg/kg wwt
	$C_{grassland}$ =	0.72 mg/kg wwt
Processing (intermediate; site specific):	C_{soil} =	2.5 * 10^{-4} mg/kg wwt
	$C_{agr,soil}$ =	2.6 * 10^{-4} mg/kg wwt
	$C_{grassland}$ =	4.3 * 10^{-4} mg/kg wwt
Processing (intermediate use):	C_{soil} =	7.0 * 10^{-4} mg/kg wwt
	$C_{agr,soil}$ =	7.1 * 10^{-4} mg/kg wwt
	$C_{grassland}$ =	1.19 * 10^{-3} mg/kg wwt
Professional use of lubricant (processing):	C_{soil} =	15.6 mg/kg wwt
	$C_{agr,soil}$ =	15.5 mg/kg wwt
	$C_{grassland}$ =	5.3 mg/kg wwt

Concentration in groundwater equals according to the Technical Guidance Document the concentration in soil porewater. PEC for groundwater under agricultural soil is calculated with EUSES (Eq. 67 of TGD) for further use in chapter 4.1. The $K_{soil\text{-}water}$ of 27.4 was applied for the calculation. Contribution of the ambient regional background concentration, which is only affected by deposition, is negligible (although included in calculation). The results are as follows.

Production (site specific): $PEC_{agr.soil,porew} = 1.05 * 10^{-4}$ mg/l

Production: $PEC_{agr.soil,porew} = 2.4 * 10^{-5}$ mg/l

Formulation of lubricant: $PEC_{agr.soil,porew} = 0.033$ mg/l

Formulation of storage aid: $PEC_{agr.soil,porew} = 0.131$ mg/l

Processing (intermediate; site specific) $PEC_{agr.soil,porew} = 1.6 * 10^{-5}$ mg/l

Processing (intermediate use): $PEC_{agr.soil,porew} = 4.5 * 10^{-5}$ mg/l

Professional use of lubricant (processing): $PEC_{agr.soil,porew} = 0.961$ mg/l

Measured data

In Israel, Muszkat et al. (1993) observed a Diphenylamine concentration of 10 µg/kg wwt in soil in samples taken at a depth of 20 cm in the unsaturated zone at a location irrigated with sewage effluent.

3.1.5 Non compartment specific exposure relevant to the food chain

Aquatic food chain

Diphenylamine is moderately bioconcentrating in fish. BCF_{fish} of 155 as derived in chapter 3.1.1 is considered as a representative value. On the basis of mammalian toxicity data Diphenylamine is classified as toxic. Therefore, an assessment of secondary poisoning is carried out.

According to the Technical Guidance Document, the level of a substance in food (fish) for predator birds and mammals is calculated from the PEC for surface water and the highest measured BCF:

$PEC_{oral,predator} = PEC_{water} * BCF_{fish} * BMF$

For Diphenylamine, the biomagnification factor BMF is 1 according to the Technical Guidance Document. $PEC_{oral,predator}$ is based on the average PEC_{water}s (given as annual averages) of the regional and local scenario (this equals to the assumption that 50 % of food is

acquired from the local recipient and 50 % from the region). Table 3.6 presents the resulting $PEC_{oral,predator}$ -values for the generic local scenarios.

Table 3.6. Results for $PEC_{oral,predator}$ for aquatic food chain.

Scenario	**$PEC_{oral,predator}$(mg/kg)**
Production (site specific)	5.78
Production	7.24
Formulation of lubricant	1.07
Formulation of storage aid	3.92
Processing (intermediate; site specific)	4.01
Processing (intermediate use)	13.4
Professional use of lubricant (processing)	4.28

Terrestrial food chain

Exposure of birds or mammals via terrestrial food-chain soil → earthworm → worm-eating birds or mammals is derived according to the TGD Eqs. 80-82. $BCF_{earthworm}$ of 31 has been estimated. It is assumed that the predator acquires 50 % of its pray from a local environment (local PECagr,soil applied) and 50 % from the region (regional PECagr,soil applied).

Table 3.7. Results for $PEC_{oral,predator}$ for terrestrial food chain.

Scenario	**$PEC_{oral,predator}$(mg/kg)**
Production (site specific)	0.012
Production	0.010
Formulation of lubricant	0.488
Formulation of storage aid	1.908
Processing (intermediate; site specific)	0.011
Processing (intermediate use)	0.011
Professional use of lubricant (processing)	13.929

3.1.6 Regional concentrations

Calculation of diffuse releases

Post harvest treatment of fruits and vegetables with a Diphenylamine containing formulation and subsequent storage takes place in the agricultural industry. 10 % of this use can be assumed to occur in the region. In Table A 3.1 of the Technical Guidance Document only a release to soil (EF = 0.05) for this scenario is considered. However, as the preparation of dip or spraying solutions containing Diphenylamine is expected to occur in a warehouse, the emissions are allocated to waste water instead of directly to soil for this first approach. As soon as specific information on this life cycle step becomes available, the appropriate target compartment will be considered. Consequently, the proposed emission factor to soil is assigned provisionally for the generic release to waste water. Assuming the default regional stp according to the Technical Guidance Document, a total emission to surface water (via stp) of 9 t/a results (For further details, see use pattern 3, in Appendix A).

Releases of Diphenylamine from consumption of fruits and vegetables is of very dispersive nature and seems to be wide spread on the basis of monitoring data from programs checking the compliance with maximum residue limits from different countries. For the release estimation, 100 % of Diphenylamine present in fruits after storage (180 t/a) is assumed to be released by washing into waste water (for further details, Appendix A, use pattern 3; the private use scenario of EUSES (in IC 1) was used to include releases from this scenario in the assessment). A refinement of this scenario may be possible based on information on the fate of Diphenylamine in treated fruits (will be updated later if relevant for the conclusions).

Allocation of releases

For regional releases, the 10 % rule for regional use, as proposed in the Technical Guidance Document, has been applied for all other life-cycle stages and uses except for production and intermediate processing.

On the basis of the information provided by industry on the size of the sites which are or were producing or processing (intermediate use only) Diphenylamine in the 90's, the division of the volume between region and continent is carried out as follows. Of the 9000 t/a production volume, 5000 t/a is addressed to the region and the rest 4000 t/a to the continent. For processing, the complete use volume of the 8950 t/a is allocated to the continent, because the only known (major) processing site is not located in the same region as the production site. It has to be kept in mind, that as the largest sites are hypothetical, there is no site specific emission data available for them and the emission estimation both in region as well as in continent is carried out with EUSES using the generic emission factors (the emission factors for each compartment are indicated in the local scenarios in Chapters 3.1.2-3.1.4). For all sources, the default 80 % connection rate to wwtp´s is assumed according to the Technical Guidance Document.

Results

Overall releases to the region and continent are presented in Table 3.8. These releases are used by EUSES as input for the calculation of the continental and regional background concentrations.

Table 3.8: Total releases (t/a).

Compartment	Continent (t/a)	Region (t/a)
Air	0.61	0.07
Surface water	54.8	7.3
Waste water	219	29.2

Considering that 89.7 % of Diphenylamine in waste water goes further through biological waste water treatment to surface water, the water compartment seems to be the main target of Diphenylamine pollution.

With the present generic assessment approach, the most significant contributors to the predicted ambient background concentrations are release from consumption of fruits, production of Diphenylamine and intermediate processing of Diphenylamine.

Regional and continental predicted environmental concentrations

The predicted environmental concentrations as calculated by EUSES with the emissions given above are presented in Table 3.10.

Table 3.10: Regional and continental predicted environmental concentrations.

	Concentration		Concentration	Unit
$PECcont_{water}$	$1.3 * 10^{-4}$	$PECregional_{water}$	$7.85 * 10^{-4}$	mg/l
$PECcont_{sediment}$	$3.46 * 10^{-3}$	$PECregional_{sediment}$	0.0198	mg/kg wwt
$PECcont_{air}$	$3.29 * 10^{-9}$	$PECregional_{air}$	$1.94 * 10^{-8}$	mg/m3
$PECcont_{agrsoil}$	$9.92 * 10^{-4}$	$PECregional_{agrsoil}$	0.0116	mg/kg wwt
$PECcont_{agrsoilporew}$	$6.15 * 10^{-5}$	$PECregional_{agrsoilporew}$	$7.21 * 10^{-4}$	mg/l
$PECcont_{natsoil}$	$1.76 * 10^{-6}$	$PECregional_{natsoil}$	$1.04 * 10^{-5}$	mg/kg wwt

Measured data

In the Netherlands, Piet and Morra (1983) monitored levels of organic substances in surface waters. Diphenylamine was detected at the level of 1 µg/l in raw Rhine water but not detected in tap water after passage through the bank filtration system.

Diphenylamine has been detected in milk of animals (cow, sheep, goat, water buffalo) raised in patches in Italy and France. Two publications of Moio et al. (1993, 1996) show concentrations up to approx. 30 ppb. Diphenylamine comes probably partly from a natural source (vegetation grazed) and partly from anthropogenic sources (via atmospheric deposition or sludge application). A contribution of Diphenylamine to the milk flavour could not be approved.

3.2 EFFECTS ASSESSMENT: HAZARD IDENTIFICATION AND DOSE (CONCENTRATION) - RESPONSE (EFFECT) ASSESSMENT

3.2.1 Aquatic compartment

3.2.1.1 Toxicity to Vertebrates

Fish:

The acute toxicity of diphenylamine to *Pimephales promelas* (fathead minnow) was examined by Geiger et al. (1990) using a flow-through test system supported by chemical analysis (HPLC). The short-term study was conducted with 20 Fish per treatment level (age:31d, length: 18.6 mm weight: 0.09 g). Fish were exposed at about 25°C for 96 h in dechlorinated laboratory water or unfiltered lake water (pH 7.8, dissolved oxygen content 7.3 mg/l, water hardness 48.0 mg/l $CaCO_3$). Five concentrations (1.1 - 6.0 mg/L) of test solution were applied by diluting a stock solution of diphenylamine. A 96 h-LC_{50} of 3.79 mg/l (confidence limits 3.47-4.14) and a 96 h-EC_{50} of 3.63 mg/L (confidence limits 3.31-3.99) were determined.

Tonogai et al. (1982) examined the correlation between TLm (Median Tolerance Limit) values and partition coefficients of 44 organic nitrogen compounds as well as 37 kinds of nitrogen-containing dyestuffs. Therefore, their acute toxicity to fish was investigated according to Japan Industrial Standards (JIS K01 02). The chemical substances were dissolved in water and neutralized with 0.01 N NaOH or HCl, if necessary. Ten fish of *Oryzias latipes* (Red Killifish) per trial were kept in 2 L of deionized water at 25 °C. The test fish were of the same age (length: about 2 cm, weight: about 0.2 g). After 24 and 48 h, the LC_{50} was determined. Based on nominal concentrations of diphenylamine a 24 h-LC_{50} of 4.0 mg/L and a 48 h-LC_{50} of 2,2 mg/L were obtained from this study.

A 48 h-LC_{50} of 5.1 mg/l for *Oryzias latipes* (Red Killifish) was derived from a further acute toxicity test according to Japan Industrial Standards. Ten fish per level were kept in 4 L of test water at 25 °C. No more relevant details are given.

Relevant test data are summarised in the following table.

Table 3.11: Toxicity to fish

Species/life-stage	Endpoint	Result (mg/l)	Method	Reference
Pimephales promelas (juven.)	48 – 96 h LC_{50} 96 h EC_{50} (behav.)	3.79 3.63 (effective conc.)	APHA, Wash. (1980) flow-through	Geiger et al. (1990)
Oryzias latipes (juven.)	24 h LC_{50} 48 h LC_{50}	4.0 2.2 (nominal conc.)	JIS K01 02 static	Tonogai et al. (1982)
	48 h LC_{50}	5.1	JIS K 01 02	CITI (MITI)

		(nominal conc.)	no details given	(1992)
Danio rerio	48 h LC_0	4.0	UBA test proposal	Pluta (1990)
(formerly *Brachy-*	72 - 96 h LC_0	3.0	semi-static	
danio rerio)	24 - 48 h LC_{50}	4.87		
(subadult)	72 - 96 h LC_{50}	4.58		
	24 - 96 h LC_{100}	6.0		
	14 d LC_0	$\geq$3		
	14 d NOEC (behav.)	0.1		
	14 d LOEC (behav.)	0.2		
		(nominal conc.)		

The symptoms observed in the tests with *Pimephales promelas* and *Danio rerio* were: depressed locomotor behaviour, loss of schooling, agony, termination of feeding (14 d test), and darkening in coloration. The transient character of all these symptoms points to a considerable capacity of fish for physiological adaptation to Diphenylamine.

In the 14 d test, growth development was not monitored. The LC_0 for the test corresponds to the maximum exposure concentration.

Toxicity to early life stages of frogs

The toxicity of agrochemicals (including diphenylamine) to tadpoles of *Rana brevicauda porosa* was examined by Nishiuchi (1989). For diphenylamine, a LC_{50} > 100 mg/l was reported.

The relatively low Diphenylamine toxicity (compared to that in fish) was confirmed by Fort et al.(1999). These studies had been prompted by reports on high incidence of amphibian mortality and malformation in and around certain ponds in Minnesota and Vermont (USA). Both lethal and teratogenic potential of Diphenylamine were investigated by using the frog embryo teratogenesis assay – Xenopus (FETAX). Therefore, a 4-d embryo-larval development test and a 30-d limb development test were conducted with 10-12 concentrations of diphenylamine. 20 blastula-stage embryos of *Xenopus laevis* were placed in 60-mm plastic petri dishes containing control or treatment solutions. 40 embryos per treatment level and control were tested. All solutions were renewed every 24 h (4-d test) or 48 h (30-d test). After 5 days, the larvae of the 30-d study were transferred to 5-L aquaria containing 2 L of the appropriate test solution.While no deaths were observed up to the limit of aqueous solubility of Diphenylamine, an EC_{50} of 39.6 mg/l for teratogenic effects was determined. In the embryos exposed for 4 d, a maldeveloped gut and muscular kinking occurred. In addition, visceral hemorrhage was observed. In contrast to those findings, 30 d exposure did not result in abnormal limb development. As minimum concentration to inhibit growth (monitored by length measurements), a value of 50 mg/l was reported.

Since there were obviously different endpoints of short-term / long-term tests with non-comparable results and a comparatively (to fish) low sensibility of *Xenopus* this 30 d results

cannot be used as a 3rd long-term test to lower the assessment factor according to Technical Guidance Document (see below

3.2.1.2 Toxicity to Invertebrates

For invertebrates, toxicity tests with *Daphnia magna* are principally available. The short term toxicity of diphenylamine to *Daphnia magna* was examined by Murin et al. (according OECD 1992).20 Daphnids less than 24 h old per level were exposed to a dilution serie of the test substance. Concentrations of diphenylamine were measured as NVOC (non volatile organic carbon). A 48 h-EC_{50} of 2.0 mg/L (convidence limits 1.69-2.46) for *Daphnia magna* was determined.

A 24 h-EC_{50} of 2.3 mg/L for *Daphnia magna* was reportet by Bayer AG (1986a). The acute toxicity test was conducted under static conditions according UBA test proposal (1984). The Daphnids were exposed at 20°C for 24 h. No more details are given.

Dave et al. studied the sediment and water phase toxicity of diphenylamine to the crustaceans *Daphnia magna* and *Nitocra spinipes* (marine). Ageing effects as a result of hydrolysis or biodegradation as well as toxicity activation by ultraviolet light were also assessed. Culturing and toxicity testing followed standard methods (SIS 1991; ISO 1996). Tests were conducted with and without addition of 10 % (wet wt) clean sediment. Potassium dichromate was used as reference toxicant.. Prior to addition of test organisms, all solutions or suspensions were allowed to settle for 1-2 h.. *Nitocra spinipes* was exposed to a dilution serie of the test substance. As dilution water 7 psu-seawater (practical salinity units: $^0/_{00}$ or g/L) was used. Five adults of *Nitocra spinipes* (2-4 weeks old) per plate were incubated in darkness at 20 °C (± 2). Survival was recorded after 96 h. 20 Daphnids (6-24 h old) per dish (2 replicates) were exposed to diphenylamine at 20 °C (light/dark cycle:12 h). Measured values for dissolved oxygen were above 80% of air saturation during the test period. Immobilisation was recorded after 24 and 48 h. 96 h-LC_{50} values of 3.7 mg/L (water) and 1.22 mg/L (sediment) for *Nitocra spinipes* were derived. 48 h-EC_{50} values of 1.2 mg/L (water) and 3.2 mg/L (sediment) for *Daphnia magna* were determined. The toxicity of diphenylamine to crustaceans was activated by UV light. 96 h-LC_{50} values of 1.0 mg/L (water) and 0.86 mg/L (sediment) for *Nitocra spinipes* as well as 48 h-EC_{50} values of 0.14 mg/L (water) and 0.6 mg/L (sediment) for *Daphnia magna* were obtained after exposure to UV light. Ageing in water and sediment indicated decreasing toxicity of test substance.

The chronic toxicity of diphenylamine was examined according OECD 202 part two (Bayer 1986 c). Four concentrations (0.05, 0.16, 0.5, 1.58 mg/L) of test solution were applied by diluting a stock solution of diphenylamine. As dilution water filtered water of a gravel-pit was used. Effluent of a sewage treatment plant was added to the test medium (dilution rate 1:40). 10 daphnids per treatment level and 20 daphnids per control were testet over a period of 21 days (one daphnid per vessel). For 0.5 mg/L, a decrease of 71.3 % in the mean fecundity rate was determined. At 1.58 mg/L, high mortality even in adults and pronounced reduction in egg production occurred (mean values: 20 eggs per surviving female, comparing to 140 eggs in the controls). Therefore, a NOEC of 0.16 mg/L was obtained.

Table 3.12. Toxicity to Crustaceans

Endpoint	Result (mg/l)	Species	Method	Reference
48 h EC_{50}	2.0 (effective conc.)	*Daphnia magna*	OECD 202, part one	Murin et al. (1997)
24 h EC_{50} 48 h EC_{50}	0.96 0.31 (effective conc.)		US-EPA, CFR Ch. 1 (1992)	Danish EPA
24 h EC_0 24 h EC_{50} 24 h EC_{100}	1.5 2.3 7 (nominal conc.)	*Daphnia magna*	UBA test proposal (1984)	Bayer AG (1986 a) not assignable!!!
water: 96 h LC_{50} 96 h LC_{50} (plus UV-Light) ***sediment:*** 96 h LC_{50} 96 h LC_{50} (plus UV-Light)	 3.7 1.0 1.22 0.86	*Nitocra spinipes*	SIS 1991	Dave et al. (2000)
water: 24 h EC_{50} 48 h EC_{50} 48 h EC_{50} (plus UV-Light) ***sediment:*** 24 h EC_{50} 48 h EC_{50} 48 h EC_{50} (plus UV-Light)	 4.0 1.2 0.14 6.6 3.2 0.6	*Daphnia magna*	ISO (1996)	Dave et al. (2000)
21 d NOEC (reprod. inh.)	0.16 (effective conc.)	*Daphnia magna*	OECD 202, part two	Bayer AG (1986 c)

3.2.1.3 Toxicity to Algae

The most relevant data resulted from the two following tests.

The toxicty of diphenylamine to the freshwater algae *Scenedesmus subspicatus was* determined by Bayer AG (1992). The test was conducted according EEC (C3) Draft 1992. Eight test concentrations ranging nominally from 0.02 to 4.55 mg/L were applied by diluting a stock solution of diphenylamine. The test substance was dissolved in deionized water by using an ultra sonic bath (1 h). Analytical determination of test substance (0-72 h) was performed on test media from the highest concentration with and without algae. A 72 h-ErC_{50} of 1.5 mg/L and a 72 h-EbC_{50} of 0.18 mg/L were derived for *Scenedesmus subspicatus*. The NOEC was 0.02 mg/L (Dunnett-test).

Murin et al (1997) examined the toxicity of diphenylamine to *Pseudokirchneriella subcapitata* after 72 h of Exposure (OECD 201). The test was performed in triplicates with one blank for each concentration and six controls. Algae were exposed to a dilution serie of the test substance under continous shaking. Cell density was adjusted to 4.6 $*10^3$ on day 0. Concentrations of diphenylamine were measured as NVOC (non volatile organic carbon).Table 3.13. Toxicity to algae

Species	Endpoint	Result (mg/l)	Method	Reference
Scenedesmus subspicatus	72 h EC_5 72 h EC_{10} 72 h EC_{50} 72 h EC_{90} 72 h EC_{100} (growth rate reduct.)	0.02 0.06 1.5 40 100 (nominal conc.)	67/548/EEC (draft, 1992)	Bayer AG (1993)
Pseudokirchneriella subcapitata (formerly *Selenastrum capricornutum)*	72 h NOEC 72 h EC_{50} (growth rate reduct.)	0.37 2.17	OECD 201	Murin et al. (1997)

In a further 72 h growth inhibition test with *Scenedesmus subspicatus*, an EC_{50} of 0.048 mg/l based on cell count was determined (Bayer AG, 1986 b). However, since that test is poorly documented (raw data not available, method not reported) these results are not considered in the PNEC derivation .

As reported by Fan et al. (1995), in another green alga (*Haematococcus pluvialis*) the Diphenylamine impact on cell multiplication is less pronounced. In this species, no significant growth inhibition (measured by cell counts) could be observed during 96 h exposure to 5.1 mg/l. The result was confirmed by supplementary measurement of chlorophyll content, dry weight, and oxygen evolution and uptake. However, that Diphenylamine concentration inhibited specifically the conversion of beta-carotene to keto-carotenoids (e. g., astaxanthin).

This specific effect mechanism (interference with carotinoid synthesis) had also been reported by other authors, e. g., by Harker & Young (1995).

In the case of *Haematococcus* and other photosynthetically active taxa the inhibitory Diphenylamine impact on carotinoid production is ecologically significant since forced astaxanthin generation is seen as protection mechanism of the photosynthetic apparatus against unfavourable light conditions.

3.2.1.4 Determination of $PNEC_{aqua}$

Results from acute toxicity tests with species from 3 trophic levels are available. The most sensitive fish from standard tests is *Oryzias latipes* (EC_{50} = 2.2 mg/l). The lowest EC_{50} for *Daphnia magna* is 0.31 mg/l and for algae 1.5 mg/l (*Scenedesmus subspicatus)*.

Reliable long-term NOECs are available for invertebrates (*Daphnia magna*) and several algae species.

For fish and other vertebrates only prolonged tests results are available which are not regarded as long-term tests and which are difficult to evaluate (transient change in behaviour).

Thus, the assessment factor is set at 50 for the aquatic compartment as data from valid long-term tests on 2 trophic levels are available. The lowest no effect concentration is determined for *Scenedesmus subspicatus* with a 72h-EC_{10}/ NOEC of 0.06 mg/l.

The PNECaqua is calculated as follows:

$PNEC_{aqua}$ = 60 µg/l : 50 = **1.2 µg/l**

3.2.1.5 Sediment

No information on toxic effects in sediment dwelling animals or benthic macroflora is currently available. However, in view of the high toxicity of Diphenylamine to a broad spectrum of taxa covering all trophic levels, the slow microbial degradation, the (calculated) suspension-water partitioning coefficient, and the maximum concentrations estimated from $Clocal_{water}$ values, a provisional $PNEC_{sediment}$ is derived.

$$PNEC_{sediment} = \frac{Ksusp\text{-}water \times PNEC_{WATER} \times 1000}{RHOsusp}$$

$$\mathbf{PNEC_{sediment}} = \frac{23.6 \times 0.0012 \text{ mg/l} \times 1000}{1150} = \mathbf{0.0246\ mg/kg}\ \text{(wet weight)}$$

3.2.1.6 Toxicity to micro-organisms

Hockenbury et al. (1977) tested the inhibition of NH_3 oxidation of *Nitrosomonas sp.* by Diphenylamine. They did not find any effects at a concentration of 100 mg/l. However, this concentration is well above the water solubility of Diphenylamine and no information is given on the use of a solvent. The test is not to be used for $PNEC_{wwtp}$ derivation.

In a test according to OECD 209 (Murin et al., 1997) respiration was inhibited to 50 % at a concentration of 18.7 mg/l and to 20 % at a concentration of 3.8 mg/l (water accommodated fraction of Diphenylamine).

The protozoan *Tetrahymena pyriformis* was exposed at 28 °C to Diphenylamine in a population growth test (Epstein et al., 1967). Visual scoring of growth inhibition led to a EC_{50} of 25 mg/l (nominal value).

Results of the studies above are listed in Table 3.14.

Table 3.14. Inhibition of microorganisms

Testorganisms	**Type**	**Result**	**Reference**
Activated sludge	3 h-EC_{20} 3 h-EC_{50}	3.8 mg/l 18.7 mg/l respiration inhibition GLP, OECD 209	Murin et al. (1997)
Tetrahymena pyriformis	48 h-EC_{50}	25 mg/l growth inhibition	Epstein et al. (1967)

The lowest effect data for organisms important to WWTPs is given for activated sludge (EC50 = 18.7 mg/l).

According to Technical Guidance Document an assessment factor of 100 is applied.

$PNEC_{wwtp}$ = 18.7 mg/l / 100 = 0.187 mg/l

3.2.2 Atmosphere

No ecotoxicological data are available for this environmental compartment.

3.2.3 Terrestrial compartment

3.2.3.1 Toxicity to higher plants

So far, valid effect values relating to exposure of higher plants in standardized soil tests suitable for derivation of a PNECsoil have not been reported.

The following results are given for additional information on effects of Diphenylamine in higher plants.

In experiments conducted by Filmer & Rhodes (1985) Diphenylamine turned out to be a relatively strong sprout-growth suppressant in potato tubers. Exposure to 2–5 mg/kg (tub.) for 10 weeks at 10 °C 1ed to a 50 % reduction in sprout growth, 20 mg/kg produced almost complete inhibition. Diphenylamine was applied by spraying or painting dissolved in acetone. It has to be considered that under natural conditions Diphenylamine is produced (together with other sprouting inhibitors) in small amounts by the tuber itself as a basic adaptation mechanism to external growth conditions. The same holds for the natural occurrence in onions as another hibernation organ (Karawya et al., 1984; see also information on "Natural occurrence" in chapter 3.1.0).

In earlier efforts aimed at prevention of fungal rots in fruit (apples) sprays containing up to 4 000 mg Diphenylamine/l were applied to the trees. There are no reports on any collateral damage to the plants caused by Diphenylamine. Considering all available information, higher plants are not expected to possess a high Diphenylamine sensitivity. Therefore, further investigation on plants is considered not necessary for the derivation of PNECsoil.

3.2.3.2 Toxicity to invertebrates

The earthworm *Eisenia fetida* was exposed to Diphenylamine concentrations ranging from 100 – 1000 mg/kg d.w. in a artificial soil test carried out according to GL OECD 207 (Bayer AG, 1992). The following results were obtained in a soil containing 10 % organic matter (nominal Diphenylamine-values;.):

14 d NOEC (weight)	= 178 mg/kg d.w
14 d LC_0	= 178
14 d LC_{50}	= 269
14 d LC_{100}	= 562

Normalisation to standard soil with 3.4 % organic matter according to Technical Guidance Document leads to a 14 d NOEC of 61 mg/kg d.w., and to a 14 d LC_{50} of 91 mg/kg d.w., respectively. Considering the given water content in the soil substrate the respective value for 14 d LC_{50} is 62.8 mg/kg wet weight.

Information about the sensitivity of further soil-dwelling invertebrates is lacking.

3.2.3.3 Determination of $PNEC_{soil}$

The only tested organism has been Eisenia fetida with a 14 d LC_{50} of 62.8 mg/kg wet weight.

With an assessment factor of 1 000 according to Technical Guidance Document, the resulting PNEC comes to

$PNEC_{soil}$ = 62.8 mg/kg / 1 000 **= 62.8 µg/kg wwt.**

Equilibrium partition method

According to the Technical Guidance Document, the risk assessment has to be performed additionally on the basis of the equilibrium partition method if only one test result with soil dwelling organisms is available. Subsequently, the lowest $PNEC_{soil}$ calculated is chosen for $PEC/PNEC_{soil}$ ratios in risk characterisation.

Application of the equation (72) of the Technical Guidance Document results

$$PNEC_{soil} = \frac{K_{soil\text{-}water} \times PNEC_{water} \times 1\,000}{RHOsoil}$$

$$\mathbf{PNEC_{soil}} = \frac{27.4 \times 0.0012 \text{ mg/l} \times 1\,000}{1\,700 \text{ kg/m}^3} = \mathbf{19.3\ \mu g/kg\ wwt.}$$

From these calculations follows that the latter value for PNECsoil is to be considered in risk characterisation.

3.2.4 Non compartment specific effects relevant to the food chain

Diphenylamine shows a moderate to high bioconcentration factor. BCF_{fish}-values up to 253 are reported (CITI 1992).

On the basis of the mammalian toxicity data, Diphenylamine is classified as toxic.

According to the Technical Guidance Document it is assumed that the available test data with laboratory animals can give an indication on the possible risk of the chemicals to top-predators in the environment. The NOAELs found in long-term studies with repeated dosing of a substance have to be converted into a food concentration by using the ratio between body weight and daily food intake as conversion factor. In the Technical Guidance Document, App. VII, conversion factors for several laboratory test species are given.

In a repeated dose toxicity study with rats conducted over 104 weeks the lowest available NOEL of 6.7 mg/kg bw/d was found (De Eds 1963, cited in Bayer/ URC, 2000). This NOEL has to be converted into a food concentration by using a conversion factor of 20. With this a NOEC of 134 mg/kg food can be derived.

According to the Technical Guidance Document, for the calculation of the PNECoral an assessment factor of 30 has to be applied to a NOEC-value based on a chronic study. Hence, a **$PNEC_{oral}$** of **4.47 mg/kg food** is derived for the risk assessment.

3.3 RISK CHARACTERISATION

3.3.1 Aquatic compartment

Waste water treatment plant

Risk ratios derived are given in Table 3.15. The $PNEC_{stp,micro-organisms}$ is 187 µg/l.

Table 3.15. Risk ratios for waste water treatment plant

Scenario	**$PEC_{stp,micro-organisms}$ (µg/l)**	**PEC:PNEC**
Production (site specific)	$3.59 * 10^3$	19*
Production	$2.2 * 10^3$	12*
Formulation of lubricant	149	0.8
Formulation of storage aid	598	3
Processing (intermediate; site specific)	$2.49 * 10^3$	13
Processing (intermediate use)	$42 * 10^3$	225
Professional use of lubricant (processing)	$4.38 * 10^3$	23

* Production closed in the EU 15

** Based on the volume stated in chapter 2.1

Result

Conclusion (i) There is need for further information and/or testing

This applies for all other scenarios except for formulation of lubricant. Up to date information on the tonnage for each use, size of the industrial sites and size of waste water treatment plants for production and processing of intermediates as well as site specific emission data or measured data is needed (see also conclusions for aquatic environment below). The further conclusions for the only known producer site and intermediate processing site are included in the Appendices B1 and B2. PNECmicro-organisms can be lowered by further testing (now AF of 100 has been applied). However, it is first necessary to obtain better data on the exposure before any testing is conducted

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

This applies for formulation of lubricant.

Aquatic environment

$PNEC_{aqua}$ of 1.2 µg/l has been estimated. The risk ratios for the **regional environment** are as follows. Surface water: $PECreg_{water}$: $PNEC_{aqua}$ = 0.8 µg/l : 1.2 µg/l = **0.5** . For sediment, both the $PECreg_{sediment}$ and the $PNEC_{sediment}$ were derived using equilibrium partitioning approach from corresponding values for surface water and thus the same risk ratio as for surface water results. Table 3.16 shows the risk ratios for the local scenarios.

Table 3.16. Aquatic risk characterisation for the local scenarios

Scenario	**$PEClocal_{water}$ (µg/l)**	**$PEClocal_{water}$:$PNEC_{aqua}$**
Production (site specific)	90.6	26 *
Production	113	94 *
Formulation of storage aid	59.7	50
Formulation of lubricant	14.9	13
Processing (intermediate; site specific)	62.9	52
Processing (intermediate use)	209	174
Professional use of lubricant (processing)	437	365

* Production closed in the EU 15

Since both PEClocal- and PNEC-values for sediment are derived from aquatic values using the equilibrium partitioning method, the risk ratios for sediment in local scenarios are equal to risk ratios for water.

Result

Conclusion (i) There is need for further information and/or testing

The conclusion applies to all industrial categories for which local environmental concentrations have been predicted. Up-to-date information on the tonnage of all uses is needed. Further conclusions on the only known production and intermediate processing sites are included in the Appendices B1 and B2. For the rest of the European use volume of 10 000 t/a not covered by the only known producer and importer, a confirmation is needed whether it

is completely imported or produced in additional European sites. It is very probable, that more than one processing sites are located in Europe. Information on their size, waste water treatment and effluent dilution rate is necessary. Information on the size and waste water treatment of lubricant formulation sites is necessary as well. In addition, more information on the end-uses of lubricants containing Diphenylamine is needed in order to be able to allocate the tonnage in lubricant use to several generic use scenarios. There is no information available whether there are any formulation sites of storage aid in Europe or not. Confirmation for this issue is needed. If any formulation sites are located in Europe, site specific data on their size, emissions and waste water treatment is needed. Hardly any new measured data is available from aquatic environment. Measured data from water and sediment are needed in order to be able to compare the model results with the reality.

After better use and emission data have been received, the risk ratios could be further reduced by approximately a factor of two in case Diphenylamine would be confirmed to be inherently biodegradable. The available biodegradation data indicates that this might be the case, but no such studies are available, which would allow to assume inherent biodegradation in this version. Therefore, a simulation test could be considered. In addition, a chronic fish-study would reduce the risk ratio in local scenarios at the most by a factor of 5 (AF for derivation of PNECaqua would be reduced from 50 to 10).

3.3.2 Atmosphere

No ecotoxicological data are available for this environmental compartment. Considering the atmospheric half-life of < 2 h of Diphenylamine, the low volatility, the highest $PEClocal_{air}$ of 0.1 µg/m^3 and a $PECregional_{air}$ of 4 * 10^{-8} mg/m^3 a risk for this compartment is not to be expected and the performance of ecotoxicological tests is not considered necessary.

3.3.3 Terrestrial compartment

PNECsoil of 19.3 mg/kg wwt has been estimated. With the $PECregional_{agr,soil}$ of 11.6 µg/kg wwt , a PEC:PNEC of 0.6 results. Table 3.17 presents the risk characterisation ratios for the local scenarios.

Table 3.17: Risk characterisation for agricultural soil.

Scenario	**PEC:PNEC**
Production (site specific)	1 * 10^{-4} (* (**
Production	3 * 10^{-5} (* (**
Formulation of lubricant	27
Formulation of storage aid	110
Processing (intermediate; site specific)	2 * 10^{-5} (*

Processing (intermediate use)	$6 * 10^{-5}$ (*
Professional use of lubricant (processing)	803

(* PECgrassland has been used for deriving the PEC:PNEC –ratio. It is the highest of the PECs for soil when no sludge application occurs.

(** Production closed in the EU 15

Result

Conclusion (i) There is need for further information and/or testing

This conclusion applies for formulation of lubricant and storage aid and professional use of storage aid. Information on the size and waste water treatment of lubricant formulation sites is necessary. In addition, more information on the end-uses of lubricants containing Diphenylamine is needed in order to be able to allocate the tonnage in lubricant use to several generic use scenarios. There is no information available whether there are any formulation sites of storage aid in Europe or not. Confirmation for this issue is needed. If any formulation sites are located in Europe, site specific data on their size, emissions and waste water treatment is needed.

A secondary alternative is to conduct a biodegradation simulation test (see the conclusions above for the aquatic compartment), the results of which may lower the estimate for PECsoil. In addition, either new terrestrial chronic ecotoxicity data or an improvement of PNECaqua is needed.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

This conclusion applies for production and processing. Deposition has been assumed to be the only input path to agricultural soil for these industrial categories.

3.3.4 Non compartment specific effects relevant to the food chain

$PNEC_{oral}$ of 4.47 mg/kg has been derived. Table 3.18 shows the RCRs for the secondary poisoning route for the aquatic food chain and Table 3.19 for earthworm-based food chain.

Table 3.18: Risk assessment – secondary poisoning for the aquatic food chain.

Scenario	**$PEC_{oral,predator}$ (mg/kg)**	**PEC/PNEC-ratio**
Production (site specific)	5.78	1.3 *
Production	7.24	1.6 *
Formulation of lubricant	1.07	0.2
Formulation of storage aid	3.92	0.9
Processing (intermediate; site specific)	4.01	0.9
Processing (intermediate use)	13.4	3
Professional use of lubricant (processing)	4.28	0.96

* Production closed in the EU 15

Result

Conclusion (i) There is need for further information and/or testing

This conclusion applies for the known production site, generic production scenario and generic scenario for intermediate use. Further conclusions on the only known production site are included in the Appendix B1. For the rest of the European use volume of 10 000 t/a not covered by the only known producer, a confirmation is needed whether it is completely imported or produced in additional European sites. It is very probable, that more than one processing sites are located in Europe. Information on their size, waste water treatment and effluent dilution rate is necessary.

After better data on tonnage and emissions have been received, the risk ratios could be further reduced by approximately a factor of two in case Diphenylamine would be confirmed to be inherently biodegradable. The available biodegradation data indicates that this might be the case, but no such studies are available, which would allow to assume inherent biodegradation in this version. A simulation test could be considered.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

This conclusion applies for formulation of lubricant and storage aid, the known intermediate processing site and professional use of lubricant.

Table 3.19: Risk assessment – secondary poisoning for the terrestrial food chain.

Scenario	**$PEC_{oral,predator}$ (mg/kg)**	**PEC/PNEC-ratio**
Production (site specific)	0.012	0.003 *
Production	0.011	0.002 *
Formulation of lubricant	0.488	0.1
Formulation of storage aid	1.908	0.4
Processing (intermediate; site specific)	0.011	0.002
Processing (intermediate use)	0.011	0.002
Professional use of lubricant (processing)	13.929	3.12

* Production closed in the EU 15

Result

Conclusion (i) There is need for further information and/or testing

This conclusion applies for professional use of lubricant. More information on the end-uses of lubricants containing Diphenylamine is needed in order to be able to allocate the tonnage in lubricant use to several generic use scenarios. Refinement of regional PEC for agricultural soil may reduce the ratio. Presently, due to EUSES –model sludge from all industrial categories is included in the regional scenario. This technical problem may be possible to be circumvented (will be done if reduction of risk will not follow from information gathering).

Additionally, the risk ratios could be further reduced by approximately a factor of two in case Diphenylamine would be confirmed to be inherently biodegradable. The available biodegradation data indicates that this might be the case, but no such studies are available, which would allow to assume inherent biodegradation in this version. A simulation test could be considered.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

This conclusion is drawn for production, intermediate processing, processing of storage aid, formulation of storage aid and formulation of lubricants.

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 General discussion

Diphenylamine is an important intermediate for the production of antioxidants, antiozonants, phenothiazine, dyes and other products. It is also used in the post-harvest treatment, as an antioxidant for lubricants, as propellants and in explosives.

Diphenylamine is currently produced by two companies in the European Union. The EU market volume is about 10 000 t/a (see chapter 2). Most of the diphenylamine is processed as a chemical intermediate (approximately 97.5%).

Diphenylamine is used as the active substance in storage aid in the post harvest treatment. Diphenylamine is registered at present as a component of plant protection products in Ireland, U.K., France, Spain, Portugal, Italy and Greece. There are no preparations containing diphenylamine permitted for application as storage aid for food produced in Germany.

According to specific information from industry, alkylated diphenylamine lubricant oils (petroleum or synthetic oils) contain a small amount of unreacted diphenylamine. diphenylamine has been reported to be used also in its unreacted form as antioxidant in lubricant oils. However, there is contradicting information from industry whether this use is still an intended use. The Nordic Product Register SPIN and the Finnish Product Register show that altogether 3.3 t/a are presently registered for lubricants use in Sweden, Denmark and Finnland. The products in the Finnish Product Register contain < 1 % diphenylamine.

In the past diphenylamine was used as an additive to gas oil (UBA, 2002). Until August 2002, each country in the EU had the possibility to mark their low tax fuel as needed. After this date, EU legislation (2001/574/EC, notified under document number C(2001)1728) prescribed the use of Solvent Yellow 124 (derivate of aniline) as the primary marker to identify this fuel and diphenylamine is no longer listed as a possible marker. France was in Europe the single user of diphenylamine as a dye marker in gas oil and marine diesel until August 2002 (Rudd, 2002).

For detailed information about the use of diphenylamine see chapter 2.

Diphenylamine is a colourless solid (vapour pressure 0.03 Pa at room temperature) with a floral odour.

The routes of exposure are by inhalation and by skin contact at the workplace.

The route of exposure to consumers is the oral intake by eating fruits and other vegetable foods, which have been preserved with diphenylamine; but also dermal exposure is possible by the use of lubricants.

4.1.1.2 Occupational exposure

The exposure assessment generally aims at assessing exposure levels representing the reasonable worst case situation. The reasonable worst case is regarded as the level of exposure which is exceeded in a small percentage of cases over the whole spectrum of likely circumstances of use for a specific scenario.

The assessment of inhalation exposure is mainly based on measured exposure levels from which – if possible – 90^{th} percentiles are derived as representing reasonable worst case situations. If available, only data measures later than 1992 are used in exposure assessment. Scenarios are clustered as far as possible to make the description transparent. If quantitative exposure data are not available, model estimates are used.

Beside inhalation exposure, dermal exposure is assessed for each scenario. Two terms can be used to describe dermal exposure:

Potential dermal exposure is an estimate of the amount of a substance landing on the outside of work wear and on the exposed skin.

Actual dermal exposure is an estimate of the amount of a substance actually reaching the skin.

Within the framework of existing substances there is an agreement between the EU member states, to assess – as a rule – dermal exposure as exposure to hands and parts of the forearms. In this, the main difference between both terms – potential and actual - is the protection of hands and forearms by work wear and – more important – the protection by gloves. Within this exposure assessment, the exposure-reducing effect achievable by gloves is only considered if information is provided indicating that, for a certain scenario, gloves are a widely accepted protective measure and that the gloves are fundamentally suitable for protection against the substance under consideration. As a measure for the latter, tests according to DIN EN 374 are taken as a criterion. For most downstream uses it is commonly known that gloves are not generally worn. In these cases, dermal exposure is assessed as actual dermal exposure for the unprotected worker. Since quantitative information on dermal exposure is often not available, the EASE model is mostly used for assessing dermal exposure.

The following national occupational limits (Ariel, 2002) are given:

COUNTRY	**8h TWA, mg/m³**	**STEL, mg/m³**	**Source**
Austria	5	10	MAK Liste 2001
Belgium	10		Exposure Limit Values 2002
Denmark	5		Arbejdstilsnet 2002
France	10		Occ. Exposure Limits 1999

Germany	5		TRGS 900; 2002
Ireland	10	20	Occ. Exposure Limits 2001
Italy	10		Occ. Exposure Limits 2001
Norway	5		Arbeidstilsynet 2001
Spain	10		Limites Exposicion profesional 2000
Sweden	4	12	Occ. Exposure Limits 2000
Switzerland	10		Grenzwerte am Arbeitsplatz 2001
The Netherlands	0.7		MAC List 2002
United Kingdom	10	20	Occ. Exposure Limits 2002
USA	10		ACGIH 2002

The following scenarios are regarded to be relevant for occupational exposure:

1. Production of diphenylamine and further processing (4.1.1.2.1)

2. Use of lubricants (4.1.1.2.2).

At present it is assumed, that approximately 2 % (~ 200 t/a) of the produced diphenylamine are used as the active substance in storage aids (solution or spray) in the post harvest treatment of e.g. apples and pears to prevent the formation of storage scald on fruit. There is no specific information available on European formulation facilities.

There are no preparations containing diphenylamine permitted for application as strorage aid for food produced in Germany. Diphenylamine has been registered as plant protection product nationally so far in Ireland, UK, France, Spain, Portugal, Italy and Greece. Two companies have notified diphenylamine under the Council Directive concerning placing plant protection products on the market 91/414/EEC ("PPP-Directive"), so assessment in this framework is not considered to be necessary.

4.1.1.2.1 Scenario 1: Production of diphenylamine and further processing

This scenario covers the production of diphenylamine, the use as a chemical intermediate and the formulation of lubricants in the petrochemical industry.

The production of diphenylamine can be performed by selfcondensation of aniline in the presence of a small amount of a strong mineralic acid, such as anhydrous hydrochloric acid (0.5 wt. % of aniline), or Lewis acids, such as ferrous chloride or ammonium bromide at elevated temperature and pressure (Moore, 1978). The technical substance is manufactured in closed systems by vacuum distillation at increased temperature. The substance is additionally purified for the use as fruit storage aid by distillation under vacuum and following crystallization.

A closed system is used to transfer liquid material into a storage tank. The same system is used to transfer material from the storage tank for processing diphenylamine to rubber

antioxidants. Diphenylamine is pumped from the storage tank to the reactors through a mass flow-meter where reactors and diphenylamine storage tank are balanced.

Diphenylamine is produced in two forms for selling: as flakes and as a liquid. Therefore there are two ways to transport the substance: as flakes in 25 / 50 kg sacks or the liquid in tank trucks. To avoid crystallization (melting point 53°C) heated pipelines (T> 60°C) and heated tank trucks are necessary.

An estimated amount of 97.5 % of the total EU quantity are used as an intermediate.

According to industry, a small amount of diphenylamine is used in lubricant oils in a concentration of 1 %. The formulation of lubricant oils takes place in the chemical / petrochemical industry. There is contradicting information regarding to whether diphenylamine is merely an impurity in alkylated diphenylamine antioxidants for lubricant oils, or whether it is as itself used as a primary constituent. Based on the specific information available from industry, it is assumed, that 50 t/a is used in petroleum or synthetic lubricant oils for this purpose. The amount of 5000 t/a lubricant oil produced results assuming the 1 % fraction for diphenylamine.

For the large-scale chemical industry high standards of control at the workplaces are assumed to be practised even if the containment is breached, e.g. during filling, cleaning, maintenance, repair works and taking of samples. Inhalation exposure in other fields is normally minimised by technical equipment (e.g. special designed filling stations, local exhaust ventilation).

Inhalation Exposure

Workplace measurements

The following table shows the occupational exposure figures in the different working areas of the production of diphenylamine and further processing to rubber antioxidants. Data were provided by the only producing company and by the company which terminated the production in 2001.

4.1.1.2.1 A: Workplace data of diphenylamine during production and further processing (1999, 2000)

Job category / activities	Years of measure-ment	Number of samples	Range of measure-ment data [mg/m³]	Mean [mg/m³]	90th-percentile [mg/m³]	95th-percentile [mg/m³]
8h time-weighted averages						
Company A						
Production	1989-1999	5	< 0.5 – 0.5	< 0.5	-	-
Flaking	1989-1999	3	< 0.5	< 0.5	-	-
Sack filling	1989-1999	12	0.08 –1.21	0.42	1.05	-
Tank truck filling	1989-1999	3	< 0.5 – 0.85	< 0.5	-	-
Company B				Median		
Production (rubber antioxidants)	1988-1992 1992-1999	63 25	0.1 - 161.2 0.1 - 1.0	0.92 0.14	- -	1.65 -
Bagging of DPA flakes	1992-1999	11	< 0.1 – 0.4	-	0.3	-

The companies provided measurement data with very limited information: The analysis method and the corresponding limit of detection are unknown and information on the cause for the partly very high measurement values up to 161.2 mg/m³ (measurement duration up to 420 min, the collective comprises also values about 50 mg/m³), the use patterns, the duration of exposure relevant activities, and the collective of exposed workers is lacking. The 95th percentile of this measurement collective amounts to 1.65 mg/m³.

Measurement data from 1992 – 1999 are between 0.1 – 1 mg/m³. However, it was possible to extract data from this measurement collective which refer to the activity "bagging of diphenylamine-chips". The highest measurement value is 0.4 mg/m³ (duration of measurement up to 60 min) and the 90th percentile is 0.3 mg/m³. These data correspond to those provided by the company which terminated the production. Thus, they can be regarded as plausible.

Because the measurement data are not very detailed, model estimations are performed.

EASE estimation

EASE for Windows 2.0, Aug. 1997 was used.

EASE estimation for the production of liquid diphenylamine and further processing in the large-scale chemical industry (high level of protection):

Input parameters: T = 80 °C, closed system, LEV (local exhaust ventilation) present, vapour pressure ~ 2 Pa

Level of exposure: 0.7 mg/m³ (0 – 0.1 ppm)

Another EASE estimation is described for taking samples during the production, but the exposure level is identically.

Input parameters: T = 20 °C, closed system, significant-breaching, LEV (local exhaust ventilation)

Level of exposure: 0.7 mg/m³ (0 – 0.1 ppm)

EASE estimation for the production of solid diphenylamine (flakes) in the large-scale industry (high level of protection):

Input parameters: T = 20 °C, exposure-type is dust, low dust technique, LEV (local exhaust ventilation) present

Level of exposure: 0 – 1 mg/m³

Conclusions

Inhalation exposure has to be assessed for the production of liquid and solid diphenylamine in fields with high levels of protection in the large-scale chemical industry.

Model estimates (0.7 - 1 mg/m³) and measured exposure levels are in the same range (90th percentile: 1.05 mg/m³).

For the assessment of health risks from daily inhalation exposure to diphenylamine an 8-h time weighed average concentration (8-h TWA) of 1 mg/m³ (model estimation) should be taken. The level is regarded to represent the reasonable worst case situation.

Dermal exposure

For assessing actual dermal exposure levels, it has to be considered that the substance is manufactured and further processed primarily in closed systems and that the use of PPE (personal protective equipment) is highly accepted in the large-scale chemical industry. The extent of protection by PPE (here gloves) depends inter alia on the suitability of the recommended material with regard to the permeation properties of substance. Contact to the liquid substance is avoided, because it is handled at temperatures above 60°C.
For the handling of powdery substances, as a rule, the suitability of the gloves can be assumed and low levels of daily dermal exposure are to be expected. However, in spite of this, dermal exposure may occur due to e.g.

- unintended contamination during the handling of used gloves
- limited protection of suitable gloves at real working conditions (e.g. mechanical stress).

Since no measurement results are available, an attempt is made to quantify dermal exposure for the above mentioned situations in application of the EASE model. Taking into account that intermediate dermal contact may occur, however, this situation could be described by the scenario:

Input parameters: Non dispersive use, direct handling, intermittent

Level of exposure: 0.1 – 1 mg/cm²/day

Considering an exposed area of 210 cm^2 (surface area for sampling) the model yields an exposure level of 21 – 210 mg /person/day. For the use of suitable gloves a protection efficiency of 90% is assumed leading to exposure levels of 2.1 – 21 mg/ person/day. The upper value is regarded to represent the reasonable worst case. Contact to the liquid substance is avoided, because it is handled at temperatures above 60°C.

Conclusions

For assessing the health risks from daily dermal exposure in the area of production handling the solid (flakes) and the liquid substance is considered. Due to the melting temperature of 53°C, the liquid substance is transferred and filled at elevated temperatures (T> 60°C). As a consequence worker avoided any dermal contacts. For handling the solid (flakes, contact the cooled, solidified substance), dermal exposure is assessed to 21 – 210 mg/person/day. The use of suitable gloves reduces dermal exposure to 10% leading to exposure of 2.1 – 21 mg/person/day. The upper value is regarded to represent the reasonable worst case.

Exposure to the eyes is largely avoided by using eye protection.

4.1.1.2.2 Scenario 2: Use of lubricants

According to industry, a small amount of diphenylamine is used in lubricant oils in a concentration of 1 %. Based on the specific information available from industry it is assumed, that 50 t/a is used for this purpose. Leading to an amount of 5.000 t/a lubricant oil.

Industry has not provided any specific information on the uses of lubricant oils. According to the Nordic Product Register and The Finnish Product Register, the substance is used in the classes ‘Sale, maintenance and repair of motor vehicles and motorcycles; Retail sale of automotive fuel’ and ‘Manufacture of machinery and equipment’.

The term "lubricant" applies to products based predominantly on mineral oils or on synthetic oils, which are intended as lubricants, power and heat transmission media, engine and process oils, and metal working fluids.

Metal working fluids (cooling lubricants) are applied by continuous jet, spray, mist or by hand dispenser. Skin contact occurs during preparation or draining of the fluids, handling workpieces, from splashes during machining, changing and setting of tools and during maintenance and cleaning activities. There will be significant potential for skin contact. In addition, inhalable aerosols can be generated during machine operations.

For activities without the formation of aerosols the inhalation exposure to diphenylamine is considered to be negligible (non-volatile substance, vapour pressure 0.03 Pa).

Inhalation Exposure

Workplace measurements

No workplace measurements are available.

Analogous data can be taken from data of metal working fluid aerosols from the German Berufsgenossenschaften-Institute for Occupational Safety and Health (BGIA, 2004). The study (1997-2002) shows aerosol data of 2 mg/m³ (95^{th} percentile, number of measurements = 2592).

Taking into account that lubricants contain 1 % diphenylamine the inhalation exposure is estimated to 0.02 mg/m³. It is to be assumed, that exposure relevant activities are performed daily during the entire length of the shift.

Conclusions

For the use of diphenylamine in lubricants detailed information is not available. Especially information on the composition of the solution, the duration and frequency of exposure, the collective of exposed worker and on the use pattern is missing.

It is assumed that only during the use of metal working fluids in the form of aerosols inhalation exposure occurs. For the assessment of health risks from daily inhalation exposure to diphenylamine an 8-h TWA of 0.02 mg/m³ should be taken.

Dermal exposure

Taking into consideration that personal protective equipment is not generally worn during e.g. decanting or draining of lubricants or by cleaning works, the estimation of dermal exposure levels is performed for the unprotected worker.

Therefore dermal exposure is assessed according to the EASE model:

Input parameters: wide dispersive use, direct handling, extensive

Level of exposure: 5 – 15 mg/cm²/day.

The estimation is performed for a formulation containing 1 % diphenylamine and an exposed area of 840 cm² (area corresponds to the surface of the hands). The estimated exposure levels amount 42 – 126 mg/person/day.

Conclusions

For assessing the health risks from daily dermal exposure during the use of lubricants (scenario 2), an exposure level of 42 – 126 mg/person/day should be taken. The upper level is regarded to represent the reasonable worst case situation.

4.1.1.2.3 Summary

Diphenylamine is produced as a liquid and as flakes by two companies in the EU.

Two scenarios were established:

Scenario 1: Production of diphenylamine and further processing

Scenario 2: Use of lubricants

Relevant inhalation and dermal exposure levels are given in table 4.1.1.2.3 A and 4.1.1.2.3 B, respectively.

Measurement values regarding the production of diphenylamine as liquid and as flakes were provided by the two above mentioned companies. Since the producing company provided only limited information to the measurement values, model estimates were performed additionally. The outcome supports the assessed exposure level. Dermal contact with the liquid substance is limited because the substance is handled at temperatures above 60°C. For handling diphenylamine-flakes, the use of suitable gloves is considered leading to reduced dermal exposure.

Diphenylamine is used as the active substance in storage aid in the post harvest treatment. Diphenylamine is registered at present as a component of plant protection products in Ireland, U.K., France, Spain, Portugal, Italy and Greece. Two companies have notified diphenylamine under the Council Directive concerning placing plant protection products on the market 91/414/EEC ("PPP-Directive"), so assessment in this framework may not be necessary.

According to industry, a small amount of diphenylamine is used in lubricant oils (scenario 2). There is contradicting information regarding to whether diphenylamine is merely an impurity in alkylated diphenylamine antioxidants for lubricant oils, or whether it is as itself used as a primary constituent. Dermal exposure occurs during draining of the fluids, handling workpieces, from splashes during machining and during maintenance and cleaning activities. In addition, inhalable aerosols can be generated during machine operations. For activities without the formation of aerosols the inhalation exposure to diphenylamine is considered to be negligible.

4.1.1.2.3 A: Summary of inhalation exposure data (reasonable worst case) of diphenylamine which are relevant for occupational risk assessment

Scenario number, Area of production and use	Form of exposure	Activity	Duration [h/days]	Frequency [days/year]	Shift average concentration [mg/m³]	Method	Short-term concentration [mg/m³)	Method
Production, formulation and use as a chemical intermediate								
1) Production of diphenylamine and further processing	dust	-flaking -sack filling -cleaning	shift length (assumed)	daily	1.0	EASE estimation and workplace measurements	-	-
Use of diphenylamine								
2) Use of lubricants, metal working fluids	aerosol	-machining of metal	shift length (assumed)	daily	0.02	analogy data	-	-

-
-

4.1.1.2.3 B: Summary of dermal exposure data (reasonable worst case) of diphenylamine which are relevant for occupational risk assessment

Scenario number, Area of production and use	Form of exposure	Activity	Frequency [days/year]	Contact level [1)]	Level of exposure [mg/cm²/day]	Exposed area [cm²]	Shift average concentration [mg/person/day]	Method (use of gloves)
Production, formulation and use as a chemical intermediate								
1) Production of diphenylamine and further processing	flakes dust	-flaking -filling -cleaning	daily	intermittent	1	210	21	EASE (suitable gloves[2)])
Use of diphenylamine								
2) Use of lubricant, metal working fluids (containing 1 % diphenylamine)	aerosol	- draining - decanting - cleaning	daily	extensive	15	840	126	EASE (without gloves)

1) according to the EASE model
2) Dermal contacts with the liquid substance are avoided, because of the temperature of 60°C. For suitable gloves a protection efficiency of 90% is taken into consideration

4.1.1.3 Consumer exposure

According to the information from chapter 2, lubricants contain a small amount of unreacted diphenylamine (< 1%). In BfR data base (BfR, 2005) are notified two lubricants with concentrations of <0.1 % and 0.23 % diphenylamine, respectively, for use at the workplace. It can be assumed that also consumers have contact with these lubricants and this contact would be lead to dermal exposure.

Additionally, in the BfR data base (BfR, 2005) there is currently only one notification for use of DPA as a trace constituent of a solvent for artists paint (<0.01 %) . This use is considered to be negligible for the risk assessment.

According to information from chapter 2 the European Union has set maximum residue limits (MRLs) for diphenylamine in its legislation (Commission Directive 2000/57/EWG). The MRL for apples is 5 mg/kg, for pears 10 mg/kg and for all other commodities 0.05 mg/kg (see also Rückstands-Höchstmengenverordnung, 2003).

Oral exposure may occur by eating of foods preserved with diphenylamine containing fungicides.

Oral exposure

According to above mentioned regulation concerning the MRLs for diphenylamine, children (age 2 - 5 years, average body weight 16.5 kg) eating 0.588 kg vegetable foods corresponds to an average consumption amount of 0.195 kg of apples, 0.010 kg of pears and 0.383 kg of other vegetable foods including processes or in juice every day (Banasiak et al., 2005) would be exposed to an amount of 0.0677 mg/kg bw/d.

Adult women, age 55-64 years, average body weight approximately 68 kg (AUH, 1995) eating 0.982 kg vegetable foods corresponds to an average consumption amount of 0.131 kg of apples, 0.013 kg of pears including juice (not taken into consideration apples and pears in processed form because no data are available), other fruits and juice 0.204 kg and 0.634 kg of other vegetable foods every day, would be exposed to ≈ 0.0122 mg/kg bw/d.

The average of the estimated daily consumption amount was determined by the German National Food Consumption Study 1985 – 1988 (Adolf et al., 1995). The age group (women 55-64 years) was chosen because this group shows the highest fruit consumption amounts per day .

Dermal exposure:

Dermal exposure may occur when touching fruits or vegetables which have been preserved with diphenylamine. The area of exposure is represented by the surface of hands. The amount is considered to be negligible due to short time of exposure (touching).

For the estimation of dermal exposure due to use of lubricants the following equation is chosen:

$U_{der,pot} = C_{der} \times TH_{der} \times AREA_{der} / BW$ (mg/kg BW/d)

In a worst-case scenario it is assumed, that both palms (AREA $_{der}$ = 420 cm^2) have contact with lubricant. TH $_{der}$ = the thickness of product layer on skin account 0.01 cm and C $_{der}$ = dermal concentration of substance on skin 1 %. The amount of substance that can potentially be taken up (U $_{der,pot}$) is calculated to be 0.7 mg/kg bw/d assuming a body weight of 60 kg.

Inhalation exposure

Considering the vapour pressure of the substance (0.033 Pa at 20 °C) inhalation exposure can be neglected.

Conclusion

The amount of diphenylamine on skin per day due to use of lubricants can reach a value up to ~ 42 mg, thus resulting in an external dermal exposure of 0.7 mg/kg bw/d assuming a body weight of 60 kg.

Oral exposure due to eating fruits and other vegetable foods per day can lead up to an intake of 0.0122 mg/kg bw/d for a female adult (age 55 – 64, average body weight approximately 68 kg). Children (age 2 -5 years, body weight 16.5 kg) would be exposed to an amount of 0.0677 mg/kg bw/d.

4.1.1.4 Indirect exposure via the environment

Releases of DPA into the environment following production, formulation and processing were calculated in chapter 3. As stated there is only limited information for the current situation available.

The indirect exposure of humans via environment, i.e. through food, drinking water and air is considered to be low. The regional total daily intake is 0.5 µg kg^{-1} d^{-1}.

4.1.1.5 (Combined exposure)

4.1.2 Effects assessment: Hazard identification and Dose (concentration) - response (effect) assessment

4.1.2.1 Toxico-kinetics, metabolism and distribution

Absorption

Orally administered diphenylamine is rapidly absorbed in man, rat, rabbit (Alexander et al. 1965), in dog (DeEds 1963) and in cow (Gutenmann & Lisk 1975). At least 68 – 89 % of orally applied diphenylamine is absorbed in mammals (JMPR, 1998).There are no data on skin absorption, but from calculation of dermal flux values (based on physical properties) it could be derived that the compound has potential for dermal toxicity (Fiserova-Bergerova et al, 1990) which is underlined by a dermal study in rabbits, where indications for dermal absorption after occlusive dermal application of diphenylamine could be observed: after dermal exposure to diphenylamine in distilled water at doses of 0, 100, 500 or 1000 mg/kg bw per day for 6h per day, gross necropsy revealed dark-red foci in the stomachs of rabbits at the intermediate and high dose (studies by Siglin, 1992, which are cited by the EPA report, the original publication was not available). There are no data on pulmonary absorption.

Distribution

There are no data on distribution available. However, extensive distribution can be assumed from the target tissues identified after acute, short- and longterm toxicological studies. After oral exposure, kidneys, liver, spleen and the haematopoietic system have been identified as the targets for the toxic effects of diphenylamine in different animal species (e.g. DeEds, 1963).

Metabolism and excretion

Animal data

Metabolism in rats

Male white rats received a single i.p. injection of 5 mg diphenylamine (dissolved in propylene glycol). Urine was collected for 24 h and examined for diphenylamine, 2-hydroxydiphenylamine, 4-hydroxydiphenylamine and 4,4’-dihydroxydiphenylamine by thin-layer chromatography. 4-Hydroxydiphenylamine and 4,4’-dihydroxydiphenylamine were found. The same metabolites were also determined in the bile of male white rats after i.v. injection of 2 mg diphenylamine (dissolved in 50% aqueous ethanol). The bile was collected for 6 h (Alexander et al., 1965).

Uniformly ring labelled ^{14}C-Dipenylamine was given orally in corn oil to groups of five male and five female Sprague-Dawley rats as a single dose of 5 mg/kg bw, as a single dose of 5 mg/kg bw preceded by 5 mg/kg bw per day of non-radioactive diphenylamine for 14 days or as a single oral dose of 750 mg/kg bw. Excreta were analysed by high-performance liquid

chromatography, thin layer chromatography or mass spectral techniques. The following 12 different metabolites were identified at all doses:

- 4,4'-dihydroxydiphenylamine (unconjugated and as the O-sulfate and O,O'-disulfate)
- 4-hydroxy-diphenylamine (unconjugated and as the O-glucuronide, N-glucuronide, O-sulfate and O,N-diglucuronide)
- indophenol (unconjugated and as the O-sulfate)
- 3-hydroxydiphenylamine
- 2-hydroxydiphenylamine

These metabolites plus parent compound accounted for 82 – 92 % of the dose in excreta and were mainly found as their sulfate and glucuronide conjugates. Differentiation between urinary and fecal metabolites is not given (studies by Wu, 1993 which are cited in the JMPR report, the original publication was not available). The proposed metabolic pathwas for diphenylamine in the rat is given in figure 4.1.

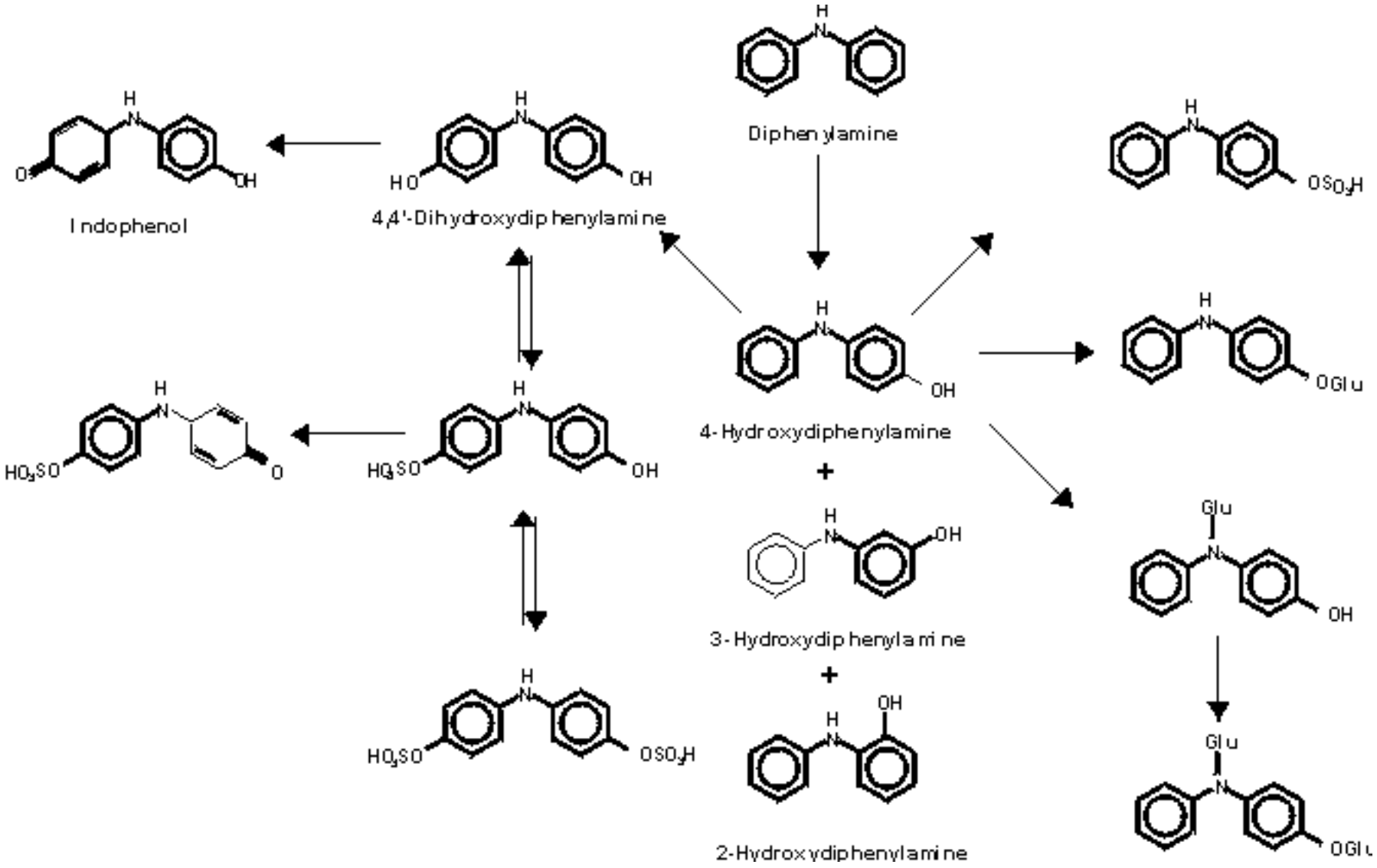

Diphenylamine
Indophenol
4,4'-Dihydroxydiphenylamine
4-Hydroxydiphenylamine
3-Hydroxydiphenylamine
2-Hydroxydiphenylamine
Glu

Figure 4.1: Metabolic scheme of diphenylamine in rats

Nitrosation of diphenylamine

Standard feed containing 0.01% diphenylamine and 0.15% sodium nitrite was given to 24 rats. The animals were sacrificed in groups of six after 30, 60, 120, and 180 minutes. The diphenylnitrosamine concentration was then determined in the pyloric stomach contents. After 120 min. a maximum amount of 37 µg diphenylnitrosamine/g stomach contents was found (Sander et al. 1968).

Female Wistar rats received a single oral dose of 850 mg diphenylamine vs. 1000 mg N-nitrosodiphenylamine in corn oil/kg bw by gavage. The urine was collected for 36 h and then analyzed. 4-hydroxydiphenylamine was one of the major metabolites of diphenylamine. The amounts of diphenylamine were relative low (0.02-0.05 µmol/rat/36 h). In the study with N-nitrosodiphenylamine the authors found also nitrite (about 15 µmol/rat) and nitrate (about 150-250 µmol/rat) (Appel et al. 1984a).

Hepatic levels of 8-OH-2-deoxyguanosine relative to 2-deoxyguanosine after treatment with diphenylamine were investigated via HPLC analysis of liver DNA (Lodovici et al. 1997). Rats were treated per os with doses of 0.9 to 1.4 mg diphenylamine/kg bw/d on ten consecutive days. Levels of 8-OH-2-deoxyguanosine were dose dependent increased relative to controls over the whole dose range tested. The authors conclude from this result hat diphenylamine generates reactive oxygen species capable of inducing cell genetic damage.

Excretion in rats

Male white rats received an i.p. injection of 5 mg ^{14}C- diphenylamine/kg bw and urine was collected in 24h intervalls. Analysis of ^{14}C in urine was performed by scintillation counting without identification of metabolites. 75% of the dose was excreted in the urine within 48 h. An intravenous dose of 5 mg ^{14}C- diphenylamine/kg bw (in 50% aqueous ethanol) resulted in the excretion of 25% of the activity in bile of male white rats after 6 h. Analysis of ^{14}C in bile was performed by scintillation counting without identification of metabolites (Alexander et al. 1965).

Uniformly ring labelled ^{14}C-Dipenylamine was given orally in corn oil to groups of five male and five female Sprague-Dawley rats as a single dose of 5 mg/kg bw, as a single dose of 5 mg/kg bw preceded by 5 mg/kg bw per day of non-radioactive diphenylamine for 14 days or as a single oral dose of 750 mg/kg bw. Urine, faeces, and cage washes were collected 4, 8, 12, and 24 h after dosing and at 24-h intervals up to 168 h thereafter. Radioactivity which was recovered in the urine after 168 h represented 68 – 89 % of the dose, the total recovery of radioactivity after 168 h accounted for 94 – 105 % of the dose. The distribution of recovered radioactivity between urine, feces and cage washes after the different dosing regimes is given in table 4.1. The mean percent of the dose in residual carcass and tissues was 0.41% in males

and 0.28 % in females at a single dose of 750 mg/kg bw and 0.14 – 0.28 % of the dose at the other dosages (studies by Wu, 1993 which are cited in the JMPR report, the original publication was not available).

Table 4.1: Recovery of radioactivity after oral administration of uniformly 14C-ringlabelled diphenylamine to Sprague-Dawley rats.

Dosing regime	Radioactivity in urine [% of the dose]	Radioactivity in feces [% of the dose]	Radioactivity in cage washes [% of the dose]
5 mg/kg bw	male: 81 female: 72	male: 9.1 female: 16	male: 9.2 female: 11
5 mg/kg bw preceded by 5 mg/kg bw nonradioactive diphenylamine for 14 days	male: 89 female: 68	male: 7.6 female: 21	male: 7.7 female: 12
750 mg/kg bw	male: 75 female: 73	male: 15 female: 8.8	male: 4 female: 11

Metabolism in rabbits

A male rabbit received five oral doses of 1 g diphenylamine (aqueous suspension) over 9 days. The following compounds were detected in the urine: small amounts diphenylamine vs. 2-hydroxydiphenylamine, 4,4'-dihydroxydiphenylamine, and the O-sulfate and O-glucuronide (as the tri-O-acetylmethyl ester) conjugates of 4-hydroxydiphenylamine (Alexander et al. 1965).

Metabolism in dogs

Groups of Beagle dogs (two males and two females) were given 0, 0.01, 0.1, or 1.0% diphenylamine in their feed for two years. The following metabolites were found in the urine and faeces: 4-hydroxydiphenylamine, 4-diphenylamine conjugates from sulfate vs. glucuronide, and 4,4'- dihydroxydiphenylamine. No differentiation between fecal and urinary metabolites and no relation to the administered dose is given. After hydrolysis, 4-hydroxydiphenylamine was identified in the bile (DeEds 1963).

Excretion in cow

A Holstein dairy cow was given 5 ppm diphenylamine in a feeding study for four days. Milk samples were taken in the morning and evening for 10 days and combined for a single daily analysis. Urine and faeces were also collected and examined daily. No traces of

diphenylamine were seen in the milk or urine, up to 1.4% of the total dose (0.45 g) was excreted in the feces. The gaschromatographic method used would not have been able to detect hydroxylated derivates of diphenylamine (Gutenmann & Lisk 1975).

Human data

Following a single dose of 100 mg diphenylamine administered orally to two humans, unchanged diphenylamine and two metabolites were identified in the urine (collected over 24 h after administration): 4-hydroxydiphenylamine and 4,4'-dihydroxydiphenylamine; 2-hydroxydiphenylamine and N-hydroxydiphenylamine however, were not detected (method: thin-layer chromatography, detection limit not reported) (Alexander et al. 1965).

In patients who had received very small amounts diphenylamine in a solution containing nitrite, N-nitrosodiphenylamine was formed in the stomach (Sander & Schweinsberg 1972). Based on the findings from in vivo rat studies and in vitro studies with rat hepatocytes (Appel et al.,1984a; 1987), however, it can be concluded, that N-nitrosodiohenylamine is likely to undergo denitrosation in turn.

In vitro studies

Incubation with S9 fraction from bovine liver in vitro led to the transformation of about 50% of the dose (5 ppm diphenylamine) within 30 minutes (Gutenmann & Lisk 1975). The gas chromatgraphic methodology applied, would not have been able to determine hydroxylated metabolites of diphenylamine. Therefore, no conclusions about the metabolites formed from diphenylamine can be drawn from these investigations.

Incubation of N-nitrosodiphenylamine with a reconstituted monooxygenase system (cytochrome P-450 and NADPH P-450 reductase, isolated from microsomes of pigs treated with phenobarbital) led to the formation of 0.18 nmol nitrite/min./nmol P-450 (Appel et al. 1984b).

The metabolism of N-nitrosodiphenylamine was investigated in phenobarbital-induced mouse liver microsomes. One metabolite was identified as diphenylamine, whereas the others were characterized as 4- hydroxydiphenylamine and its corresponding quinoneimine (Appel et al. 1987).

The in vitro oxidation of diphenylamine by pig liver microsomes to generate a nitrooxide free radical, diphenylnitroxide, in the presence of NADPH and molecular oxygen, has been investigated. The results indicated that 75% conversion of 0.22 mM diphenylamine takes place within the first 30 min. of the reaction. The formation of an oxidative product was determined by comparing the HPLC and ESR of the biosynthesized diphenylnitroxide to chemically synthesized diphenylnitroxide (Valvis et al. 1990).

Conclusion:

Orally administered diphenylamine is well absorbed from the gastrointestinal tract in man and in several animal species including rat, rabbit, dog and cow. Up to 3 % of the parent compound and approximately 80-90 % of the dose is excreted as 12 different metabolites, which include 4-hydroxydiphenylamine, 4,4'- 2 hydroxydiphenylamine and sulfate and glucuronide conjugates of these hydroxylated metabolites. In addition, indophenol has been identified as metabolite. N-hydroxylated metabolites responsible for methaemoglobinemia by aromatic amines could not be detemined. From these results it can be assumed that diphenylamine is readily metabolized and excreted and that accumulation seems to be unlikely. There are no data on dermal route of administration or exposure by inhalation. An absorption rate of 100% for the oral route is proposed to be taken for the risk characterisation, whereas dermal and inhalation absorption is assumed to be 100 % (defaults). The assumption of a default dermal absorption value of 100% is supported by the physicochemical properties of DPA (molecular weight: 169 g/mol; log Pow 3.4; water solubility: 40 mg/l). Due to the potential for absorption and the lack of experimental data, a default absorption value of 100% is also assumed for inhalative uptake.

4.1.2.2 Acute toxicity

Animal data:

Oral

Oral LD50 values were detected for male Syrian hamsters, rats and gerbils in a study conducted by Lenz and Carlton (1990): Forty male Syrian hamsters, rats and gerbils were assigned to 3 treatment groups and 1 control group with 10 animals per group. Each animal of a respective treatment group received diphenylamine (vehicle peanut oil) at 400 mg/kg bw/d, 600 mg/kg bw/d or 800 mg/kg bw/d by single daily gavage for 3 consecutive days. The control group received only peanut oil. Animals that became moribund during the study were euthanized 24 hours following the third dose and necropsied immediately. Six of ten hamsters orally given 600 mg/kg bw/d and five of ten hamsters orally given 800 mg/kg bw/d became listless and moribund within 20 hours of the first dose. The remaining 4 hamsters of the 600 mg/kg bw/d group died within 12 hours following the second dose, as did 2 hamsters orally given the second 800 mg/kg bw/d dose. No death rates were reported for the 400 mg/kg treatment group. In consequence, an oral LD50 of diphenylamine for hamsters of app. 600 mg/kg bw can be concluded. Gross renal lesions were observed in the 600 mg/kg bw/d and in the 800 mg/kg bw/d groups. The kidneys were swollen and had a diffuse, dull, brown discoloration that was visible from the capsular surface and extended through the cortex and outer medulla. Yellow-brown discoloration was the only gross renal papillary lesion in both groups. Other gross lesions were limited to the stomach and spleen (gastric ulcers, splenomegaly). No mortalities were reported for male rats and gerbils. Hence, an oral LD50 of >800 mg/kg bw/d can be assumed for these species. At necropsy, one rat treated orally with 800 mg/kg bw/d diphenylamine demonstrated renal papillary lesions, whilst no findings were reported for gerbils.

In a study similar to current EU and OECD guidelines with two groups of 10 male rats each, an oral LD50 of > 5000 mg/kg bw was reported: Doses of 3100 mg/kg bw and 5000 mg/kg bw were applied per gavage using lutrol as vehicle. After a 14 days observation period, no clinical signs and no mortality were observed in the 3100 mg/kg bw/d dosed group, while two rats died on days 3 and 4 and all of the rats demonstrated piloerection and decreased general state within the 5000 mg/kg bw/d dosed group (Bayer AG 1977).

LD50 values for male rats (2960 mg/kg bw/d) and female rats (2480 mg/kg bw/d) for diphenylamine with a purity of 99.9% are mentioned within the data and recommendations of a joint FAO/WHO meeting. Information on the method used and on clinical signs observed are not given within this report (Spanjers & Til 1982, cited in FAO/WHO 1984).

In a feeding study focusing on cystic renal lesions (Philbert et al. 1978, cf. 4.1.2.9) 20 Wistar rats and 20 Hartley guinea pigs (including 2 animals/species/per dose group, pregnant at g.d. 2-3) were given diets containing 2 or 4% diphenylamine of technical grade corresponding to an average dose of 1800 mg/kg or 3600 mg/kg body weight for rats and 2200 mg/kg or 4400 mg/kg body weight for guinea pigs. Within the first month of treatment 10 % or 60 % of the animals died, receiving 2 % or 4 % diphenylamine in the diet, respectively. Clinical signs of intoxication were significant weight loss, loss of hair, increasing pale discolouration of the fur and reduced vivacity. Pathological findings were reported for animals which survived the treatment up to 6 month and included 100 % abortions in the pregnant animals during the first week of treatment, liver necrosis, necrosis of proximal tubular epithelial cells and coritcal cysts in kidneys.

Inhalation

Animal data on acute inhalation toxicity are not available.

Dermal

A dermal LD50 of >2000 mg/kg bw/d for diphenylamine with a purity of 99.9% was detected in a study with rabbits. This information is given within the data and recommendations of a joint FAO/WHO meeting (van Beek & Bruijntjes 1982, cited in FAO/WHO 1984).

The acute dermal LD50 after a 24-h exposure to diphenylamine (purity 99.9-100.1%) was > 2000 mg/kg bw in New Zealand white rabbits of each sex. No clinical signs were noted. In rats (sex not reported) the dermal LD50 after a 24-h exposure was > 5000 mg/kg bw (Majnarich, 1991; unpublished study; cited in JMPR report, 1998).

Human data:

Human data on the acute toxicity of diphenylamine are not available.

Conclusion:

Human data on the acute toxicity of diphenylamine are not available. An oral LD50 value of approximately 600 mg/kg bw/d was detected for male Syrian hamsters and oral LD50 values exceeding 800 mg/kg bw/d for rats and male Mongolian gerbils. A dermal LD50 value of >2000 mg/kg bw/d is reported for rabbits and of >5000 mg/kg bw/d for rats. Data on the acute inhalation toxicity of the substance are not available. Based on these informations, diphenylamine is to be classified as harmful and labelled with R 22, harmful if swallowed; it is not to be labelled because of acute dermal toxicity. For the assessment of acute inhalation toxicity an animal study according to current EU or OECD guidelines is lacking.

4.1.2.3 Irritation

Animal data:

Diphenylamine caused no or only very slight skin irritation in tests with rabbits: Skin irritation potential of diphenylamine (no data on purity) was tested in the inner surface of the ears of two Albino rabbits by means of occlusive application of the substance for 24 hours (no information whether a vehicle was used or not used). There were no signs of irritation observed within a 7 days observation period (Bayer AG 1977). Application of 0.5 g undiluted diphenylamine to the intact and abraded skin of rabbits produced only very slight primary skin irritation in a test cited in a joint FAO/WHO meeting report. No further information on this study is given (van Beek 1982, cited in FAO/WHO 1984). A more recent study, cited in a JMPR report (1998), revealed no skin irritation of diphenylamine (purity, 99.9-100.1%) in rabbits (Kreuzmann, 1991, unpublished study; cited in JMPR report, 1998).

In a primary skin irritation test with purified diphenylamine in albino rabbits (Elf Atochem North America Inc. 1994a, study dated from 1982), conducted according EPA guideline, 0.5 g diphenylamine was applied on two intact and two abraded skin sites of each six rabbits. After 24 hours occlusive exposure, mild effects were observed. At the final 72 hours reading, 6 out of 24 application sites showed very slight edema (grade 1).

Data on eye irritating properties of diphenylamine are conflicting and poorly documented: Moderate conjunctival irritation was detected in a Draize eye irritation test after instillation of 50 mg of the substance into the eyes of 2 rabbits. One of the animals demonstrated slight to moderate conjunctival irritation (redness and edema) within a 7 days observation period. There is no information on reversibility or irreversibility of these effects (Bayer AG 1977). In a second study, a Draize eye irritation test with 0.1 g reagent grade diphenylamine was performed according to current EU and OECD guidelines using 3 rabbits. Corneal opacity, erythema, chemosis, secreta and iritis were classified according to the Draize score system. Analogous to the EU-classification system, a recovery time of 21 days of corneal and conjunctival damages was used to classify substances as severe irritants. In this study diphenylamine is classified as „corrosive“ to eyes because of „corneal involvement, irritation or eye damage that persists for more than 21 days after treatment“ (Sugai et al. 1990). However, neither Draize scores nor effects are given. In a third eye irritation test with 0.1 g purified diphenylamine in 6 albino rabbits (Elf Atochem North America Inc. 1994b, study dated from 1982), conducted according EPA guideline, mild-moderate effects were observed. After 24 hours, mild iritis (in one animal only, score = 1) and mild-moderate conjunctivitis

occurred. Averaged Draize scores for conjunctival erythema, 24/48/72 hours- 4/7 days: 1.2/1.2/1- 0.4/0.2 and for conjunctival chemosis: 1/1/0.5- 0.5/0.2, respectively. During the course of the observation period, these effects cleared up gradually. After 10 days, all treated eyes were normal again. In a JMPR report (1998) was mentioned that Kreuzmann (1991, unpublished study) reported corrosivity and corneal opacity after application of 0.1 g diphenylamine (purity 99.9-100.1%) to the eyes of one rabbit for seven days without rinsing.

Human data:

No data available.

Conclusion:

Human data on local irritant or corrosive properties of diphenylamine are not available. The substance caused no or only very slight skin irritation in tests with rabbits.

Data on eye irritating properties of the substance are conflicting and poorly documented, but it may be assumed that diphenylamine may pose a risk of serious damage to eyes. There exist two studies (one with documented guideline-compliance), which both report severe eye irritation caused by diphenylamine. In one of these studies irreversibility of effects after 21 days is stated. Hence, appropriate labelling with R41 "Risk of serious damage to eyes" is proposed.

4.1.2.4 Corrosivity

All studies on dermal effects demonstrated only a weak skin irritation potential (4.1.2.3). Hence, diphenylamine is not a corrosive substance.

4.1.2.5 Sensitisation

Animal data:

Diphenylamine (purity 99.9%) did not produce dermal sensitization in guinea pigs (Kiplinger, 1995, as cited in JMPR report, 1998).

No data on skin sentisation is available.

Human data:

A 44-year-old woman who worked in a circuit braker factory handling metals, plastics and greases had a vesicular and exudative eczema on the back of her hands. Patch testing (test concentration 1%, vehicle not mentioned) revealed a positive response to diphenylamine but not to p-phenylenediamine (Bazin et al. 1986). A total of 1012 eczema patients were patch tested in nine cities (test concentration: 1% in petrolatum). There were three positive patients in one city. These patients were all known to be positive to p-phenylenediamine and the reactions were regarded as cross sensitivities. It is mentioned that exposure is possible due to the use of perfume ingredients where diphenylamine is included at a concentration of 0.1% (Calnan 1978). There was an outbreak of dermatitis in a group of 16 men involved in the quality control and analysis of fine organic chemicals, including diphenylamine. Eleven of the workers (the remaining four people were tested with another substance) were patch tested with a concentration of 1% diphenylamine in methanol. None of the workers showed a positive response (Slovak 1980). A maximization test carried out on 30 volunteers produced no sensitization reactions (test concentration 1% in petrolatum; Epstein 1976).

Respiratory sensitisation

No information on the sensitising potential of the substance at the respiratory tract is available. However, diphenylamine is not suspected to be a potent respiratory sensitiser in humans according to the fact that during all the years of use no notice of specific case reports has been given.

Conclusion:

Diphenylamine (purity 99.9%) did not produce dermal sensitization in guinea pigs (Kiplinger, 1995, as cited in JMPR report, 1998). There is one case of one woman where a contact allergy could be demonstrated. Other studies with 11 or 1012 patients did not demonstrate a skin sensitization that could be attributed to diphenylamine. Cross sensitization to p-phenylenediamine has not been demonstrated in the woman who reacted positive to diphenylamine and cross sensitivities were suspected in three positive patients. In a maximization test carried out on 30 volunteers no sensitization reactions were produced. These data demonstrate that in humans the substance has a weak or no sensitizing potential. Though the occurrence of cross reactions to p-phenylenediamine is rare, it should not be dismissed. However, based on the overall negative data on people exposed as consumers or as workers the risk phrase 43 - May cause sensitization by skin contact - is not warranted. In addition, in human volunteers the substance produced no contact allergy.

4.1.2.6 Repeated dose toxicity

Animal studies

Oral

Gavage studies

Male Sprague Dawley derived rats (5 controls and 6 dosed) were treated (gavage) with one dose of 338 mg/kg bw/d of diphenylamine over a period of 21 days by gavage. The animals showed necrosis in 20% of the kidney papillae, increased kidney weights and loss of urine concentrating ability (Hardy 1974).

In a guideline conform oral 28 day study Fischer rats of both sex received 111, 333 and 1000 mg/kg bw/d diphenylamine by gavage. Thirty-six animals were divided into 6 groups of equal numbers, 4 groups being used for treatment and the remainder for investigation of recovery. Inhibition of body weight gain, increase of liver, spleen and kidney weights and anemia were observed in the high dose group in both sexes. The same group demonstrated mucosal hyperplasia in the forestomach, dilatation, degeneration or necrosis of renal tubules in the corticomedullary junction and hyperplasia in the bone marrow histopathologically. Slight increase of spleen, liver and kidney weights as well as slight degeneration of renal tubules were evident in several animals of the 333 mg/kg bw/d dose group. Repair of histopathologic lesions and anemia occurred after 14 days. As there were no toxic effects in the low dose group a NOAEL of 111 mg/kg bw/d could be derived under these experimental conditions (Yoshida et al. 1989).

Diet studies

Rats

Male rats received 2.5% (1250 mg/kg bw/d) diphenylamine in the diet over a period of 19 weeks. The first ultramorphological detectable renal lesions were electron dense material in epithelial cells of the proximal tubuli. These cells underwent degeneration. The same was seen in distal portions of the nephron together with cellular congestion and swelling of mitochondria and desquamation of cells. As consequence an obliteration of some of the tubuli occurred leading to dilatation and cyst formation as well as compression of adjacent parenchyma (Woodhouse et al. 1965).

40 Albino rats per group (20 male and 20 females) received oral doses of 0.001% (0.5 mg/kg bw/d), 0.01% (5 mg/kg bw/d), 0.1% (50 mg/kg bw/d), 0.5% (250 mg/kg bw/d) and 1.0% (500 mg/kg bw/d) of diphenylamine in a feeding study over a period of two years. Growth and body weight of the animals were not effected up to 0.1% (50 mg/kg bw/d) diphenylamine within the first 240 days of treatment. Higher diphenylamine concentrations induced significant growth retardation. At 1% (500 mg/kg bw/d) diphenylamine food consumption was reduced to 10% of the control level. Haematological investigations (day 126 and 463) showed slight anemia together with reduced haemoglobin and erythrocyte values and increased numbers of normoblasts. Mortality was not increased and did not differ from

controls. Animals of the 0.1% and 0.5% dose groups exhibited signs of dilatated renal tubuli as well as chronic interstitial nephritis (DeEds, short version 1963).

Female Albino rats (6 animals per group) received diphenylamine in a feeding study for 226 days in concentrations of 0%, 0.25% (125 mg/kg bw/d), 0.1% (50 mg/kg bw/d), 0.5% (250 mg/kg bw/d), 1.0% (500 mg/kg bw/d) and 1.5% (750 mg/kg bw/d). In concentrations of 0.5% and higher main toxic effects of diphenylamine were growth retardation, focal dilatation of renal tubuli and cyst formation in distal tubuli and collecting ducts (Thomas et al. 1957).

In a study male Sprague Dawley rats were given 1.0% (500 mg/kg bw/d) diphenylamine in food up to 76 weeks. After 2 to 6 weeks the animals developed polyurea with diluted urine. The first histological change was noted after 5 weeks, and was described as focal proliferation of distal tubular and collecting duct cells. Focal areas of medullary tubuli appeared thickened because several cells were layered upon each other. By week 10 collecting ducts showed cystic dilatations with focal necrosis and increasing cast material in the duct lumina. As the study was not designed for toxicologic purposes, a NOAEL was not derived (Evan et al. 1978).

Further investigations were groups of 40 Albino rats were fed with diphenylamine in diet in concentrations of 0.001% (0.5 mg/kg bw/d), 0.01% (5 mg/kg bw/d), 0.1% (50 mg/kg bw/d), 0.5% (250 mg/kg bw/d), and 1% (500 mg/kg bw/d) during 2 years showed a moderate growth retarding effect in males and females at 0.5 and 1% feeding level. This was considered due to lower food intake because of unpalatability. Cystic dilatations of the distal tubuli and collecting ducts occurred at 0.1%, and there was an accompanying interstitial inflammation to be observed between 0.1 and 0.5% diphenylamine in diet. Proximal tubuli were extremely rarely and glomeruli never altered. Dose levels of 0.01% (5 mg/kg bw/d, NOAEL) and below did not induce any relevant changes (Thomas et al. 1967a).

Female Sprague Dawley rats fed with 2.5% diphenylamine in diet showed gross cysts after 3-6 weeks in approximately 10% of the kidneys examined, however, all kidneys showed morphological alterations and the most consistent histological finding was dilatation of collecting ducts. Furthermore, the urine concentration ability of the kidney decreased within two weeks (Eknoyan et al. 1976).

Male Sprague Dawley rats were fed 1% (500 mg/kg bw/d) diphenylamine (% in diet) over a period of 5 to 12 months. All examined kidneys showed dilated tubuli and/or cyst formations. Lumina of tubuli and collecting ducts were often filled with cellular debris partially obstructing the lumen. The effected tubuli showed an increased intraluminal hydrostatic pressure. Histology revealed conjunctions between delated tubuli and cyst formation leading to obliteration and compression of adjacent tissue structures. This was interpreted as morphological basis for functional impairment of the kidney. Haematological parameters were not investigated (Gardner et al. 1976).

Groups of 10 male and 10 female Sprague Dawley rats received technical-grade diphenylamine in the diet at 0, 150, 1500, 7500, or 15000 ppm for 90 days, equal to doses of 0, 9.6, 96, 550, and 1200 mg/kg bw per day in males and 0, 12, 110, 650, and 1300 mg/kg bw per day in females. The frequency of darkening of the urine increased with dose, starting with one female at 1500 ppm and 100% of rats at 15 000 ppm. Haematological measures indicated

decreased erythrocyte counts and haemoglobin values, which were statistically significantly different from those of controls in animals at 7500 and 15 000 ppm at termination. The haematocrit values were statistically significantly lower than those of controls in females at the three highest doses. Small, statistically significant increases in alkaline phosphatase activity, albumin content, and albumin:globulin ratio in males and glucose and albumin content and albumin:globulin ratio in females were observed at 7500 and 15 000 ppm. The cholesterol concentration increased with dose in females and was statistically significantly different from that of controls at the three higher doses. In males, the absolute and relative weights of the liver and spleen increased with dose and were statistically significantly raised at 7500 and 15 000 ppm; the relative weights of the kidney and gonad also increased with dose and were also statistically significant at the two higher doses. In females, the absolute and relative weights of the liver increased with dose, and the change in relative weights was statistically significant at doses > 1500 ppm. The kidneys were dark in animals of each sex at 7500 and 15 000 ppm, and about 60% of the females at the high dose had dark and/or enlarged livers. The spleens of both males and females at the two higher doses were congested. Histopathological examination revealed an increased incidence of haematopoiesis and pigment in the liver, haematopoiesis, haemosiderosis, and congestion in the spleen, and pigmented kidneys in animals of each sex at 7500 and 15 000 ppm. The spleens of all females at 1500 ppm also showed an increase from minimal to slight haematopoiesis and haemosiderosis. The NOAEL was 150 ppm, equal to 12 mg/kg bw per day, on the basis of increased clinical signs of toxicity, clinical chemical changes, organ weights, and gross and histopathological appearance (Krohmer, 1992a).

In a 90-day dietary feeding study rats (10 male and 10 female) received a diet containing 0.01, 0.03, 0.1, 0.3 and 1 % diphenylamine according to dosages of 5, 15, 50, 150 and 500 mg/kg bw/d. No indication of the rat strain used was given in the available summarized study report (full report was not available). For results, only data on growth, average hematological values in females and average organ weights at autopsy were shown.
Male rats at the 0.03 and 0.01% levels showed no evidence of adverse effects when judged by appearance, behavior, growth, food consumption, mortality, final body weight and organ weights and gross and microscopic examination of lung, heart, liver, kidney, spleen and testes. In female rats, liver weights were statistically significant increased at all dose levels tested and spleen weights were increased at 0.03% and higher dose levels. Liver weights of male rats were increased at 0.1% diphenylamine and higher dosages. At 0.3% liver, kidney and spleen appeared brown in color and congestion as well as an increase in weight was noted for spleen. After 50 days both male and female rats at the 1.0% level were severely affected (weakness, growth retardation, increase of liver and spleen weights, central lobular necrosis in the liver, increased nephritis in the kidneys, congestion in the spleen). Additionally, in male rats the mortality rate was increased at the 1% level due to upper respiratory infections and average hematological values were changed in females compared to controls. A LOAEL of 0.01% (5 mg/kg bw/d) could be derived from this study based on liver weight effects in female rats. Since the weight change in females was only weak and was not accompagnied by functional adverse effects, this LOAEL will not taken into consideration for the risk characterisation of diphenylamine (Dow Chem, 1958).

Dogs

Groups of 4 Beagle dogs (2 males and 2 females) received diphenylamine by feed in concentrations of 0.01% (8 mg/kg bw/d), 0.1% (77 mg/kg bw/d), and 1.0% (769 mg/kg bw/d) over a period of two years. The doses were calculated by the rapporteur on the basis of the

species-specific equations for food consumption as given in Annex VI of the revised TGD. Up to 400 days growth (body weight) was not effected in the two low dose groups. High dosed animals showed a clear inhibition of body weight gain. Thus, the dose of 77 mg/kg bw/d is considered as NOAEL. In the 1.0% diphenylamine group there was a decrease of haemoglobin and erythrocytes and there were hints of sulphurbromphthalein-retention. In the same group organ weights of spleen, kidney and liver were enhanced. Histopathology revealed signs of accumulation of bilirubin in hepatocyctes as well as slight haemosiderosis in spleen, kidney, liver, and bone marrow (DeEds, short version 1963).

Groups of four pure-bred beagle dogs of each sex received technical-grade diphenylamine (purity, > 99%) in gelatin capsules at doses of 0, 10, 25 or 50 mg/kg bw per day for 90 days. They were observed for deaths, clinical signs, body weight, food consumption, ophthalmological, haematological, clinical chemical, and urinary parameters, organ weights, and gross and histopathological appearance. There were no deaths, and no treatment-related changes were seen in any of the above parameters. Statistically significant increases were seen, however, in some clinical chemical parameters including albumin content, the albumin:globulin ratio in maes, and bilirubin content in females at the high dose. These effects may have been incidental. The NOAEL was 50 mg/kg bw/day, the highest dose tested (Krohmer, 1992b).

Groups of 4 Beagle dogs (2 males and 2 females) were treated orally with diphenylamine in concentrations of 0.01% (2.5 mg/kg bw/d), 0.1% (25 mg/kg bw/d), and 1.0% (250 mg/kg bw/d) in their diet for 2 years. Mid and high dose animals developed marked growth retardation after one year. A dose dependent anemia was seen in the same treatment groups, being pronounced in the high dose group and moderate in the mid dose group. After two years the erythrocytes of the dogs on the 1.0% diet showed a moderate decreased resistance to hypotonicity. Liver function as a result of sulfobromophthalein testing at days 618 to 627 indicated a moderate degree of liver damage in the 1.0% group.Thus, the NOAEL of this study is 2.5 mg/kg bw/d based on a slight decrease in the haemoglobin and red blood cell content at 25 mg/kg bw/d. These animals also showed an increased organ weight of the liver with perilobular fatty changes and increased lipid content, a mild haemosiderosis of the spleen, kidneys and bone marrow, and a slight increase in kidney weight (Thomas et al. 1967b).

Mice

A total of 1200 Charles River CD-1 mice (both sexes) received diphenylamine in diet in concentrations of 0 ppm, 50 ppm (7.5 mg/kg bw/d), 100 ppm (15 mg/kg bw/d), and 250 ppm (37.5 mg/kg bw/d) up to 92 weeks. No effect of diphenylamine exposure was noted on growth, clinical conditions, survival, pattern of spontaneous disease, principal haematology (including methaemoglobin) and any kind of histopathology in relevant tissues. The only obvious effects were Heinz bodies in erythrocytes of the animals receiving the diet with 250 ppm (0.025%) diphenylamine. After 5 weeks of recovery Heinz bodies were still not in the range of representative controls. The same strain of mice fed with a diet containing 0 ppm, 5 ppm (0.75 mg/kg bw/d), 10 ppm (1.5 mg/kg bw/d), 50 ppm (7.5 mg/kg bw/d), 100 ppm (15 mg/kg bw/d), 250 ppm (37.5 mg/kg bw/d), and 1000 ppm (150 mg/kg bw/d) diphenylamine for 12 weeks had Heinz bodies in dose groups of 50 ppm diphenylamine and higher. The Heinz bodies occurred within 1-2 weeks. A temporary depression of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in erythrocytes were noticed in mice

fed with 1000 ppm (0.1%) (Ford et al. 1972). A NOAEL of 1.5 mg/kg bw/d can be derived from this study which is only as an abstract available.

Groups of 15 male and 15 female Swiss-derived CD-1 mice received technical-grade diphenylamine in the diet at 0, 10, 52, 260, or 5200 ppm for 90 days, equal to doses of 1.7, 94, 440, and 920 mg/kg bw per day in males and 2.1, 110, 560, and 1100 mg/kg bw per day in females. Haematology indicated dose-related decreases in erythrocyte counts and haematocrit in animals at the two higher doses that were statistically significantly different from controls. The values for mean corpuscular haemoglobin, mean corpuscular volume, and mean corpuscular haemoglobin content increased with dose and were statistically significantly different from those of controls in animals at the two higher doses; the mean corpuscular haemoglobin content was also statistically significantly increased in males at 525 ppm. The reticulocyte counts increased with dose and were statistically significantly different from those of controls at the high dose. In males, the absolute and relative weights of the liver and spleen increased with dose and were statistically significantly different from those of controls at the two higher doses; the relative weights of the kidney and heart were statistically significantly different from those of controls in mice at the high dose. In females, the absolute and relative weights of the spleen increased with dose and were statistically significantly different from those of the controls in animals at the two higher doses; the absolute and relative weights of the liver and the relative weights of the kidney were statistically significantly different from those of controls in females at the high dose. Necropsy of females revealed dark, enlarged spleens at the three higher doses, dark livers at the two higher doses, and dark kidneys at the highest dose. In males, necropsy showed dark, enlarged spleens and dark livers at the two higher doses. Histopathological examination of the liver showed increased pigment deposition and slight haematopoiesis in animals of each sex at the two higher doses. The spleen showed haemosiderosis and congestion at the three higher doses, reaching incidences of 14/15 or more at the two higher doses; the severity of spleen haematopoiesis was also increased at the three higher doses. The kidneys showed pigment deposition at the two higher doses. Cystitis was observed in 9/15 males at the high dose and in 2/15 females at 2625 ppm and 8/14 females at 5250 ppm. The cellularity of the bone marrow was increased at the two higher doses. The NOAEL was 10 ppm, equal to 1.7 mg/kg bw per day, on the basis of changes in haematological parameters and findings at necropsy (Botta, 1992).

Inhalation (rat/mouse/dog/other)

No data.

Dermal

Rabbits

Groups of five male and five female New Zealand white rabbits received repeated dermal applications of technical-grade diphenylamine dissolved in distilled water at doses of 100, 500, or 1000 mg/kg bw per day. The material was applied daily for 6 h to an area of clipped skin corresponding to about 10% of the body surface and kept under occlusion for 21 consecutive days, with terminal sacrifice on day 22. Two additional groups of five rabbits of

each sex served as vehicle controls. There were no deaths or treatment-related effects on clinical signs, body weights, food consumption, or haematological end-points. The only possible treatment-related effects on clinical chemistry were on sodium and potassium concentrations; females at all three doses had depressed sodium values, and those at the intermediate and high doses and males at the high dose had depressed potassium values with respect to controls. Gross necropsy, revealed dark-red foci in the stomachs of rabbits of each sex at the intermediate and high doses, which increased in frequency with dose: the incidences were 1/5 in males at the intermediate dose, 4/5 in those at the high dose, 1/5 in females at the intermediate dose, and 2/5 in females at the high dose. No dark-red foci were seen in the stomachs of controls or rabbits at the low dose. The NOAEL for systemic toxicity was 100 mg/kg bw per day on the basis of the presence of dark-red foci in the stomachs of males and females (Siglin, 1992).

In a dermal 90-day study two groups of Sprague Dawley rats were treated with 500 or 2000 mg/kg bw on five days per week (Mobil Oil Corp., 1994). A control group received the same treatment without test compound. Animals were housed in single cages and equipped with a collar to prevent oral uptake of the test compound. Because the study consisted of only two treated groups it is not in full concordance with the OECD test requirements. There were no signs of systemic toxicity. Treatment had no effect on body weight gain. There were some changes of parameters in serum chemistry and hematology in the treated groups. These observations occurred to some extend also in control animals and did not lay outside the range of historical control levels. A NOAEL for systemic toxicity of DPA after dermal application over a period of 90 day is 500 mg/kg bw/d based on an increase in the relative kidney weight of males at the higher dose. All treated animals exhibited dermal hyperplasia at the application side. Thus, a NOAEL could not be achieved. The LOAEL for local effects at the skin after 90 day application is 500 mg/kg bw/d.

Other information

Male Syrian hamsters, male SD rats and male Mongolian gerbils were treated with dosages of 400, 600 and 800 mg/kg bw/d diphenylamine either orally by gavage or by i.p. injection three consecutive days. 24 hours after the last application animals were sacrificed and kidneys were investigated by H and E histology. In the group of Syrian hamsters treated with 600 mg/kg bw/d six of ten animals and in the group treated with 800 mg/kg bw/d five of ten animals were found in moribund conditions after the first oral dosing and thus necropsied before schedule. Total renal papillary necrosis was found in kidneys in four of ten, seven of ten and six of ten male Syrian hamsters treated orally with doses of 400, 600 and 800 mg/kg bw/d respectively. Total papillary necrosis was also observed in five of ten and four of ten male Syrian hamsters intraperitonally treated with 600 or 800 mg/kg bw/d respectively. In two male SD rats treated orally at 800 mg/kg bw/d apex-limited necrosis of the medullary interstitial cells and vasa recta and degeneration of the renal interstitial matrix occurred. Gross and microscopic lesions were not observed in any Mongolian gerbils. It was concluded that the Syrian hamster is more susceptible to the papillotoxic effects of diphenylamine than the Sprague Dawley rat and the Mongolian gerbil (Lenz and Carlton 1990).

There are a number of studies in different animal species in which diphenylamine is used as model compound to induce polycystic renal disease (Alvarez et al. 1987; Darmady et al. 1965; Darmady and Offer 1970; Thomas et al. 1957; Safouh et al. 1970; Evan and Gardner

1976; Evan et al. 1979; Philbert et al. 1978; Powell et al. 1987; Rohrbach et al. 1993; Kronevi and Holmberg 1979; Kronevi and Holmberg 1980).

As these studies in general have been carried out with relative high dose levels and do not follow current standards of toxicological testing guidelines they do not contribute to the selection of critical effects for risk assessment.

Summary of repeated dose toxicity data in animals:

There are no data on effects after inhalation of diphenylamine.

From studies of animals fed with a diet containing diphenylamine the most sensitive indication for toxicity seem to be haematological effects such as a slight anemia and formation of Heinz bodies (Ford et al. 1972).

Heinz bodies are considered to be indicative for methaemoglobin formation.

At higher doses diphenylamine cause kidney changes generally subscribed as polycystic kidney disease, accompanied with different stages of papillary necrosis and nephritis in different species. The only guideline conform 28 day test with oral gavage application (Yoshida et al. 1989) revealed some minor weight changes on liver, spleen, and kidney as well as a slight degenerative change on renal tubulus cells in some animals. A clear NOAEL for rats could be demonstrated at 111 mg/kg bw/d for systemic effects under these experimental conditions.

In a 1998 JMPR report on diphenylamine more recent guideline conform repeated dose toxicity studies on mice and rats (Botta, 1992; Krohmer, 1992a) have been described. All of them underline haematotoxicity as the main toxic effect by diphenylamine. However, these studies are not available as original literature. The primary target organs after short- and long-term dietary exposure of diphenylamine are the hematological system and the kidneys, spleen, and liver.

In table 4.1.2.6 the NOAELs and LOAELs of many studies with repeated oral administration of diphenylamine to rats, mice, and dogs are summarized (cf. also 4.1.2.8 Carcinogenicity) including the study results taken from the JMPR report (JMPR, 1998). Comparing the LOAELs from the different studies it becomes obvious that adverse effects in rats and dogs occurred at the same doses of about 25 mg/kg bw/d. Mice seem to be less sensitive according to the results from the newer short- and long-term studies by Botta (1992, 1994a).

Table 4.1.2.6 Summary table: NOAEL and LOAEL values for diphenylamine derived from repeated dose oral toxicity studies in experimental animals

Species	Exposure route; exposure duration	NOAEL [mg/kg bw/d]	LOAEL [mg/kg bw/d]	Reference
Rat, albino	diet, 2 years	50	250	DeEds, 1963
Rat, albino	diet 2 years	50	250	Thomas, 1967a
Rat, F344	gavage, 28 days	111	1000	Yoshida, 1989
Rat, SD	diet, 90 days	12	110 (f)	Krohmer, 1992a
Rat, SD	diet, 2 years	7.5	25 (f)	Botta, 1994b
Mouse, CD-1	diet, 92 weeks	15	37.5	Ford et al., 1972
Mouse, CD-1	diet, 12 weeks	1.5	7.5	Ford et al., 1972
Mouse, CD-1	diet, 90 days	1.7	94	Botta, 1992
Mouse, CD-1	diet, 78 weeks	73	370 (f)	Botta, 1994a
Dog, Beagle	diet, 2 years	77	769	DeEds, 1963
Dog, Beagle	diet, 2 years	2.5	25	Thomas, 1967b
Dog, Beagle	gavage, 90 days	50	-	Krohmer, 1992b
Dog, Beagle	gavage, 1 year	10	25	Botta, 1994c

There is poorly documented information on a 12 week oral feeding study on mice (Ford et al. 1972, abstract only) which describes the formation of Heinz bodies in erythrocytes at doses of 7.5 mg/kg bw/d and higher without any correlative data from other investigations. No substance-related effects were reported for the dose of 1.5 mg/kg bw/d thus this dose may be considered as NOAEL for repeated dose toxicity. However, this NOAEL is contradictory to the results of a diet study on 1200 Charles river CD-1 mice with dosages of 7.5, 15, and 37.5 mg/kg bw/d for 92 weeks by the same authors. No effect of diphenylamine exposure was noted on growth, clinical conditions, survival, pattern of spontaneous disease, principal haematology (including methaemoglobin) and any kind of histopathology in relevant tissues. The only obvious effect in this longer lasting study was the formation of Heinz bodies after application of 37.5 mg/kg bw/d, whereas no effect was seen after application of 7.5 and 15 mg/kg bw/d. Thus the low value of 1.5 mg/kg bw/d from the old Ford 12 week study will be ignored for taking as NOAEL for repeated dose toxicity.

Taking together all data from animal studies with repeated oral application, it is proposed to derive the value of 7.5 mg/kg bw/d as NOAEL for adverse effects after chronic exposure from the two-year carcinogenicity study on rats (Botta, 1994b). This NOAEL is based on haematological and histological effects at dietary levels equal or greater than 25 mg/kg bw/d in female rats (LOAEL). This study was the basis for establishing the actual acceptable daily intake (ADI) of 0-0.08 mg/kg bw/d by the JMPR (1998), too.

In the JMPR report (1998) on diphenylamine a dermal study in rabbits lasting 21 days (Siglin, 1992) is described which is not available as original paper. From this study a NOAEL of 100 mg/kg bw/d can be derived. After dermal application of DPA to rats over a period of 90 days the NOAEL for systemic toxicity is 500 mg/kg bw/d based on an increase in the relative kidney weight of males at the higher dose. All treated animals exhibited dermal hyperplasia at the application side. Thus, the LOAEL for local effects at the skin after 90 day application is 500 mg/kg bw/d, whereas a NOAEL could not be derived.

It is proposed to base risk characterisation for dermal exposure (systemic effects) on the NOAEL of 500 mg/kg bw/d from the 90-day study on rats. Dark red foci in the stomach as seen in two rabbits of the 500 mg/kg bw/d group of the 21-day study were not observed on rats at the same dose in the 90-day study.

Conclusion on classification:

Formation of Heinz bodies was seen in mice after a feeding period of 12 weeks at 7.5 mg/kg bw/d diphenylamine, although without any correlative data from other investigations. Normally this fact alone would be sufficient to decide for R 48. Because further studies of long duration (subchronic, chronic) in mice and other species do not demonstrate that anaemic effects occur at dose levels requiring R 48, this R-phrase can be neglected.

The slight decreases in the haemoglobin and red blood cell content at 25 mg/kg bw/d in the dog study of Thomas et al. (1967b) are not considered to be sufficient for classification of R48 for haemolytic anemia. According to the paper by Muller et al. (2005) "Hazard assessment of chemicals inducing haemolytic anemia: An EU Regulatory perspective" a reduction in haemoglobin concentration of below 10% is generally asymptomatic. Decreases

in erythrocyte counts, haemoglobin, and haematocrit values in the 2 year study on rats (Botta, 1994b) reached statistical significance only at higher doses (140 and 290 mg/kg bw/d.

R 33 is not appropriate, since DPA does not accumulate in any mammalian tissue to a reasonable extent. Toxicokinetic studies describe a rapid and almost complete excretion after oral application (cf. 4.1.2.1).

4.1.2.7 Mutagenicity

In vitro studies

Bacterial genotoxicity assays

DPA was negative in a gene mutation test with Salmonella typhimurium tester strains TA97, TA98, TA100 and TA1535 with and without S-9 mix obtained from human or rat livers (Zeiger et al, 1988). The test was performed in accordance with OECD guideline 471. Doses up to 333 µg/plate were tested; strain-specific toxic effects occurred at doses from 33 µg/plate upwards. Negative results from a Salmonella typhimurium/mammalian microsome plate incorporation assay with tester strains TA1535, TA1537, TA1538, TA98, and TA100 were reported by EPA (1998) (original publication (Lawlor 1992) was not available). Diphenylamine was tested in the absence and presence of metabolic activation in doses of 6.67, 10.0, 33.3, 66.7 and 100 mg/plate without metabolic activation and 10.0, 33.3, 66.7, 100 and 333 µg/plate with metabolic activation. According to EPA (1998) diphenylamine was cytotoxic at the highest tested doses.

Further negative results were reported from a gene mutation test that was not conducted according to current guidelines (Litton Bionetics, 1977). DPA was tested in tester strains TA1535, TA1537, TA1538, TA98 and TA100 in doses of 0.1, 1, 10, 100 and 500 µg/plate in the presence and the absence of S-9 mix from Aroclor induced rat liver. The highest dose was cytotoxic to all tester strains. Diphenylamine was negative in a Salmonella / mammalian microsome mutagenesis pre-incubation screening assay with only one tester strain (TA1535) in doses up to 75 µg/plate without metabolic activation (Mobil Oil Corp., 1985).

Furthermore, negative results from gene mutation tests with various Salmonella typhumurium tester strains were reported by other authors; however, data presentations were inadequate for appropriate interpretation of results (Epler et al., 1978; Probst et al., 1981; Sugimura et al., 1982). Inconclusive findings were obtained by Ferretti et al. (1977) and Florin et al. (1980).

A bacterial assay for induction of SOS repair of DNA was negative; methodology and results were not described in detail (Hude et al, 1988).

Mammalian cell gene mutation assay

In a mouse lymphoma assay a negative result was obtained with DPA in the presence of S-9 mix (Amacher et al., 1980). The highest dose tested - 0.2849 mmol/l, equivalent to 48.2 µg/ml - led to a clear cytotoxic effect (relative cell survival of 22%). Small colonies were not

distinguished from large colonies in this test. The test was conducted in accordance with OECD guideline 476 unless no testing was done without S-9 mix.

Weak positive results from an L5178Y TK+/- mouse lymphoma mutation assay were reported by EPA (1998) and JMPR (1998) (original publication (Cifone 1992) was not available). Diphenylamine was tested at dose levels of 5 - 80 µg/ml with and without rat liver S9 mix. A relatively equal distribution of large and small colonies was reported by EPA (1998).

DNA repair test with mammalian cells

Screening for induction of DNA repair (unscheduled DNA synthesis, UDS) in primary cultures of rat hepatocytes was negative for a dose of 100 nmoles/ml (equivalent to 16.9 µg/ml) (Probst et al., 1981).

Test for induction of sister chromatid exchanges (SCE)

In human lymphocyte cultures DPA was tested in accordance to OECD-guideline 479 for induction of sister chromatid exchanges (SCE) in doses up to 3.5×10^{-5} mol/l (60 µg/ml) (Ardito et al., 1996). Short-term exposure for 4 h was negative with and without S-9 mix; continuous treatment without S-9 mix led to a 1.2-fold increase of the SCE frequency for the top dose. According to Ardito et al. (1996) this effect is of statistical significance; however, such a marginal increase is probably induced by an unspecific rather than by an genotoxic mechanism.

In vivo studies

Cytogenetic tests

In an in vivo chromosomal aberration test, rats were orally exposed to 0.05, 0.5 or 5.0 mg/kg bw/d DPA per day; type and duration of exposure were not given (Korolev et al., 1976). A negative result was obtained; however, due to poor description of the study and results and methodological insufficiencies (e.g., no positive control) this finding is of low reliability.

A screening for induction of sister chromatid exchanges (SCE) in mice was negative (Gorecka-Turska et al., 1983). In this assay bone marrow cells were analysed after intraperitoneal administration of 1 to 100 mg/kg bw/d DPA.

Negative results up to lethal oral gavage doses from an mouse micronucleus assay were reported by EPA (1998) and JMPR (1998) (original publication (Murli 1992) was not available). ICR mice were treated by single oral gavage of 250, 500 or 1000 mg/kg bw (males) or 375, 750 or 1500 mg/kg bw (females) diphenylamine (99.9%) and frequencies of micronucleated polychromatic erythrocytes in bone marrow cells were investigated 24, 48 or 72 hours after treatment.

In a 90-day multiple endpoint study with dermal application of DPA in doses of 500 and 2000 mg/kg bw/d to 5 male and 5 female Sprague Dawley rats (more details are provided in chapter 4.1.2.6.) no increases in micronucleated bone marrow erythrocytes were observed compared to untreated controls (Mobil Oil Corp., 1987). No changes in the ratio of polychromatic to normochromatic erythrocytes were found. Due to methodological insufficiencies (no positive control) and since no indications were given that the test compound reached the bone marrow cells this finding is of low reliability.

Host-mediated assay

In an intraperitoneal host-mediated assay with mice as host and Salmonella typhimurium TA 1950 as indicator organism (cultivated in the peritoneum of the hosts), a negative finding was obtained after oral doses ranging from 1.45 to 2.90 µmoles/kg (equivalent to 0.245 to 0.490 mg/kg bw/d). Also after co-administration of equimolar amounts of sodium nitrite, no mutagenicity was observed in the bacteria. Quantitative data on results were not given (Braun et al., 1977).

Conclusion

DPA was negative in two Salmonella gene mutation tests. Further studies indicate that DPA is not or only marginally genotoxic to mammalian cells in vitro.

Negative results from an in vivo micronucleus test indicate that no mutagenic effects are expressed in vivo. In conclusion the whole amount of data indicates that diphenylamine may not be mutagenic in humans.

4.1.2.8 Carcinogenicity

Animal studies

Oral

Gavage

Rats

20 rats per group (Sprague Dawley) received a single per os dose of 300 mg/kg bw/d diphenylamine. After 30 days and 6 months the animals were sacrificed and autopsied. There were no indications of neoplastic effects in the major organs (mammary tissues, intestinal tract, pituitary) and the mortality before end of the experiment was 2/20 in each group (Griswold et al. 1966).

Mice

NMRI outbred Albino mice, 8 weeks old, were administered with 300 mg/kg bw/d diphenylamine in soy bean oil by gavage once a week during 18 months (78 times). A positive control group receiving dimethynitrosamine (DMNA) (15 mg/kg bw/d) was equally investigated. The diphenylamine treatment group consisted of 125 mice and the control group of 30 animals. Sacrifices were at 25 and 52 weeks. Total observation time lasted 126 weeks. There were no changes in the frequency of tumors as compared to the vehicle treated controls. In the diphenylamine group 22.9% of the animals developed tumors. The most common tumor type were lymphomas (8.3%) and alveolar adenomas (16.5). The tumor incidence in the vehicle control group were 22.2% with 11.1% lymphomas and 11.1% alveolar adenomas. As a non neoplastic alteration an increased frequency of lymphohistiocytic nephritis occurred in diphenylamine treated animals. The results on tumor morphology and incidence did not reveal a diphenylamine related change of tumors compared to vehicle treated controls (Holmberg 1983).

Dogs

Four beagles of each sex received diphenylamine (purity > 99%) by gelatin capsule at a dose of 0, 10, 25, or 100 mg/kg bw per day for 52 weeks and were observed for clinical signs, body weight, food consumption, ophthalmological, haematological, clinical chaemical, and urinary end-points, organ weights, and gross and histopathological appearance. No treatment-related clinical signs were seen at termination. One dog at the intermediate dose and two at the high dose had greenish hair. There were no deaths or treatment-related effects on body weight, food consumption, or ophthalmological parameters. Haematological examination revealed decreased mean erythrocyte counts (by 11% in comparison with controls), haemoglobin (9.3%), and haematocrit (8.7%) in males at the high dose; smaller decreases in these parameters were found in females. The platelet count increased with dose in males at the 13-, 26-, 39-, and 52-week evaluation periods, becoming statistically significant at the intermediate and high doses. There was a dose-related increase in mean total bilirubin concentration, which was statistically significant for animals at the the intermediate and high doses throughout the study, in animals at the low dose at week 26, and in females only at week 39. The mean cholesterol concentration appeared to increase with dose at all evaluation periods but was statistically significantly increased only in males at the high dose at week 13

(by 68%) and in females at the high dose at week 39 (by 37%). The blood urea nitrogen concentration was decreased in females at the intermediate (by 16%) and high doses (by 20%) at week 52. The mean absolute and relative weights of the liver and thyroid appeared to increase with dose in males, but only the mean absolute liver weight of males at the high dose was statistically significantly increased. The mean absolute and relative weights of the thyroid decreased with dose in females but did not reached statistical significance at any dose. There were no treatment-related gross or histopathological changes. The NOAEL for toxicity was 10 mg/kg bw per day on the basis of small clinical chemical changes (Botta, 1994c).

Diet

Rats

Two-year feeding of 0.5 and 1.0% diphenylamine to male and female Albino rats (20 males and 20 females per group) did not induce test compound related neoplastic alterations. It was concluded by the investigators that the incidence of observed tumors (not further specified) was due to senility of the rats at autopsy and not related to treatment (Thomas et al. 1967a).

Groups of 60 male and 60 female Sprague-Dawley rats received diets containing technical-grade diphenylamine (purity > 99%) at concentrations of 0, 200, 750, 3750, or 7500 ppm for males and 0, 150, 500, 2500, or 5000 ppm for females for up to two years, equal to 0, 8.1, 29, 150, and 300 mg/kg bw per day for males and 0, 7.5, 25, 140, and 290 mg/kg bw per day for females. Groups of 10 rats of each sex per group were killed at a one-year interim sacrifice. No treatment-related effects on mortality rates were observed; however, the study was terminated at 102 weeks because of increased mortality rates in controls and animals at the low dose: survival among males was 22% at 0 and 200 ppm and 55% at 7500 ppm. Survival thus seemed to increase with dose, and an analysis of the survival data indicated a statistically significant negative trend for mortality as the dose increased in animals of each sex; the mortality rates at the two higher doses were statistically significantly lower than those for the control group. A dose-related decrease in body weight and body-weight gain was seen throughout most of the study, which reached statistical significance at the two higher doses. The body-weight gains of males at the two higher doses were depressed to 95 and 87% of the control values at 78 weeks and equal to those of controls at 102 weeks; in females, the corresponding values were 78 and 56% of controls at 78 weeks and 80 and 61% at 102 weeks. There was no decrease in food consumption, except during the first week; at other times, food consumption appeared to have increased, possibly due to food wastage. There were no treatment related effects on ophthalmological parameters.

Haematological examination revealed dose-related decreases in erythrocyte counts, haemoglobin, and haematocrit in animals at the two higher doses throughout treatment. The decreases reached statistical significance for erythrocyte count and haemoglobin in males at 3750 and 7500 ppm in week 26 and at termination and for erythrocyte counts, haemoglobin, and haematocrit in females at 2500 and 5000 ppm through most of the treatment and at termination. Although the erythrocyte counts, haemoglobin, and haematocrit were decreased in males at 750 ppm and in females at 500 ppm, the decreases reached statistical significance only sporadically during treatment. The mean corpuscular volume and mean corpuscular haemoglobin content were significantly different from those of controls in males at the three higher doses and in females at the two higher doses.

Dose-related, statistically significant increases in the absolute and relative weights of the spleen were seen in females at the two higher doses at interim sacrifice and at termination. A similar effect on spleen weights was observed in males, except that the changes in rats at 3750 ppm were not statistically significant. The relative weight of the liver was statistically significantly increased in females at 5000 ppm at termination and at 3750 and 5000 ppm at interim sacrifice; no increase in liver weights was observed in males. The increases in spleen and liver weights are consistent with the haematological effects of the compound. Findings of dark and/or enlarged spleens at necropsy in males at > 750 ppm and in females at 500 ppm are probably related to the haematological effects. Microscopic examination revealed treatment-related effects in the kidney, liver, spleen, and bone marrow. The incidence of pigment deposition in the kidney increased in a dose-related fashion and reached 44/50 in males and 44/52 in females at the high dose that were found dead or moribund or were sacrificed at termination. The incidences of haematopoiesis and pigment deposition in the liver increased in a dose-related fashion and reached 21/50 and 27/50 in males and 41/52 and 45/52 in females at the high dose that were found dead or moribund or sacrificed at termination. Erythroid hyperplasia was seen at the higher dose but not in controls or at the low dose. The incidence of congestion of the spleen increased in a dose-related fashion and reached incidences of 50/50 in males and 47/52 in females at the high dose that were found dead or moribund or sacrificed at termination. These findings are all related to the observed haematological effects. No treatment-related increase in tumour incidence was observed. The NOAEL for toxicity was 150-200 ppm, equal to 7.5 mg/kg bw per day, on the basis of changes in haematological parameters and in the histopathological appearance of the spleen, kidney, and liver (Botta, 1994b).

Mice

Groups of 60 CD-1 mice of each sex received diets containing technical-grade diphenylamine (purity > 99%) at concentrations of 0, 520, 2600, or 5200 ppm for up to 78 weeks, equal to 0, 73, 370, and 760 mg/kg bw per day for males and 0, 90, 460, and 940 mg/kg bw per day for females. Ten mice of each sex per dose were sacrificed at 52 weeks. The incidence of penile prolapse increased with dose, affecting seven males at 2625 ppm and 17 at 5250 ppm by 78 weeks. The frequency of unkempt appearance also increased with dose, with a higher incidence among males. The mortality rate increased with dose, becoming statistically significantly different from controls for males at 2625 and 5250 ppm by 52 weeks. The deaths were attributed mainly to cystitis among males and amyloidosis in females. The mean body-weight gains were 87, 86, and 91% of control values for males at 5250 ppm and 104, 93, and 93% of control values for females at 5250 ppm at 13, 52, and 78 weeks, respectively. The body-weight gains of males at 5250 ppm and occasionally animals at 2625 ppm were statistically significantly decreased throughout the study (mainly through week 58). The body-weight gain of females at 5250 ppm was significantly decreased during the first three weeks and then occasionally for the remainder of the study. Mean food consumption was statistically significantly decreased during the first week of treatment in males at the high dose and remained increased throughout treatment for males at the two higher doses, with occasional statistically significant difference from controls.

At the interim haematological evaluation at 52 weeks, males showed dose-related decreases in haematocrit and erythrocyte counts that reached statistical significance at 2625 and 5250 ppm; and mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular

haemoglobin content increased with dose and reached statistical significance in males at these doses. Females showed dose-related decreases in haematocrit that reached statistical significance at > 525 ppm; their erythrocyte counts decreased in a dose-related fashion and reached statistical significance at 2625 and 5250 ppm. Mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin content increased with dose, the latter two reaching statistical significance at 2625 and 5250 ppm and the mean corpuscular volume at 5250 ppm. At termination at 78 weeks, males and females showed dose-related decreases in haematocrit and erythrocyte counts that reached statistical significance at 2625 and 5250 ppm; reticulocyte counts, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin content increased with dose and reached statistical significance in males at these doses.

At the interim necropsy, darkened spleens were seen at 2625 ppm and at 5250 ppm. Darkened livers and pale kidneys were seen at 5250 ppm. At terminal necropsy, darkened and enlarged spleens were seen and darkened livers were seen at 2625 ppm and at 5250 ppm. Dose-related increases in the absolute and relative weights of the spleen, liver, and heart were seen in animals of each sex at the interim and final sacrifices. At interim sacrifice, the absolute weights of the spleen and liver of males at 2625 and 5250 ppm were statistically significantly different from those of controls, while the increases in the relative liver weights reached statistical significance only at the highest dose. In females, the absolute and relative weights of the spleen were statistically significantly increased only at the highest dose. The absolute and relative weights of the heart were statistically significantly increased in females at 5250 ppm; the increases in males were not statistically different from those in controls. At final sacrifice, the absolute and relative weights of the spleen and liver of males at 2625 and 5250 ppm were statistically significantly increased. In females, the absolute and relative weights of the spleen were significantly increased only at 5250 ppm,; the differences in relative liver weight reached statistical significance at 2625 and 5250 ppm. The absolute and relative weights of the heart were statistically significantly increased in animals of each sex at 5250 ppm.

Histopathological examination revealed treatment-related effects in the kidney, liver, spleen, bone marrow, urinary bladder, and penis. The incidences of haematopoiesis and pigment deposition in the liver were increased at the intermediate and high doses. Of mice at 2625 ppm that were found dead or moribund or were sacrificed at termination, 19/51 males and 24/51 females showed liver haematopoiesis and 15/51 males and 37/51 females showed liver pigmentation; higher incidences of these effects were seen at the high dose. The incidences of spleen congestion and haemosiderosis were increased in all treated mice. Of the mice at the low dose found dead or moribund or sacrificed at termination, 11/50 males and 8/50 females showed spleen congestion and 8/50 males and 35/50 females showed spleen haemosiderosis; highe incidences of these effects were seen at the higher doses. The frequency of pigment deposition was increased in males at the high dose and in females at the intermediate and high doses. Among females found dead or moribund or sacrificed at termination, pigment was found in 5/51 at the intermediate dose and 7/51 at the high dose. Among males found dead or moribund or sacrificed at termination, 5/51 at the intermediate dose and 7/51 at the high dose showed pigment accumulation; additionally, 5/54 males had pyelonephritis. Although the incidence of spleen haematopoiesis did not increase with dose, the severity scores increased from minimal or slight in controls to mixed scores including moderate or marked at the high dose. The severity of haematopoiesis in the spleen increased from minimal to slight at 525 ppm in contrast to the largely minimal level in controls. Additionally, bone-marrow cellularity changed from predominantly moderate in controls to marked at the higher doses.

The incidences of urinary bladder cystitis and dilatation increased with dose, reaching statistical significance at the intermediate and high doses. Among mice at the intermediate dose that were found dead or moribund or were sacrificed at termination, 24/51 males and 13/48 females had cystitis and 18/51 males and 13/48 females had dilatation; higher values were seen at the high dose. The incidence of balanoposthitis apparently increased with dose; however, no statistical analysis was available. In females at 5250 ppm, the incidence of amyloidosis in the thyroid, adrenals, kidneys, stomach, small intestine, ovaries, and uterus was increased. The incidence of tumours was comparable in the treated and control groups. The NOAEL for toxicity was 525 ppm, equal to 73 mg/kg bw per day, on the basis of decreased body-weight gain, decreased survival, and significant haematological and gross and microscopic pathological alterations (Botta, 1994a).

Charles River CD-1 Albino mice of both sexes (150 per group) were fed diets containing 0, 0.005, 0.01, and 0.025% diphenylamine over a time period of 92 weeks. No effects of diphenylamine exposure were noted on the nature and incidence of histopathological changes in particular the times of appearance and incidence of tumors (Ford et al. 1972, Abstract).

Dogs

In these studies Beagle dogs were fed diets containing 0.01, 0.1, and 1% diphenylamine over a time period of 24 months. The group size consisted of 2 dogs per sex. At all dose levels no neoplastic alterations were observed and all animals survived until the termination of the study (DeEds 1963; Thomas 1967b).

Short term in vivo and in vitro studies

After a single intra gastrig (gavage) application of 400 mg/kg bw/d diphenylamine male Wistar rats did not show an enhanced mitotic activity of adrenocortical cells measured 36 hours later. Compared with carcinogenic compounds such as aflatoxin these results these results were considered negative in this rapid screening test (Danz and Urban 1979).

The same authors reported a similar experiment on male Sprague Dawley rats, where 500 mg/kg bw/d diphenylamine as a single dose (gavage) did not alter the mitotic index of adrenicortical cells after 36 hours (Danz and Urban 1980).

A host-mediated in vivo-in vitro assay for chemical carcinogenesis was performed as follows: Pregnant Syrian golden hamsters were injected i.p. with 0.5 to 2.0 mg/100g bw diphenylamine at the 10th to 11th day of gestation. At the 13th day of gestation, 48 to 72 hours after injection of the mothers, fetuses were excised. A portion of the fetal cells were investigated for transformation and cytogentic alterations and some cells were injected into compatible hosts (irradiated hamsters of the same strain) and scored for tumor development. Diphenylamine treatment did not induce any positive results under these experimental conditions. In fact in the context of the experimental setting of the cited paper, diphenylamine served as a negative control substance (DiPaolo et al. 1973).

In an in vitro transformation assay normal rat kidney cells were exposed to various dilutions of diphenylamine (dose range from 2.5 - 20.0 µg/ml medium) with or without prior metabolic activation. After 24 hours the test system was infected with murine sarcoma virus (MS; oncorna derived). The enhancement of the transformation frequency as compared to control

cultures receiving virus alone was measured. Without prior metabolic activation, diphenylamine caused a modest depression in frequency of viral transformations, ranging from 0.8 of the control value at 2.5 µg/ml to 0.4 of the control at 20 µg/ml. Prior metabolic activation by rat liver S9 fractions resulted in an insignificant 2.5-fold enhancement in frequency of viral transformation (Wilson and Khoobyarian 1982).

In a review paper on cell transformation by chemical agents the following experiment on diphenylamine is reported. Primary Syrian hamster embryo cells were treated with dilutions of diphenylamine (concentration range: 6 - 100 µg/ml) for 2 to 18 hours. Following treatment the cells were inoculated with Simian adeno virus SA7 and incubated for 3 hours at 37 C. The SA7-induced transformation rate of cellular DNA in Syrian hamster embryo cells was found to be enhanced by prior exposure to diphenylamine starting at a concentration of 25 µg/ml (Casto 1983, cited in Heidelberger et al. 1983).

Human data:

not available

Conclusion:

Although there are almost no guideline conform long term bioassays on a potential carcinogenic action of diphenylamine available, quite a number of older investigations using several strains of rats and mice and even dogs do not report any diphenylamine related neoplastic alterations. Survival rate and toxicity did not interfere with the interpretation concerning the endpoint tumor development. Since in these studies non neoplastic toxic effects have been clearly detected as being related to diphenylamine treatment, it could be suggested that signs of neoplastic proliferative activity would have become evident under these experimental conditions. In addition the majority of short term in vivo and in vitro tests equally do not show evidence for transforming activity of diphenylamine. The only exception is mentioned in a review by Costa et al. 1983, where at concentrations of 25 µg/ml the transformation rate of SA7 virus infected hamster embryo cells was enhanced. But as there are other transformation tests which do not show the same effect of diphenylamine, in fact in some experiments an inhibition occurred on diphenylamine treated cells, and in view of the negative genotoxic activity of diphenylamine, the overall results do not show evidence for carcinogenicity. In a JMPR document from 1998 containing a complete report on DPA based on recent guideline conform long term investigations in mice and rats (Botta et al. 1994a, 1994b) no evidence for increased tumor incidence was found. In an one year study in beagle dogs with bolus application by the same author (Botta 1994c) equally no neoplastic alterations could be found.

4.1.2.9 Toxicity for reproduction

Human data:

No data available.

Animal data:

Fertility impairment

Guideline-according generation studies are presently not available.

In an unpublished two-generation reproductive toxicity study by Rodwell, 1993, Sprague-Dawley rats (28 per sex/group) received diphenylamine (99.8%) in the diet at dose levels of 0, 500, 1500, or 5000 ppm (0, 40, 115, or 399 mg/kg bw/d for F_0 males and 0, 46, 131, or 448 mg/kg bw/d for F_0 females, respectively, during premating). Compound- related systemic toxicity was observed in a dose related manner among both sexes and generations at all dose levels. In general, females were more affected than males and F_1 animals were more affected than F_0 animals. Clinical signs (bluish colored fluid in the cage and bluish colored staining of the coat in both sexes, and swelling of mammary gland(s) or palpable lateral-ventral masses, primarily in females) were evident at 5000 ppm. Body weight was decreased at 1500 and 5000 ppm. At 5000 ppm, there was a 6-9% decrease in body weight values, as compared to control, for F_0 males, 5-8% for F_0 females, 22-28% for F_1 males, and 11-23% for F_1 females. At 1500 ppm, there was a 5-8% decrease in body weight values from controls for F0 females, 7-9% for F_1 males, and 5% for F_1 females. Food consumption (g/animal/day) was also decreased at 1500 and 5000 ppm. Kidney, spleen, and liver appeared to be the target organs as evidenced by weight differences from control at 5000 ppm in males and at 1500 and 5000 ppm in females and gross and microscopic findings at all dose levels in both sexes. Gross findings included enlarged and blackish-purple spleens. Microscopic findings included brown pigment in the proximal convoluted tubules of the kidney, hepatocytic hypertrophy, brown pigments in the Kupffer cells of the liver, congestion and hemosiderosis of the spleen. The systemic toxicity NOAEL was less than 500 ppm (40 mg/kg bw/d in males and 46 mg/kg bw/d in females). The LOAEL was less than or equal to 500 ppm based on gross pathological findings in the spleen (enlarged, discolored), and on microscopic findings in the kidney (brown pigment in the proximal convoluted tubule), liver (hepatic hypertrophy and brown pigment in the Kupffer cells), and spleen (congestion and hemosiderosis).

Developmental toxicity was observed at 1500 and 5000 ppm, as evidenced by significantly decreased body weight for F_1 pups at 5000 ppm throughout lactation (11-25 % less than control), for F_2 pups at 5000 ppm from LD 4 through 12 LD 21 (10%-29% less than control), and for F pups at 1500 ppm on LD 14 (10%) and LD 21 (12%). The developmental toxicity NOAEL was 500 ppm (46 mg/kg bw/d for maternal animals) and the LOAEL was 1500 ppm (131 mg/kg bw/d for maternal animals) based on decreased F2 pup body weight in late lactation. Reproductive toxicity was noted as smaller litter sizes at birth (significant for the F_2 litters) in both generations at 5000 ppm. The reproductive toxicity NOAEL was 1500 ppm (131 mg/kg bw/d for maternal animals) and the LOAEL was 5000 ppm (448 mg/kg bw/d for maternal animals), based upon decreased litter size in both generations (cited from EPA RED report, 1998 and JMPR report, 1998; original publication not available).

Within a two year feeding study on chronic toxicity (c.f. 4.1.2.6) with Albino rats (Slonaker-Addis strain), additional satellite groups of animals were investigated for effects of diphenylamine on reproductive performance (Thomas et al. 1967a; DeEds et al. 1963). It is reported that groups of 12 females and 3 males each were fed commercial laboratory chow

containing diphenylamine (minimum purity by cryoscopy: 99.9%) at concentrations of 0.0, 0.1, 0.25, and 0.50% (according to an intake of approximately 50, 125, and 250 mg/kg bw/d). The animals were assigned to these diets when 5 weeks old and mated over a period of three weeks (4 females and one male) at the age of 100 days. After all litters from the first mating had been weaned, rats were remated. In addition to this, offspring from the first mating was mated once to yield a second generation. During this exercise there were no data collected on parental/maternal conditions as for example on clinical signs, weight gain and/or on food consumption. Only few parameters were evaluated (number of litters at birth, mean litter size at birth, number of litters at weaning, mean litter size at weaning, mean offspring weight at weaning on p.n. day 21) from which results were provided in the report. From these data no effects were revealed in the F0 generation (first and the second mating) and the F1 generation for the numbers of litters born and for postnatal mortality of the offspring in all dose groups in comparison to their concurrent controls. The overall and most consistent findings were indications for reduced litter sizes in the offspring of the treated animals in comparison to the controls (F0 first mating: 6.3 pups/litter versus 8.3 pups/litter, $p<0.05$ / F0 second mating: 6.6 pups/litter versus 9.6 pups/litter, $p<0.01$ / F1 generation: 7.0 pups/litter versus 8.6 pups/litter) and for reduced body weight gain of the offspring during lactation at dietary levels of 0.5% diphenylamine. Mean pup weights of weanlings of the first mating were lower in the high dose group than in those of controls (2 and 2.5 grams for females and males). Mean pup weights of weanlings of the high dose group of the second mating were not significantly reduced compared to those of controls but were lower than those of the lower dose groups (8 and 9 grams in femals and males). Mean pup weights of the second generation were statistically significantly reduced by 0.5% diphenylamine ($p<0.01$) From the main study there are some suggestions that the observed offspring body weight effects may be related to inadequate food intake of their dams during gestation and lactation, since lower food intake and subsequently reduced body weight gain was reported from the main study for nonpregnant females at dietary levels of 0.5 and 1.0% diphenylamine (c.f. 4.1.2.6). Although this study provides only limited information and does not meet the requirements of a standard 2 generation study, for the purpose of risk characterization a NOAEL of 0.25% (according to an intake of approximately 125 mg/kg bw/d) may be derived based on the findings of smaller litters and reduced postnatal growth observed at the highest tested dietary level.

In a guideline conform oral 28 day study (c.f. 4.1.2.6) with F344 rats (Yoshida et al., 1989) organ weights of gonads had been determined and in addition to gonads also prostates, uteri and vaginae had been processed for histopathological evaluation. At the highest tested dose level of 1000 mg/kg bw/d, for which anemia and body weight gain inhibition was observed among animals of both sexes, absolute right and left testes weights were slightly but statistically significantly decreased (r: $p<0.05$; l: $p<0.01$) in comparison to the controls. Relative left but not right testes weights were slightly but statistically significantly ($p<0.01$) increased in comparison to the controls. No effects on absolute/relative testes weights were revealed for daily doses of 333 and 111 mg/kg bw/d. Weights of female gonads were not reported. Histopathological evaluation did not give evidence for any treatment-related effects.

Developmental toxicity:

Data from guideline-according developmental toxicity studies are presently not available.

In an unpublished teratology study by Rodwell, 1992, pregnant female Sprague-Dawley rats (25/group) received diphenylamine (99.9%) in corn oil by oral gavage at dose levels of 0, 10, 50, or 100 mg/kg bw/d from gestation day six through gestation day 15 inclusive; dams were sacrificed on gestation day 20. None of the rats died during the study. Maternal toxicity was evidenced by increased splenic weights, enlarged spleens and blackish-purple colored spleen in the dams at 100 mg/kg bw/d. The maternal toxicity NOAEL was 50 mg/kg bw/d and the LOAEL was 100 mg/kg bw/d. No developmental toxicity was seen at any dose level. The developmental toxicity NOAEL was equal to or greater than 100 mg/kg bw/d, the highest tested dose (cited form EPA RED report, 1998 and JMPR report 1998; the original study was not available).

Developmental toxicity was observed in an unpublished two-generation reproductive toxicity study in Sprague-Dawley rats by Rodwell, 1993, at 1500 and 5000 ppm, as evidenced by significantly decreased body weight for F_1 pups at 5000 ppm throughout lactation (11-25 % less than control), for F_2 pups at 5000 ppm from LD 4 through 12 LD 21 (10%-29% less than control), and for F_2 pups at 1500 ppm on LD 14 (10%) and LD 21 (12%). Details of the study are reported in the fertility impairment section. The developmental toxicity NOAEL was 500 ppm (46 mg/kg bw/d for maternal animals) and the LOAEL was 1500 ppm (131 mg/kg bw/d for maternal animals) based on decreased F2 pup body weight in late lactation (cited from EPA RED report, 1998 and JMPR report, 1998; original publication not available).

From a teratogenicity study in rabbits (Edwards et al. 1983; FAO 1984) it is reported that groups of pregnant New Zealand rabbits (16 to 18 mated females per group) were administered suspensions of purified diphenylamine (99.9% purity) via gastric intubation at dose levels of 0, 33, 100 and 300 mg/kg bw/d on gestation days 7 to 19 inclusive. Dosages were based on a preliminary study with 6 females/dose group, in which there was initial weight loss at dosages of 200 and 400 mg/kg bw/d and a greater divergence at 600 mg/kg bw/d. Vehicle controls received 1% methylcellulose. Animals were observed daily for clinical signs and mortality. Body weights were taken and food intake was measured at days 1, 7, 9, 11, 15, 20, 24, and 29 of gestation. On gestation day 29, does were sacrificed and examined for macroscopic pathological changes. Ovaries and uteri were examined immediately to determine number of corpora lutea, number and distribution of live young, number and distribution of embryonic/fetal deaths, individual fetal weights, and for macroscopic fetal abnormalities. Fetuses were examined for visceral and skeletal anomalies. Green discoloration of the urine was observed in the does of all dose groups, but in particular at the 100 and 300 mg/kg bw/d dose levels. At 300 mg/kg bw/d mean food consumption was reduced and animals showed a slight initial mean weight loss from the start of treatment. Subsequently mean weight gain remained lower than among controls. No such effects were seen at dosages of 33 and 100 mg/kg bw/d. There were no other signs of toxicity or mortality considered treatment-related. Pregnancy rates were unaffected by treatment. Macroscopic findings were unremarkable at terminal necropsy. Litter size, litter weight, pre- and post-implantation loss and mean fetal weight were not affected by diphenylamine. There were no malformations or anomalies observed which are considered compound-related. The incidence of visceral and skeletal anomalies was unaffected by treatment at doses of diphenylamine up to and including 300 mg/kg bw/d. Based on the findings of reduced food intake and reduced body weight gain at the highest dose level, a NOAEL/maternal toxicity of 100 mg/kg bw/d and a NOAEL/developmental toxicity of equal or higher than 300 mg/kg bw/d is determined for this study in the absence of developmental effects.

An investigation with diphenylamine was incorporated in a validation study of the Chernoff-Kavlock screening assay modified for the application in rats (Wickramaratne 1987) for which only few data were presented. Diphenylamine was administered at a maximum daily dose (Limit Test, OECD) of 1000 mg/kg bw/d orally to a group of 12 pregnant rats (Wistar-derived Alpk:Ak strain) during g.d. 7 to 17. Maternal observations were restricted to body weights on days 1, 7-17, and 22 of gestation. Offspring observations were restricted to litter weights of live pups on days 1 and 5 post-partum and the number of live and dead pups on these days. No specific examinations for malformations were conducted. While mean maternal weight gain in diphenylamine treated dams was obviously reduced in comparison to saline treated controls during the dosing period as well as during the whole period of pregnancy, no effects were reported for litter size and postnatal survival of the pups. Reduced weight gain in terms of lower mean % weight gain per litter during p.n. day 1-5 was assessed by the authors as an indication for *fetotoxicity*. This "result", however, has to be doubted, since mean pup weights on p.n. day 1 and 5 did not differ from those of saline treated controls.

In a further study focusing on the nephrotoxic properties of diphenylamine the in utero induction of cystic tubular renal lesions was investigated (Crocker et al. 1972, Crocker et al. 1970). Pregnant Sprague-Dawley rats were treated during the late fetal period (g.d. 14 until term) with different preparations of diphenylamine (commercial aged diphenylamine, chromatographically pure diphenylamine) and with three different impurities isolated from shelf aged commercial diphenylamine either via diet (1.5% or 2.5% commercial diphenylamine in rat chow) or by gavage as solutions in 70% alcohol (daily doses of 20 mg commercial diphenylamine or purified diphenylamine, or 50 µg of the three isolated contaminants). Maternal observations were restricted to histology of the kidneys which was unaffected in both the diet and the gavage treated groups. It was reported that in both of these treatment groups the number of offspring per litter varied from 3 to 17 and that there was a high degree of cannibalism of the newborns by the mothers of some litters. No renal lesions were induced in the offspring after treatment with chromatographically pure diphenylamine, whereas cystic tubular lesions were observed after treatment with commercial diphenylamine as well as with one of the impurities (later identified as N,N,N´-triphenyl-p-phenylenediamine), indicating the latter to represent the active nephrotoxic compound in commercial aged diphenylamine.

In a further feeding study also focusing on cystic renal lesions (Philbert et al. 1978) 20 Wistar rats and 20 Hartley guinea pigs (including 2 animals/species/per dose group pregnant at g.d. 2-3) were given diets containing 2 or 4% diphenylamine of technical grade, which in the pregnant animals resulted in 100% abortions during the first week of treatment. The results of this study are not considered for the evaluation of developmental toxicity since under the conditions of this study diphenylamine treatment revealed to be acutely toxic with more than 50% of all animals of the dosed groups dying during the first few weeks on diet.

Summary and conclusion:

There are no human data available on reproductive toxicity of diphenylamine. Data from investigations in laboratory animals are limited to studies with the oral route of administration. From the available data obtained from studies with rats it appears that impairment of reproductive capability and capacity is unlikely to occur from treatment with diphenylamine at dosages that do not interfere with food intake and body weight gain of the parental animals. From the available data obtained from two developmental studies in two

species (rats and rabbits) any specific embryo-/fetotoxic or teratogenic potential is not indicated even at maternally toxic dosages. In a two generation study cited by the EPA, 1998, no teratogenic effects were observed up to maternal oral doses of 448 mg/kg bw/ d. A NOAEL for developmental toxicity of 46 mg/kg bw/d was deduced based on a growth retardation in the F2 generation during late lactation at doses of 131 and 448 mg/kg bw/d. Maternal toxicity was observed at this doses with respect to reduced body weight, decrease in food consumption and pathological findings in spleen, kidney and liver. With respect to any nephrotoxic properties of diphenylamine developmental studies performed with rats did not reveal the induction of any renal lesions in the offspring. From the available animal data a NOAEL for fertility of 131 mg/kg bw/d, a NOAEL for developmental toxicity of 46 mg/kg bw/d and a LOAEL for developmental toxicity of 131 mg/kg bw/d is recommended to be used for risk characterisation purposes.

4.1.3 Risk characterisation

4.1.3.1 General aspects

Orally administered diphenylamine is well absorbed from the gastrointestinal tract in man and in several animal species including rat, rabbit, dog and cow. Up to 3 % of the parent compound and approximately 80-90 % of the dose is excreted as 12 different metabolites, which include 4-hydroxydiphenylamine, 4,4'- 2 hydroxydiphenylamine and sulfate and glucuronide conjugates of these hydroxylated metabolites. In addition, indophenol has been identified as metabolite. N-hydroxylated metabolites responsible for methaemoglobinemia in aromatic amines could not be detemined. From these results it can be assumed that diphenylamine is readily metabolized and excreted and that accumulation seems to be unlikely. There are no data on dermal route of administration or exposure by inhalation. An absorption of 100% for the oral route is proposed to be taken for risk characterisation purposes, whereas dermal and inhalation absorption is assumed to be 100% (defaults). The assumption of a default dermal absorption value of 100% is supported by the physicochemical properties of DPA (molecular weight: 169 g/mol; log Pow 3.4; water solubility: 40 mg/l). Due to the potential for absorption and the lack of experimental data, a default absorption value of 100% is also assumed for inhalative uptake.

Human data on the acute toxicity of diphenylamine are not available. An oral LD50 value of approximately 600 mg/kg bw/d was detected for male Syrian hamsters. Oral LD50 values exceeding 800 mg/kg bw/d were determined for rats and male Mongolian gerbils. Dermal LD50 values of >2000 mg/kg bw/d are reported for rabbits and of >5000 mg/kg bw/d for rats. Data on acute inhalation toxicity are not available.

Human data on the local irritant or corrosive properties of diphenylamine are not available. The substance caused no or only very slight skin irritation in tests with rabbits. Data of eye irritating properties of the substance are conflicting and poorly documented, but it can be assumed that diphenylamine may pose a risk of serious damage to eyes.

Diphenylamine did not produce dermal sensitization in guinea pigs. There is one case of one woman where a contact allergy could be demonstrated. Other studies with 11 or 1012 patients did not demonstrate a skin sensitization that could be attributed to diphenylamine. Cross sensitization to p-phenylenediamine has not been demonstrated in the woman who reacted positive to diphenylamine. In a maximization test carried out on 30 volunteers no sensitization reactions were produced. These data demonstrate that in humans the substance has a weak or no skin sensitising potential. Diphenylamine is not suspected to be a potent respiratory sensitiser.

From subchronic studies of animals fed with a diet containing diphenylamine the most sensitive indication for toxicity seem to be haematological effects such as a slight anemia and formation of Heinz bodies. Heinz bodies are considered to be indicative for methaemoglobin formation. At higher doses diphenylamine cause kidney changes generally subscribed as polycystic kidney disease, accompanied with different stages of papillary necrosis and nephritis in different species.

The primary target organs after long-term dietary exposure of animals to diphenylamine are the hematological system and the kidneys, spleen, and liver. Comparing the LOAELs from the different studies it becomes obvious that adverse effects in rats and dogs occurred at the same doses of about 25 mg/kg bw/d. Mice seem to be less sensitive according to the results from the new short- and long-term studies.

Taking together the data from all animal studies with repeated oral application, the value of 7.5 mg/kg bw/d was proposed as NOAEL for adverse effects after chronic exposure from the two-year carcinogenicity study on rats (Botta, 1994b). This NOAEL is based on haematological and histological effects at dietary levels equal or greater than 25 mg/kg bw/d in female rats (LOAEL). This study was the basis for establishing the actual ADI of 0-0.08 mg/kg bw/d by the JMPR (1998).

The short report (abstract) of a study on formation of Heinz bodies in mice after a feeding period of 12 weeks at 7.5 mg/kg bw/d diphenylamine will not be taken forward for risk characterisation purposes on repeated dose toxicity.

A dermal study in rabbits lasting 21 days revealed dark-red foci in the stomachs of rabbits of each sex at the doses of 500 and 1000 mg/kg bw/d. A NOAEL of 100 mg/kg bw/d can be derived from this study. After dermal application of DPA to rats over a period of 90 days the NOAEL for systemic toxicity is 500 mg/kg bw/d based on an increase in the relative kidney weight of males at 2000 mg/kg bw/d. All treated animals exhibited dermal hyperplasia at the application side. Thus, the LOAEL for skin effects from this study is 500 mg/kg bw/d, whereas no NOAEL could be derived. It is proposed to base risk characterisation for dermal exposure (systemic effects) on the NOAEL of 500 mg/kg bw/d from the 90-day study on rats.

DPA was negative in two Salmonella gene mutation tests. Further studies indicate that DPA is not or only marginally genotoxic to mammalian cells in vitro. Negative results from an in vivo micronucleus test indicate that no mutagenic effects are expressed in vivo. In conclusion the whole amount of data indicates that diphenylamine may not be mutagenic in humans.

In a JMPR document (1998) containing a complete report on DPA based on recent guideline conform long-term investigations on mice and rats no evidence for increased tumor incidences was found. In a one year study in beagle dogs with bolus application neoplastic alterations could be not found, too. A number of older investigations using several strains of rats and mice and even dogs do not report any diphenylamine related neoplastic alterations. Survival rate and toxicity did not interfere with the interpretation concerning the endpoint tumor development. Since in these studies non neoplastic toxic effects have been clearly detected as being related to diphenylamine treatment, it could be suggested that signs of neoplastic proliferative activity would have become evident under these experimental conditions. In addition the majority of shortterm in vivo and in vitro tests equally do not show evidence for transforming activity of diphenylamine. The overall results support the assumption that there was no indication on carcinogenic effects to diphenylamine.

There are no human data available on reproductive toxicity of diphenylamine. Data from investigations in laboratory animals are limited to studies with the oral route of administration. From the available data obtained from studies with rats it appears that impairment of reproductive capability and capacity is unlikely to occur from treatment with diphenylamine at dosages up to 131 mg/kg bw/d that do not interfere with food intake and body weight gain of the parental animals. From the available data obtained from two

developmental studies in two species (rats and rabbits) any specific embryo-/fetotoxic or teratogenic potential is not indicated even at maternally toxic dosages. In a two generation study cited by the EPA, 1998, no teratogenic effects were observed up to maternal oral doses of 448 mg/kg bw/ d. An NOAEL/developmental toxicity of 46 mg/kg bw/d was deduced based on a growth retardation in the F2 generation during late lactation at doses of 131 and 448 mg/kg bw/d. Maternal toxicity was observed at these doses with respect to reduced body weight, decrease in food consumption and pathological findings in spleen, kidney and liver. With respect to any nephrotoxic properties of diphenylamine developmental studies performed with rats did not reveal the induction of any renal lesions in the offspring. From the available animal data a NOAEL/fertility of 131 mg/kg bw/d, a NOAEL/developmental toxicity of 46 mg/kg bw/d and a LOAEL/developmental toxicity of 131 mg/kg bw/d is recommended for use for risk characterisation purposes.

4.1.3.2 Workers

4.1.3.2.1 Introductory remarks

Diphenylamine is a colourless solid with the very low vapour pressure of 0.00033 hPa at room temperature. For occupational risk assessment the MOS approach as outlined in the revised Technical Guidance Document is applied. This occupational risk assessment is based upon the toxicological profile of diphenylamine (chapter 4.1.2) and the results of the occupational exposure assessment (chapter 4.1.1.2). The threshold levels identified in the hazard assessment are taken forward to this occupational risk assessment.

This introductory remark specifies the route-specific information on diphenylamine absorption and compares the route-specific diphenylamine results of experimental animal testing in order to describe the relative toxic potency for the oral and dermal route of exposure. In addition, a short introduction to the MOS approach is given.

Absorption and bioavailability for different routes of exposure

For the majority of toxicological endpoints diphenylamine data originate from oral studies. Since workers are predominantly exposed either by inhalation or by skin contact, route to route transformation is an essential step in the occupational risk assessment.

Orally administered diphenylamine is well absorbed in man, rat, rabbit, dog and in cow. Based on the available absorption data, an oral absorption percentage of 100% is taken forward to risk characterisation.

There are no experimental data on absorption by inhalation. As a default, air-borne concentrations of diphenylamine are assumed to be completely absorbed by the respiratory tract system (100% absorption by inhalation).

There are no specific data on dermal absorption. Based on physico-chemical properties of diphenylamine the default for dermal absorption is 100% (see hazard assessment).

In general, route-to-route extrapolation is considered to be a poor substitute for route-specific toxicity data. For diphenylamine, relevant oral and dermal toxicity data are available (see hazard assessment for repeated dose toxicity). The adjusted oral and dermal NOAELs are compared in order to describe the relative route-specific potency of diphenylamine (table 4.1.3.2.A).

Comparing the adjusted NAELs it is evident that toxic potency of diphenylamine is considerably smaller for the dermal route of administration compared to the oral route. Using the broader concept of bioavailability (instead of absorption) a dermal bioavailability of about 5% (4.2%) and a 100% oral bioavailability are used for risk characterisation. From these considerations it is evident, that relative route-specific absorption (oral/dermal) does not match the corresponding relative bioavailability. Specific reasons for these differences are not known.

Table 4.1.3.2.A: Route-specific toxic potency of diphenylamine

Route of application	Species	Duration frequency	NOAEL in mg/kg/d	Adjustment for Frequency	Adjustment for Duration	Adjusted NAEL (2-year, 5d/w)	Bioavailability
Oral	rat	2-year 7d/w	7.5	7/5	-	10.5	100%
Dermal	rat	90-day 5d/w	500	-	1/2	250	4.2%

Occupational exposure and internal body burden

In table 4.1.3.2.B. the exposure levels of table 4.1.1.2.2 are summarised and the route specific and total internal body burdens are identified. Risk assessment for combined exposure requires the calculation of a total internal body burden; to this end the derived route-specific percentages of bioavailability are used (100% for inhalation exposure and 4.2% for dermal exposure).

Table 4.1.3.2.B: Occupational exposure levels and internal body burden (diphenylamine)

Exposure scenario	Inhalation	Dermal contact		Internal body burden		
				Inhalation(1)	Dermal(2)	Combined
	mg/m³	mg/p/d	mg/kg/d	mg/kg/d		
1. Production of diphenylamine and further processing	1.0	21	0.3	0.14	0.013	0.15
2. Use of lubricant, metal working fluids (with 1% diphenylamine)	0.02	126	1.8	0.003	0.076	0.08

(1) based on the assumption of 100% bioavailability by inhalation; breathing volume of 10 m³ per shift and a body weight of 70 kg
(2) based on the assumption of 4.2% dermal bioavailability and a body weight of 70 kg

MOS Approach

The MOS approach for human risk characterisation is described in detail in chapter 4 of the draft revision of the Technical Guidance Document (ECB ....). The following chapter only contains a short introduction to the MOS approach used. The basic principle of the MOS approach is a comparison of scenario-specific MOS values (the relationship between the experimental NOAEL respectively the adjusted starting point and the exposure level) with a reference MOS (product of various assessment factors).

MOS calculation and the adequate starting point

Basically, MOS values are calculated as quotient of a relevant NOAEL from experimental animal testing or human studies and actual workplace exposure levels. In specific situations, the MOS approach requires to convert the original NOAEL into an adequate starting point or corrected NOAEL previously to MOS calculation in order to be directly comparable to the exposure assessment. If the route of application in animal or human studies is different from the actual occupational exposure, the dose units of the experimental data should be converted to the dose unit of the exposure data. Additionally, possible differences in bioavailability between routes, as well as possible differences in bioavailability between animals and humans should be accounted for the calculation of the corrected NOAEL. For diphenylamine, a bioavailability of 100% is taken for the oral route and for inhalation exposure. Based on the comparison of oral and dermal experimental toxicity data, a dermal bioavailability of about 5% (4.2%) is calculated (see table 4.1.3.2.A).

For worker risk assessment, the corrected inhalatory NOAEC accounts for the difference of the standard respiratory volume (6.7 m³) and the respiratory volume for light activity (10 m³). If the experimental exposure schedule differs from actual exposure in terms of the frequency of exposure within a week (e.g. a 7-day experimental exposure versus a 5-day working week), this aspect additionally may be accounted for in the calculation of the adequate starting point (corrected NOAEL).

MOS values are calculated for different routes of exposure and for different toxicological endpoints. The routes of exposure specifically considered in worker risk assessment are inhalational exposure and dermal contact.

In addition, for risk assessment of combined exposure (inhalational exposure and dermal contact) an adequate internal NOAEL is derived from external NOAELs and specific information on route-specific absorption. For MOS calculation, the adjusted internal starting point is divided by the internal body burden. Depending on route-specific exposure and absorption, exposure by inhalation and/or dermal exposure may contribute to the internal body burden. With respect to the possible outcome of an assessment for combined risks, interest focuses on scenarios with conclusion ii at both exposure routes. Based on theoretical considerations, combined exposure will not increase the most critical route-specific risk component more than twice.

Reference MOS

The MOS values calculated have to be compared with a reference MOS. The reference MOS is an overall assessment factor, which is obtained by multiplication of individual assessment factors. The Technical Guidance Document emphasises several aspects which are involved in the extrapolation of experimental data to the human situation. For these assessment factors, default values are recommended. It is important to point out that any relevant substance-specific data and information may overrule the defined default values.

Interspecies extrapolation is split up in one subfactor for differences in basal metabolic rate (allometric scaling factor) and one factor for other interspecies differences (variability factor). For the allometric scaling factor it is assumed that equitoxic doses (when expressed in mg/kg/d) scale with body weight to the power of about 0.75. This results in specific scaling factors for different animal species. For the rat the factor is 4, for the mouse it is 7. No such default factor is indicated for inhalation data (in mg/m³) because they implicitly scale according to metabolic rate. To account for additional interspecies differences a default value of 2.5 was agreed in the TGD working group.

For workers, an adjustment factor for intraspecies differences of 5 is recommended. Based on an evaluation of empirical data by Schneider et al. (2004) it is anticipated that a factor of 5 will be sufficient to protect the major part of the worker population (about 95%).

For chemical substances it is usually expected that the experimental NOAEL will decrease with increasing duration of application. Furthermore, other and more serious adverse effects may appear with prolonged exposure duration. For duration adjustment, a default factor of 6 is proposed for extrapolation from a subacute to chronic exposure. The duration adjustment factor is lower (a factor of 2) for the transition from subchronic experimental exposure to chronic exposure.

The Technical Guidance Document defines two further adjustment factors (uncertainty in route-to-route extrapolation and dose-response relationship including severity of effect). In specific cases these factors may be different from one. For diphenylamine, no such factors are used. In this context, it is pointed out, that all considerations on diphenylamine-specific route-to-route extrapolation are covered by the proposed percentages of bioavailability.

Comparison of MOS and reference MOS

The MOS values for different toxicological endpoints and different exposure scenarios are compared with the substance- and endpoint-specific reference MOS. MOS values clearly above the reference MOS do not lead to concern, whereas MOS values that are clearly below the reference MOS are cause for concern. There may be various risk-related aspects which are not covered by default assessment factors. These additional qualitative aspects should be carefully considered when performing a risk assessment and should have adequate influence on finding of conclusions.

Critical Exposure Levels

In a parallel procedure, which gives identical but more direct results, the adjusted toxicological starting point is directly divided by the reference MOS. As a result, an exposure level (in mg/m³ or mg/kg/day) is identified, which may serve as a direct trigger for decisions when compared with the occupational exposure levels. In the context of this risk assessment report this trigger value is called "critical exposure level". Concern will be expressed for scenarios with occupational exposure levels higher than the relevant "critical exposure level".

4.1.3.2.2 Occupational risk assessment

Acute toxicity

Systemic effects

Human data on the acute toxicity of diphenylamine are not available. Animal data show an oral LD50 value of approximately 600 mg/kg for male Syrian hamsters and oral LD50 values exceeding 2,000 mg/kg for rats and male Mongolian gerbils.

For rats, a dermal LD50 of greater than 5,000 mg/kg is reported. A dermal LD50 of greater than 2,000 mg/kg is described in a study with rabbits. No clinical signs were noted in the rabbit study. Acute dermal toxicity is less pronounced than acute oral toxicity.

Data on acute inhalation toxicity are not available.

Sublethal toxicity occuring at lower doses is considered as a more rational starting point for acute toxicity than mortality data. Findings from e.g. maternal toxicity in developmental toxicity studies may provide additional relevant information for acute risk assessment. Maternal toxicity in rats seems to be more pronounced than maternal toxicity in rabbits (Rodwell 1992, Edwards et al. 1983). Pregnant rats were exposed for 10 days in a feeding study. Maternal toxicity was evidenced by enlarged spleens at 100 mg/kg/day. The corresponding NOAEL was 50 mg/kg/day. For pregnant rabbits, the corresponding NOAEL was 100 mg/kg/day. The rat data indicate, that haematotoxicity could be considered as an acute, primary effect of diphenylamine.

The use of the NOAEL of 50 mg/kg/day (pregnant rats, 10-day exposure, enlarged spleen) for acute risk assessment may be more appropriate than the use of the available mortality data.

For comparison: the NOAEL for repeated dose toxicity is established to be 7.5 mg/kg/day (see chapter 4.1.2.6).

The calculation of the internal starting point has to account for an oral absorption percentage of 100%. Thus, the oral NOAEL of 50 mg/kg/day is identical to the internal starting point.

Inhalation

The internal starting point of 50 mg/kg/day has to be converted into an inhalatory NAEC (rat, in mg/m³). Absorption (and bioavailability) by inhalation is assumed to be 100%. The internal starting point is divided by 0.384 m³/kg/day (default respiratory volume for the rat for 8 hours) and multiplied by a factor of 6.7/10 (ratio of worker respiratory volumes under standard conditions and under conditions of light activity). Correspondingly, the inhalation starting point is calculated to be 87 mg/m³ (50 x 1 x 1/0.384 x 6.7/10).

The default factor for interspecies differences (rat, inhalation) is 2.5 (the factor for allometric scaling is already implicitly applied). For intraspecies differences, the default factor of 5 is chosen. The reference MOS for inhalation is calculated to be 12.5 (2.5 x 5). The corresponding "critical exposure level" for inhalation exposure is 7 mg/m³ (87 / 12.5).

Dermal exposure

Because of the assumption of 4.2% dermal bioavailability the internal starting point of 50 mg/kg/day (equivalent to 4.2% of the dermal exposure) is converted to an adequate dermal starting point of 1190 mg/kg/day.

The default factor for interspecies differences (rat, dermal) is 4 x 2.5. For intraspecies differences, the default factor of 5 is chosen. The reference MOS for dermal contact is calculated to be 50 (4 x 2.5 x 5). The corresponding "critical exposure level" for dermal exposure is 24 mg/kg/day (1190/50).

Combined exposure

The internal starting point is 50 mg/kg/d. The reference MOS is identical to the dermal reference MOS of 50. The corresponding internal "critical exposure level" results in 1 mg/kg/day.

Table 4.1.3.2.B: MOS values for acute toxicity (spleen enlargement) of diphenylamine

	Inhalation			Dermal			Combined		
Starting point for MOS calculation	87 mg/m^3			1,190 mg/kg/d			50 mg/kg/d		
Reference MOS	12.5			50			50		
Critical exposure level	7 mg/m^3			24 mg/kg/d			1 mg/kg/d		
	Exposure (mg/m^3)	MOS	Conclusions	Exposure (mg/kg/d)	MOS	Conclusions	Internal body burden (mg/kg/d)	MOS	Conclusions
1. Production of diphenylamine and further processing	1.0	87	ii	0.3	3,967	ii	0.15	333	ii
2. Use of lubricant, metal working fluids (with 1% diphenylamine)	0.02	4,350	ii	1.8	661	ii	0.08	625	ii

For acute toxicity (spleen enlargement) the MOS approach clearly indicates no concern for both exposure scenarios.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

Irritation/Corrosivity

Dermal irritation

Human data on local irritant or corrosive properties of diphenylamine are not available. According to the results of two skin irritation tests, diphenylamine caused no or only very slight dermal irritation in rabbits. There is no concern for dermal irritation at the workplace.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

Eyes

Data on eye irritating properties of the substance are conflicting and poorly documented, but it may be assumed that diphenylamine may pose a risk of serious damage to eyes. There exist two guideline-compliant studies which both report severe eye irritation caused by diphenylamine. In one of these studies irreversibility of effects after 21 days is stated.

Conclusion ii is proposed on the grounds that control measures exist which can minimise exposure and risk of severe irritation to the eyes, thereby reducing concern. However, these

controls must be implemented and complied with to reduce the risk of severe irritation to the eyes.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

Inhalative irritation

There are no animal data available concerning respiratory tract irritation of diphenylamine. Also no case reports on respiratory irritation from situations of human exposure are noted. Dermal irritation data do not indicate that the substance may cause serious effects at the site of initial contact. A risk relevant damage of the airways by acute irritation properties is therefore not anticipated. There is no reason for concern.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

Sensitisation

Skin sensitisation

Based on experimental data (dermal sensitisation study in guinea pigs) and human evidence (see chapter 4.1.2.5) diphenylamine is not considered to be a skin sensitiser. There is no reason for concern.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

Respiratory sensitisation

No information on the sensitising potential of the substance at the respiratory tract is available. However, diphenylamine is not suspected to be a potent respiratory sensitiser in humans according to the fact that during all the years of use no notice of specific case reports has been given. There is no concern with respect of respiratory sensitisation at the workplace.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

Repeated dose toxicity

Local effects

Dermal irritation in rabbits (acute exposure) is only very slight. In a dermal 90-day rat study treated animals exhibited dermal hyperplasia at the application side. The LOAEL for this local effect is 500 mg/kg/day. There are no data available, which express the LOAEL in the unit mg/cm^2. As local effects depend on the surface area concentration of a substance, and not on the internal body burden, for a recalculation from "mg/kg/day " into "mg/cm^2" default values are used (rat body weight 0.25 kg and assuming that 10% (40 cm^2) of a total body surface area of 400 cm^2 was exposed). This gives a value of 3.1 mg/cm^2 diphenylamine (500 mg/kg/day x 0.25 kg x 1/40 cm^2). For both dermal exposure scenarios (exposure levels in the range of 0.1 – 0.15 mg/cm^2, see chapter 4.1.1.2) the margin of safety for these chronic local effects (dermal hyperplasia) is considered to be sufficiently high in order to reach a conclusion of no concern.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

Systemic effects

Diphenylamine has been extensively tested for repeated dose toxicity in experimental animals (rats, mice and dogs) via the oral route of administration. Haematotoxicity proved to be the main toxic effect of diphenylamine. The primary target organs are the haematological system and the kidney, spleen and liver.

Taking together all data from animal studies with repeated oral application, it is proposed to derive the value of 7.5 mg/kg/day as NOAEL for adverse effects after chronic exposure from the two-year carcinogenicity study on rats (Botta, 1994b). This NOAEL is based on haematological and histological effects at dietary levels equal or greater than 25 mg/kg/day in female rats (LOAEL).

Repeated dose toxicity of diphenylamine was additionally tested in a dermal 90-day rat study. The corresponding NOAEL of 500 mg/kg/day (systemic effects) is additionally used for dermal risk assessment.

The calculation of the internal starting point has to account for an oral absorption percentage of 100% and a worker-specific adjustment factor of 7/5 (experimental frequency of exposure is 7 days/week, workers are exposed 5 days/week). Thus, the oral NOAEL of 7.5 mg/kg/day is converted to an internal starting point of 10.5 mg/kg/day (7.5 x 1 x 7/5).

Systemic effects by inhalation

The internal starting point of 10.5 mg/kg/day has to be converted into an inhalation NAEC (rat, in mg/m³). Absorption (and bioavailability) by inhalation is assumed to be 100%. The internal starting point is divided by 0.384 m³/kg/day (default respiratory volume for the rat for 8 hours) and multiplied by a factor of 6.7/10 (ratio of worker respiratory volumes under standard conditions and under conditions of light activity). Correspondingly, the inhalation starting point is calculated to be 18.3 mg/m³ (10.5 x 1 x 1/0.384 x 6.7/10).

The default factor for interspecies differences (rat, inhalation) is 2.5 (the factor for allometric scaling is already implicitly applied). For intraspecies differences, the default factor of 5 is chosen. The reference MOS for inhalation is calculated to be 12.5 (2.5 x 5). The corresponding "critical exposure level" for inhalation exposure is 1.5 mg/m³ (18.3/12.5).

Systemic effects by dermal exposure

Because of the assumption of 4.2% dermal bioavailability the internal starting point of 10.5 mg/kg/day (equivalent to 4.2% of the dermal exposure) is converted to an adequate dermal starting point of 250 mg/kg/day.

The default factor for interspecies differences (rat, dermal) is 4 x 2.5. For intraspecies differences, the default factor of 5 is chosen. The reference MOS for dermal contact is calculated to be 50 (4 x 2.5 x 5). The corresponding "critical exposure level" for dermal exposure is 5 mg/kg/day (250/50).

Alternatively dermal risk assessment can be performed by directly using the dermal NOAEL of 500 mg/kg/day (rat, 90-day study, 5 days/week). The experimental NOAEL of 500 mg/kg/day is directly used as dermal starting point. The default factor for interspecies differences (rat, dermal) is 4 x 2.5. For intraspecies differences, the default factor of 5 is chosen. For duration adjustment, the default factor of 2 is used. The corresponding reference MOS is 100. These considerations result in a dermal "critical exposure level" of 5 mg/kg/day (500/100).

Both calculations (those based on internal or external doses for the dermal route of exposure) yield an identical result of 5 mg/kg/day for the "critical exposure level". The results have to be identical, because the experimental information on potency differences (about 5% dermal bioavailability) is accounted for when starting the risk assessment with the oral toxicity data.

Systemic effects by combined exposure

The internal starting point is 10.5 mg/kg/day. The reference MOS is identical to the dermal reference MOS of 50. The corresponding internal "critical exposure level" results in 0.2 mg/kg/day.

Table 4.1.3.2.B: MOS values for repeated dose toxicity of diphenylamine, systemic effects

	Inhalation			Dermal			Combined		
Starting point for MOS calculation	18.3 mg/m^3			250 mg/kg/d			10.5 mg/kg/d		
Reference MOS	12.5			50			50		
Critical exposure level	1.5 mg/m^3			5 mg/kg/d			0.2 mg/kg/d		
	Exposure (mg/m^3)	MOS	Conclusions	Exposure (mg/kg/d)	MOS	Conclusions	Internal body burden (mg/kg/d)	MOS	Conclusions
1. Production of diphenylamine and further processing	1.0	18	ii	0.3	833	ii	0.15	70	ii
2. Use of lubricant, metalworking fluids (with 1% diphenylamine)	0.02	915	ii	1.8	139	ii	0.08	131	ii

With respect to repeated dose toxicity (systemic effects) there is no concern for both exposure scenarios and for all routes of exposure.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

Mutagenicity

DPA was negative in two Salmonella gene mutation tests. Further studies indicate that DPA is not or only marginally genotoxic to mammalian cells in vitro .

Negative results from an in vivo micronucleus test indicate that no mutagenic effects are expressed in vivo. In conclusion the data indicate that diphenylamine may not be mutagenic in humans.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

Carcinogenicity

Diphenylamine has been tested for potential carcinogenicity in various long-term bioassays with rats, mice and dogs. Based on the interpretation of the overall results, diphenylamine is not considered to be a carcinogen in experimental animals (see chapter 4.1.2.8). Correspondingly, there is no concern for workers as to this toxicological endpoint.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

Reproductive toxicity

Fertility impairment

There are no human data available on reproductive toxicity of diphenylamine. Data from investigations in laboratory animals are limited to studies with the oral route of administration. Based on available rat data (see chapter 4.1.2.9) it appears that impairment of reproductive capability and capacity (i.e. decreased litter size) only occurs at dosages that interfere with food intake and body weight gain of the parental animals. From repeated dose toxicity studies there are no indications for adverse effects to gonads. From the available animal data a NOAEL for fertility impairment of 131 mg/kg/day is recommended for risk characterisation purposes.

In order to avoid redundant MOS calculations, reference is made to the calculations for repeated dose toxicity. The NOAEL for fertility impairment is about 18-times higher (131/7.5) than the experimental NOAEL for repeated dose toxicity. Because of identical adjustment factors the relationship for the adequate starting points is the same as for the experimental NOAELs. Because of identical route-specific reference MOS's the same relationship (factor 18) is true for the corresponding critical exposure levels (fertility impairment versus RDT). Because of the quality of the reproductive effects, no additional "severity" factor is used.

Table 4.1.3.2.C: MOS values for fertility impairment of diphenylamine

	Inhalation			Dermal			Combined		
Starting point for MOS calculation	319 mg/m^3			4,357 mg/kg/d			183 mg/kg/d		
Reference MOS	12.5			50			50		
Critical exposure level	26 mg/m^3			87 mg/kg/d			3.6 mg/kg/d		
	Exposure (mg/m^3)	MOS	Conclusions	Exposure (mg/kg/d)	MOS	Conclusions	Internal body burden (mg/kg/d)	MOS	Conclusions
1. Production of diphenylamine and further processing	1.0	319	ii	0.3	14,523	ii	0.15	1,220	ii
2. Use of lubricant, metal working fluids (with 1%diphenylamine)	0.02	15,950	ii	1.8	2,420	ii	0.08	2,288	ii

With respect to fertility impairment conclusion ii is applied for both occupational exposure scenarios.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

Developmental toxicity

Available data from developmental toxicity studies in two species (rats and rabbits) do not indicate any specific embryo-/fetotoxic or teratogenic potential even at maternally toxic dosages. In a two-generation study (EPA 1998) no teratogenic effects were observed up to maternal oral doses of 448 mg/kg/day. A NOAEL for developmental toxicity of 46 mg/kg/day is based on growth retardation in the F2 generation during late lactation at doses of 131 and 448 mg/kg/day. Maternal toxicity was observed at this doses with respect to reduced body weight, decrease in food consumption and pathological findings in spleen, kidney and liver.

Again, in order to avoid redundant MOS calculations, reference is made to the calculations for repeated dose toxicity. The NOAEL for developmental toxicity (growth retardation) is about 6-times higher (46/7.5) than the experimental NOAEL for repeated dose toxicity. Because of identical adjustment factors the relationship for the adequate starting points is the same as for the experimental NOAELs. Because of identical route-specific reference MOS's the same relationship (factor 6) is true for the corresponding critical exposure levels (developmental toxicity versus RDT). Because of the quality of the reproductive effects, no additional "severity" factor is used.

Table 4.1.3.2.D: MOS values for developmental toxicity of diphenylamine

	Inhalation			Dermal			Combined		
Starting point for MOS calculation	112 mg/m^3			1,524 mg/kg/d			64 mg/kg/d		
Reference MOS	12.5			50			50		
Critical exposure level	9 mg/m^3			31 mg/kg/d			1.2 mg/kg/d		
	Exposure (mg/m^3)	MOS	Conclusions	Exposure (mg/kg/d)	MOS	Conclusions	Internal body burden (mg/kg/d)	MOS	Conclusions
1. Production of diphenylamine and further processing	1.0	112	ii	0.3	5,080	ii	0.15	427	ii
2. Use of lubricant, metal working fluids (with 1% diphenylamine)	0.02	5,600	ii	1.8	846	ii	0.08	800	ii

Experimental testing of diphenylamine did not result in embryotoxic, fetotoxic or teratogenic effects. Based on these data, diphenylamine is not classified as a reprotoxic substance. However, at maternally toxic doses growth retardation of the offspring is observed. For this adverse effect, the outlined MOS approach did not result in concern.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

4.1.3.2.3 Summary of occupational risk assessment (diphenylamine)

Risk characterisation for workers is complicated by the situation that data on oral absorption (100%) and the default value for dermal absorption (100%) cannot by itself explain the route-specific potency differences for diphenylamine toxicity. Comparison of oral and dermal experimental results for repeated dose toxicity of diphenylamine indicates a relatively low toxic potency for the dermal route of exposure. Based on the comparison of the adjusted NAELs for oral and dermal repeated dose toxicity (see introduction workers) a 100% oral bioavailability and a dermal bioavailability of about 5% is taken forward to risk characterisation. This relationship of relative oral and dermal potency is assumed to be valid for all toxicological endpoints of diphenylamine. Without specific data to the contrary, the default value of 100% absorption by inhalation is interpreted in the sense of 100% bioavailability.

Based on the available data on diphenylamine toxicity and exposure to diphenylamine the risk assessment for workers does not result in any concern (table 4.1.3.2.E).

Table 4.1.3.2.E: Endpoint-specific overall conclusions for diphenylamine

Toxicological endpoints		Conclusion
Acute toxicity	inhalation	ii
	dermal	ii
	combined	ii
Irritation/ Corrosivity	dermal	ii
	eye	ii
	acute respiratory tract	ii
Sensitisation	skin	ii
	respiratory	ii
Repeated dose toxicity	inhalation, local	ii
	dermal, local	ii
	inhalation, systemic	ii
	dermal, systemic	ii
	combined, systemic	ii
Mutagenicity		ii
Carcinogenicity	inhalation	ii
	dermal	ii
	combined	ii
Fertility impairment	inhalation	ii
	dermal	ii
	combined	ii
Developmental toxicity	inhalation	ii
	dermal	ii
	combined	ii

Tables 4.1.3.2.F (inhalation) and 4.1.3.2.G (dermal contact) try to visualize the risk profile of diphenylamine. According to the specific arrangement of exposure scenarios and critical exposure levels for different toxicological endpoints you will find the relatively high risk characterisation ratios (exposure divided by critical exposure level) in the left upper corner, the relatively low risk characterisation ratios in the bottom right corner of the tables. This table may help to reach consistent conclusions for different endpoints and scenarios.

Table 4.1.3.2.F: Ranking of health risks for workers (inhalation)

Exposure scenario		Exposure (mg/m³)	Repeated dose toxicity (systemic)	Acute toxicity	Developmental toxicity	Fertility impairment
			Critical exposure level in mg/m³			
			1.5	7	9	26
1.	Production of diphenylamine and further processing	1.0	ii	ii	ii	ii
2.	Use of lubricant, metal working fluids	0.02	ii	ii	ii	ii

Table 4.1.3.2.G: Ranking health risks for workers (dermal contact)

Exposure scenario		Exposure (mg/kg/d)	Repeated dose toxicity (systemic)	Acute toxicity	Developmental toxicity	Fertility impairment
			Critical exposure level in mg/kg/d			
			5	24	31	87
1.	Production of diphenylamine and further processing	1.8	ii	ii	ii	ii
2.	Use of lubricant, metal working fluids	0.3	ii	ii	ii	ii

4.1.3.3 Consumers

CONSUMER EXPOSURE

There are no measured data on exposure to humans available. It can be assumed that the exposure of consumers to diphenylamine is primarily due to oral exposure from eating fruits and other vegetable foods which are treated with diphenylamine. This exposure can lead up to an intake of 0.0122 mg/kg bw/d for a female adult (age 55 - 64, average body weight approximately 68 kg). Children (age 2-5 years, body weight 16.5 kg) would be exposed to an amount of 0.0677 mg/kg bw/d. The oral exposure is covered by the legislation on plant protection products. Therefore, no risk characterisation for this intake will be performed in this section.

Dermal exposure of consumers is possible by the use of lubricants. An external dermal exposure of 0.7 mg/kg bw/d is estimated assuming a body weight of 60 kg.

EFFECTS

Acute toxicity

Following the exposure assessment, consumers are not expected to be exposed to diphenylamine in the range of hazardous doses which can be derived from acute oral or dermal toxicity figures based on animal LD 50. Therefore, the substance is of no concern for the consumer in relation to dermal toxicity. Information about inhalation toxicity is not available. However, considering the low vapoure pressure of the substance inhalation exposure can be neglected.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

Irritation / Corrosivity

Human data on the local irritant or corrosive properties of diphenylamine are not available. The substance caused no or only very slight skin irritation in tests with rabbits.

Data of eye irritating properties of the substance are conflicting and poorly documented, but it can be assumed that diphenylamine may pose a risk of serious damage to eyes.

Taking into account the intended use of lubricants (kind of exposure: two palms) and the amount of the substance which might be brought possibly into contact with eyes it can be concluded that there is no concern for eye irritation.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

Sensitization

Diphenylamine did not produce dermal sensitization in guinea pigs. One case is reported where one woman demonstrated a contact allergy. Other studies with 11 or 1012 patients did not demonstrate a skin sensitization that could be attributed to diphenylamine. Cross sensitization to p-phenylenediamine has not been demonstrated in the woman who reacted positive to diphenylamine and cross sensitivities were suspected in three positive patients. In a maximization test carried out on 30 volunteers no skin sensitization reactions were produced. Diphenylamine is not suspected to be a potent respiratory sensitiser

It can be concluded that diphenylamine does not induce skin sensitization in humans.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

Repeated dose toxicity

Oral exposure

The primary target organs after short- and long-term dietary exposure of diphenylamine to rats, mice and dogs are the hematological system and the kidneys, spleen, and liver. Comparing the LOAELs from the different studies it becomes obvious that adverse effects in rats and dogs (LOAEL) occurred at the same doses of about 25 mg/kg bw/d. Mice seem to be less sensitive according to the results from new short- and long-term studies by Botta (1992, 1994a). Taking together the data from animal studies with repeated oral application, the value of 7.5 mg/kg bw/d was derived as NOAEL for adverse effects after chronic exposure from the two-year carcinogenicity study on rats (Botta, 1994b). This NOAEL is based on haematological and histological effects at dietary levels equal or greater than 25 mg/kg bw/d in female rats (LOAEL).

Dermal exposure

In the JMPR report (1998) on diphenylamine a dermal study in rabbits lasting 21 days (Siglin, 1992) is described. Gross necropsy revealed dark-red foci in the stomachs of rabbits of each sex at the doses of 500 and 1000 mg/kg bw/d. A NOAEL of 100 mg/kg bw/d was derived from this study. After dermal application of DPA to rats over a period of 90 days the NOAEL for systemic toxicity is 500 mg/kg bw/d based on an increase in the relative kidney weight of males at 2000 mg/kg bw/d. All treated animals exhibited dermal hyperplasia at the application side. Thus, the LOAEL for the skin from this 90 day study is 500 mg/kg bw/d, whereas no NOAEL could be derived. The risk characterisation for dermal exposure (systemic effects) is based on the NOAEL of 500 mg/kg bw/d from the 90-day study on rats.

These figures were taken for further consideration of MOS.

For the decision on the appropriateness of MOS, the following aspects have been considered and taken into account:

- overall confidence in the database

The data taken into account for performing the risk characterization have been evaluated with regard to their reliability, relevance and completeness according to section 3.2 of the TGD. The findings of all studies are not contradictory except the one on mice by Ford et al., 1972. .

- uncertainty arising from the variability in the experimental data

The NOAEL of 7.5 mg/kg bw/d derived from the study on rats is considered to be the most appropriate value for risk assessment for oral exposure with diphenylamine. The main findings from all studies showed identical main toxic effects.

For dermal exposure (systemic effects) the NOAEL of 500 mg/kg bw/d from the 90-day study on rats is used for the risk characterisation.

There are no reasons to assume a special extent of uncertainty which has to be taken into account.

- intra- and interspecies variation

Data on kinetics of the substance do not allow to calculate the intraspecies and interspecies variability by applying modern approaches. From the excretion mechanism by the renal route it is concluded that no major species differences have to be taken into consideration as the mechanism of excretion concerns.

- the nature and severity of the effect

From subchronic and chronic studies of animals fed with a diet containing diphenylamine the most sensitive indication for toxicity seem to be haematological effects (slight anemia) and effects on kidneys, spleen and liver. There are no reasons to assume that the effects shown in the animal experiments are limited to the species tested, hence they are of relevance for humans.

- differences in exposure (route, duration, frequency and pattern)

For dermal risk characterisation of consumers the rat study is used which follows dermal exposure. Thus, the estimated dermal exposure is compared with a dermal NOAEL.

- the human population to which the quantitative and/or qualitative information on exposure applies

Considering the route of excretion there is reason to assume a special risk for the elderly and for patients with impaired renal function.

- other factors

There are no other factors known requiring a particular margin of safety.

MOS for the dermal exposure scenario

Local effects

An external dermal exposure due to use of lubricants has been estimated to be up to 0.7 mg/kg bw/d. The margin of safety between the

exposure level of 0.7 mg/kg bw/d

and the

dermal LOAEL (local) of 500 mg/kg bw/d

is judged to be sufficient taking into consideration the worst-case scenario assumption that both palms have contact with the lubricant and short times of exposure .

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

Systemic effects

The margin of safety between the

exposure level of 0.7 mg/kg bw/d

and the

dermal NOAEL (systemic) of 500 mg/kg bw/d

is judged to be sufficient.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

Mutagenicity

Diphenylamine was negative in two Salmonella gene mutation test. Further studies indicate that diphenylamine is not or only marginally genotoxic to mammalian cells in vitro. Negative results from an in vivo micronucleus test indicate that no mutagenic effects are expressed in vivo. In conclusion, the data indicate that diphenylamine may not be mutagenic in humans.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

Carcinogenicity

In a JMPR document (1998) containing a complete report on DPA based on recent guideline conform long-term investigations on mice and rats no evidence for increased tumor incidence was found. In an one year study in beagle dogs with bolus application neoplastic alterations could also be not found. A number of older investigations using several strains of rats and mice and dogs do not report any diphenylamine related neoplastic alterations. In addition the majority of short term in vivo and in vitro tests equally do not show evidence for transforming activity of diphenylamine. The overall results support the assumption that there was no indication on carcinogenic effects to diphenylamine

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

Reproductive toxicity

There are no human data available for reproductive toxicity of diphenylamine. Data from investigations in laboratory animals are limited to studies with the oral route of administration. From the available data obtained from studies with rats it appears that impairment of reproductive capability and capacity is unlikely to occur from treatment with diphenylamine at dosages up to 131 mg/kg bw/d that do not interfere with food intake and body weight gain of the parental animals. From the data obtained from two developmental studies in rats and rabbits any specific embryo-/fetotoxic or teratogenic potential is not indicated even at maternally toxic dosages. In a two generation study (cited by the EPA, 1998) no teratogenic effects were observed up to maternal oral doses of 448 mg/kg bw/ d. An NOAEL/developmental toxicity of 46 mg/kg bw/d was deduced based on a growth retardation in the F2 generation during late lactation at doses of 131 and 448 mg/kg bw/d. Maternal toxicity was observed at these doses with respect to reduced body weight, decrease in food consumption and pathological findings in spleen, kidney and liver. With respect to any nephrotoxic properties of diphenylamine developmental studies performed with rats did not reveal the induction of any renal lesions in the offspring. From the available data a NOAEL/fertility of 131 mg/kg bw/d, a NOAEL/developmental toxicity of 46 mg/kg bw/d and a LOAEL/developmental toxicity of 131 mg/kg bw/d is recommended for use for risk characterization purposes.

Fertility

There are no guideline studies available.

From an unpublished two-generation reproductive toxicity dietary study on Sprague-Dawley rats (Rodwell, 1993) at dose levels of 0, 500, 1500, or 5000 ppm (0, 46, 131, or 448 mg/kg bw/d for F_0 females) the value of 131 mg/kg bw/d was deduced as NOAEL/fertility. In a two year feeding study on rats a NOAEL of 125 mg/kg bw/d is found.

For the decision on the appropriateness of MOS, the following aspects have been considered and taken into account:

- overall confidence in the database

The data taken into account for performing the risk characterization have been evaluated with regard to their reliability, relevance and completeness according to section 3.2 of the TGD.

- uncertainty arising from the variability in the experimental data

The oral NOAEL for fertility of 131 mg/kg bw/d from the study on SD rats is considered to be the appropriate value for risk assessment.

There are no reasons to assume a special extent of uncertainty which has to be taken into account.

- intra- and interspecies variation

Data on kinetics of the substance do not allow to calculate the intraspecies and interspecies variability by applying modern approaches. From the excretion mechanism by the renal route it is concluded that no species differences have to be taken into consideration as the mechanism of excretion concerns.

- the nature and severity of the effect

The effects are considered to be severe health effects.

There are no reasons to assume that the effects shown in the animal experiments are limited to the species tested, thus being not of relevance for humans.

- differences in exposure (route, duration, frequency and pattern)

The estimated dermal exposure is assumed to be 100% and compared with an oral NOAEL.

- the human population to which the quantitative and/or qualitative information on exposure applies

Considering the route of excretion there is reason to assume a special risk for patients with impaired renal function.

- other factors

There are no other factors known requiring a peculiar margin of safety.

MOS for the dermal exposure scenario

An external dermal exposure due to use of lubricants has been estimated be up to 0.7 mg/kg bw/d. The margin of safety between the

exposure level of 0.7 mg/kg bw/d

and the

oral NOAEL of 131 mg/kg bw/d

is judged to be sufficient taking into consideration the worst-case scenario assumption that both palms have contact with the lubricant and short times of exposure.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

Developmental toxicity

Data from guideline-according developmental toxicity studies are presently not available.

From the data obtained from two developmental studies in rats and rabbits any specific embryo-/fetotoxic or teratogenic potential is not indicated even at maternally toxic dosages. In a two generation study (cited by the EPA, 1998) no teratogenic effects were observed up to maternal oral doses of 448 mg/kg bw/d. An NOAEL/developmental toxicity of 46 mg/kg bw/d was derived based on a growth retardation in the F2 generation during late lactation at doses of 131 and 448 mg/kg bw/d.

For the decision on the appropriateness of MOS, the following aspects have been considered and taken into account:

- overall confidence in the database

The data taken into account for performing the risk characterization have been evaluated with regard to their reliability, relevance and completeness according to section 3.2 of the TGD.

No studies according to internationally acknowledged guidelines were presented.

- intra- and interspecies variation

Data on kinetics of the substance do not allow to calculate the intraspecies and interspecies variability by applying modern approaches. From the excretion mechanism by the renal route it is concluded that no species differences have to be taken into consideration as the mechanism of excretion concerns.

- the nature and severity of the effect

The effects are considered to be severe health effects per se.

There are no reasons to assume that the effects shown in the animal experiments are limited to the species tested, thus being not of relevance for humans.

- differences in exposure (route, duration, frequency and pattern)

From a two generation feeding study on rats a NOAEL of 46 mg/kg bw/d is derived. The dermal exposure is assumed to be 100% and compared with an oral NOAEL.

- the human population to which the quantitative and/or qualitative information on exposure applies

Considering the route of excretion there is reason to assume a special risk for patients with impaired renal function.

- other factors

There are no other factors known requiring a peculiar margin of safety.

MOS for the dermal exposure scenario

An external dermal exposure due to lubricants has been estimated to be up to 0.7 mg/kg bw/d. The margin of safety between the

dermal exposure level of 0.7 mg/kg bw/d

and the

oral NOAEL of 46 mg/kg bw/d

is judged to be sufficient taking into account the worst-case scenario assumption that both palms have contact with the lubricant and short times of exposure.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

4.1.3.4 Man exposed indirectly via the environment

The local scenario is based on an exposure scenario caused by the application of sewage sludge from a municipal wwtp which resulted in the highest local diphenylamine concentrations in soil and porewater and is supplemented with the calculated local exposure data (surface water and air) from site B. This is compared to an average intake due to exposure via the regional background concentrations.

However, it has to be noted, that the applied model calculations are of preliminary nature and may have to be revised as soon as further knowledge, e.g. on PECregional or the sludge application scenario becomes available.

Exposure

Model calculations for the local scenario resulted in a total daily dose of 0.0048 mg/kg bw/d (cf. 4.1.1.3). For the regional scenario a total daily dose of 0.0005 mg/kg bw/d was calculated. For the purpose of risk characterisation the highest value of 0.0048 mg/kg bw/d has been used.

Repeated dose toxicity

From different short- and long-term studies on mice, dogs and rats with oral administration of diphenylamine a NOAEL of 7.5 mg/kg bw/d (rats, oral, two-year carcinogenicity study) was derived.

Comparison indirect exposure – NOAEL

$$\frac{\text{Indirect exposure}}{\text{NOAEL}} = \frac{0.0048\ \text{mg/kg bw/d}}{7.5\ \text{mg/kg bw/d}}$$

The margin of safety between the calculated exposure and the NOAEL is judged to be sufficient. Thus, regarding of repeated dose effects the substance is of no concern in relation to indirect exposure via the environment.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

Reproductive toxicity

Fertility

The NOAEL/ fertility of 131 mg/kg bw/d from an oral rat study is considered to be the appropriate value for risk assessment.

Comparison indirect exposure – NOAEL

$$\frac{\text{Indirect exposure}}{\text{NOAEL}} = \frac{0.0048\ \text{mg/kg bw/d}}{131\ \text{mg/kg bw/d}}$$

The margin of safety between the calculated exposure and the NOAEL is judged to be sufficient. Thus, regarding of adverse effects on reproductive performance the substance is of no concern in relation to indirect exposure via the environment.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

Developmental toxicity

Based on the findings of growth retardation in the F2 generation during late lactation a NOAEL/developmental toxicity of 46 mg/kg bw/d was determined.

Comparison indirect exposure - NOAEL

$$\frac{\text{Indirect exposure}}{\text{NOAEL}} = \frac{0.0048\ \text{mg/kg bw/d}}{46\ \text{mg/kg bw/d}}$$

The margin of safety between the calculated exposure and the NOAEL is judged to be sufficient. Thus, the substance is regarding of fetotoxic and teratogenic effects of no concern in relation to indirect exposure via the environment.

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

4.1.3.5 (Combined exposure)

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

4.2.1 Exposure assessment

4.2.1.1 Occupational exposure

See chapter 4.1.1.1

4.2.1.2 Consumer exposure

4.2.1.3 Indirect exposure via the environment

4.2.2 Effects assessment: Hazard identification and Dose (concentration) - response (effect) assessment

4.2.2.1 Explosivity

Diphenylamine is not explosive

4.2.2.2 Flammability

Diphenylamine is not flammable

4.2.2.3 Oxidising potential

Due to its chemical structure, diphenylamine is not expected to possess any oxidizing properties.

4.2.3 Risk characterisation

4.2.3.1 Workers

Not applicable

4.2.3.2 Consumers

4.2.3.3 Man exposed indirectly via the environment

5 CONCLUSIONS / RESULTS

5.1 INTRODUCTION

Diphenylamine is produced or imported only by few companies within the European Union (EU). The EU 15 market volume is about 10 000 t/a. Of this amount approx. 98 % (9 750 t/a) are processed as chemical intermediate for the production of antioxidants and antiozonants widely used in the rubber industry and for lubricants.

The remaining 2-3 % of total EU tonnage are mainly used in storage aid for fruits/vegetables (approx. 2 %) and in lubricants (approximately 0.5 %).

Diphenylamine is a cream coloured solid (flake) with a sharp creosote odour. It has a low vapour pressure and a low water solubility.

5.2 ENVIRONMENT

Table 5.1 gives an overview of the uses and local scenarios for which risk was assessed. Further conclusions for the only known production site and the only known intermediate processing site are presented in the confidential Appendices B1 and B2.

Table 5.1: Overview of the scenarios and conclusions of the assessment.

		Conclusions			
Uses/Scenarios	**Tonnage**	**WWTP**	**Aquatic compartment** (water and sediment)	**Soil**	**Secondary poisoning** (aquatic/terr.)
Production (generic site)	5000 t/a	(i)	(i)	(ii)	(i) / (ii)
Production (site specific)	Confidential	(i)	(i)	(ii)	(i) / (ii)
Use as intermediate	9750 t/a)				
Processing (generic)	4000 t/a	(i)	(i)	(ii)	(i) / (ii)
Processing (site specific)	Confidential	(i)	(i)	(ii)	(ii)/ (ii)
Use in lubricants	50 t/a				
Formulation		(ii)	(i)	(i)	(ii) / (ii)
Professional use		(i)	(i)	(i)	(ii) / (i)
Use in storage aid (plant protection product)	200 t/a				
Formulation		(i)	(i)	(i)	(ii) / (ii)

		Conclusions			
Uses/Scenarios	**Tonnage**	**WWTP**	**Aquatic compartment** (water and sediment)	**Soil**	**Secondary poisoning** (aquatic/terr.)
Processing	No local scenario; releases are taken into account in the reg. and cont. concentrations				
Releases from the private use of fruits	No local scenario; releases are taken into account in the reg. and cont. concentrations				
Use in explosives	~ 0.1 %	Not assessed			
Use as stabilizer, colouring agent	traces	Not assessed			

5.2.1 Waste water treatment plant

Conclusion (i) There is need for further information and/or testing

This applies for all other scenarios except for formulation of lubricant. Up to date information on the tonnage for each use and size of the industrial sites is necessary to refine the assessment. Further conclusions for the only known production and processing sites are included in the Appendices B1 and B2. Size of waste water treatment plants for production and processing of intermediates as well as site specific emission data or measured data from effluents are needed (see also conclusions for aquatic environment below). In addition, PNECmicro-organisms may be lowered by further testing (now AF of 100 has been applied). However, it is first necessary to obtain better data on the exposure before any testing is conducted.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

This conclusion applies for the formulation of lubricant.

5.2.2 Aquatic environment (including sediment)

Conclusion (i) There is need for further information and/or testing

The conclusion applies for compartment water to all industrial categories for which local environmental concentrations could be predicted. While the equilibrium partitioning approach was used to derive both the PEC and the PNEC for sediment, same conclusions are drawn for sediment as for water compartment. Up-to-date information on the tonnage of all uses is needed. For the rest of the European use volume of the 10 000 t/a not covered by the only known producer and importer, confirmation is needed whether it is completely imported or produced in additional European sites. It is very probable, that other processing sites than the

only known one are located in Europe. Information on their size, waste water treatment and effluent dilution rate is necessary. Information on the size and waste water treatment of lubricant formulation sites is necessary as well. In addition, more information on the end-uses of lubricants containing Diphenylamine is needed in order to be able to allocate the tonnage in lubricant use to several generic use scenarios. There is no information available whether there are any formulation sites of storage aid located in Europe or not. Confirmation for this issue is needed. If any formulation sites are located in Europe, site specific data on their size, emissions and waste water treatment is needed. Hardly any new measured data is available from aquatic environment. Measured data from water and sediment are needed in order to be able to compare the model results with the reality.

After better emission data has been received, the risk ratios could be further reduced by approximately a factor of two in case Diphenylamine would be confirmed to be inherently biodegradable. The available biodegradation data indicates that this might be the case, but no such studies are available, which would allow to assume inherent biodegradation in this version. Therefore, a simulation test could be considered. In addition, a chronic fish-study would reduce the risk ratio in local scenarios at the most by a factor of 5 (AF for derivation of PNECaqua would be reduced from 50 to 10). A chronic fish test belongs to the base set of the Commission Directive 414/91/EEC for evaluation of plant protection products, and the full base set requirements will be delivered to the rapporteur Ireland by May 2004.

The conclusion applies also for sediment because same risk ratios were derived as for water compartment due to the application of equilibrium partitioning method for PEC and PNEC in sediment.

As a conclusion, at this phase, generation of further import, production, use and emission information is preferred instead of conducting any tests.

5.2.3 Terrestrial compartment

Conclusion (i) There is need for further information and/or testing

This conclusion applies for formulation of lubricant and storage aid and professional use of storage aid. Information on the size and waste water treatment of lubricant formulation sites is necessary. In addition, more information on the end-uses of lubricants containing Diphenylamine is needed in order to be able to allocate the tonnage in lubricant use to several generic use scenarios. There is no information available whether there are any formulation sites of storage aid located in Europe or not. Confirmation for this issue is needed. If any formulation sites are located in Europe, site specific data on their size, emissions and waste water treatment is needed.

A secondary alternative is to conduct a biodegradation simulation test (see the conclusions above for the aquatic compartment), the results of which may lower the estimate for PECsoil. In addition, either new terrestrial chronic ecotoxicity data or an improvement of PNECaqua is needed.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

This conclusion applies for production and processing.

5.2.4 Non-compartment specific effects relevant to the food chain

Conclusion (i) There is need for further information and/or testing

This conclusion applies for the aquatic food chain for the known production site, generic production scenario and generic scenario for intermediate use. Further conclusions on the only known production site are included in the Appendix B1. For the rest of the European use volume of the 10 000 t/a not covered by the only known producer and importer, a confirmation is needed whether it is completely imported or produced in additional European sites. It is very probable, that more than one processing sites are located in Europe. Information on their size, waste water treatment and effluent dilution rate is necessary.

For the terrestrial food chain, this conclusion applies for professional use of lubricant. More information on the end-uses of lubricants containing Diphenylamine is needed in order to be able to allocate the tonnage in lubricant use to several generic use scenarios. Refinement of regional PEC for agricultural soil may reduce the ratio. Due to EUSES –model, sludge from all industrial categories is included in the regional scenario. This technical problem may be circumvented.

After better emission data has been received, the risk ratios could be further reduced by approximately a factor of two in case Diphenylamine would be confirmed to be inherently biodegradable. The available biodegradation data indicates that this might be the case, but no such studies are available, which would allow to assume inherent biodegradation in this version. Therefore, a simulation test could be considered.

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

Regarding the aquatic food chain, this conclusion applies for formulation of lubricants and storage aid, the known intermediate processing site and professional use of lubricants.

Regarding the terrestrial food chain, this conclusion is drawn for production, intermediate processing, processing of storage aid, formulation of storage aid and formulation of lubricants.

5.3 HUMAN HEALTH

5.3.1 Workers

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

5.3.2 Consumers

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

5.3.3 Man exposed indirectly via the environment

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already

6 REFERENCES

Adolf, T., Schneider, R., Eberhardt, W., Hartmann, S., Herwig, A., Heseker, H., Hünchen, K., Kübler, W., Matiaske, B., Moch, K.J., Rosenbauer, J. (1995): Ergebnisse der Nationalen Verzehrsstudie (1985-1988) über die Lebensmittel- und Nährstoffaufnahme in der Bundesrepublik Deutschland. VERA-Schriftenreihe Band XI, Wissenschaftlicher Fachverlag Dr. Fleck, Niederkleen

Alexander, W.E., Ryan, A.J., Wright, S.E. (1965): Metabolism of diphenylamine in the rat, rabbit and man. Fd Cosmet Toxicol; **3**: 571-579

Amacher, D.E., Paillet, S.C., Turner, G.N., Ray, V.A., Salsburg, D. (1980): Point mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells. II. Test validation and interpretation. Mutation Res; **72**: 447 - 474

Anna, J., Plöger, E. and Reupert, R. (1984): Gewässerschutz, Wasser, Abwasser **65**, 315-332

Appel, K.E., Görsdorf, S., Scheper, T., Bauszus, M., Hildebrandt, A.G. (1987): Enzymatic denitrosation of diphenylamine: activation or inactivation? Arch Toxicol; **60**: 204-208

Appel, K.E., Rühl, C.S., Spiegelhalder, B., Hildebrandt, A.G. (1984a): Denitrosation of diphenylnitrosamine in vivo. Toxicol Lett; **23**: 353-358

Appel, K.E., Rühl, C.S., Hildebrandt, A.G. (1984b): Metabolic inactivation of N-nitrosamines by cytochrome P-450 in vitro and in vivo. In: N-Nitroso Compounds: Occurence, Biological Effects and Relevance to Human Cancer; Proc. VIIIth Int. Symp. on N-Nitroso Compounds, Banff 05.-09.Sept. 1983 (O'Neill IK, von Borstel RC, Miller CT, Long J, Bartsch H, Hrsg.) IARC Sci Publ 1984b; No 57, Lyon, P 443-451

Ardito, G., Bramanti, B., Bigatti, P., Lamberti, L., Dolara, P. (1996): Cytogenetic effect of thiabendazole and diphenylammine on cultured human lymphocytes: sister chromatid exchanges and cell cycle delay. J Biol Res, Boll Soc Ital Biol Sper; **72**: 171-178

Ariel (2002): Ariel Insight 2.0, For survival in a world of expanding chemical regulations, August 2002

Auer Technikum (1988): Ausgabe 12, Herausgeber: AuergesellschaftGmbH, Abt. WuÖ, Berlin 44

AUH-Report (1995): Standards zur Expositionsabschätzung; Bericht des Ausschusses für Umwelthygiene der AGLMB. Behörde für Arbeit, Gesundheit und Soziales, Hamburg

Babish, J.G., Hotchkiss, J.H., Wachs, T., Vecchio, A.J., Gutenmann, W.H. and Lisk, D.J. (1983): N-Nitrosamines and Mutagens in Rubber Nursing Nipples. Journal of Toxicology and Environmental Health, **11**, 167-177.

BAM (1997): Bundesanstalt für Materialforschung und –prüfung: Assessment of the safety relevant characteristics in February 2003

Banasiak, U., Heseker, H., Sieke, C., Sommerfeld, C., Vohmann, C., (2005): Abschätzung der Aufnahme von Pflanzenschutzmittel-Rückständen in der Nahrung mit neuen Verzehrsmengen für Kinder. Bundesgesundheitsbl-Gesundheitsforsch-Gesundheitsschutz 2005 48: 84-98

Bayer AG (1977): Institut für Toxikologie Wuppertal-Elberfeld, Löser E. Akute orale Toxizität, Prüfmuster Diphenylamin. Unpublished report 15 November 1977

Bayer AG (1977): Institut für Toxikologie Wuppertal-Elberfeld, Löser E. Untersuchung zur Haut- und Schleimhautverträglichkeit, Prüfmuster Diphenylamin. Unpublished report 15 November 1977

Bayer AG (1986a): Abteilung AV-IM-AM, WV-LE Umweltschutz/AWALU, Geb. Q18, Datenblatt zu physikalisch-chemischen Eigenschaften

Bayer AG (1986b): Unpublished test; "Bestimmung der Schwimmfähigkeit beim Wasserfloh Daphnia magna" (Verfahrensvorschlag des Umweltbundesamtes, Stand Mai 1984), Institut für Umweltanalyse und Bewertungen, 1986-07-17

Bayer AG (1986c): Unpublished test; Algeninhibitionstest mit *Scenedesmus subspicatus*, Institut für Umweltanalyse und Bewertungen, 1986-11-06

Bayer AG (1986d): Unpublished test; "Daphnia sp., Acute Immobilisation Test and Reproduction Test" OECD Guideline 202 (adopt. 04.04.1984), Institut für Umweltanalyse und Bewertungen, 1986-08-25

Bayer AG (1992): Toxicity of diphenylamine to Earthworms. Report-No. HBF/Rg 168

Bayer AG (1993): Unpublished test; Untersuchungen zum ökologischen Verhalten von Diphenylamin technische Schuppen. Institut für Umweltanalyse und Bewertungen. 04.01.1993. Prüfnummer 394 A/92. p. 20.Bayer AG (1997): unpublished data

Bayer AG (1998): Determination of Safety-Relavent Data of Diphenylamin, Identification No. 98/00129

Bayer AG (1999a): Determination of physical-chemical property data according to GLP, Surface tension, Diphenylamin, techn. Schuppen, January 25, 1999

Bayer AG (1999b): unpublished “Industrial Shadow Risk Assessment Report diphenylamine”, ch. 1-3

Bayer AG (2000): unpublished information from 20.03.2000

Bayer AG, Uniroyal Chemical (2000): unpublished “Industrial Shadow Risk Assessment Report diphenylamine”, ch. 4.1.1.0-4.1.2.9

Bazin, B.H., Foussereau, J., Cavelier, C. (1986): Allergy to diphenyldiamine from an industrial grease. Contact Dermatitis; **14**: 116

BBA (2001): Information by K. Krammer from German Biologische Bundesanstalt ; 30 May 2001

BfR (2005): Information from an internal data base of the German Federal Institute for Risk Assessment, formerly BgVV, Berlin 2005

BGIA (2004): "Einsatz von Kühlschmierstoffen bei der spanenden Metallbearbeitung", BIA Report 4/2004, HVBG

Blume, H.-P. (1990): Handbuch des Bodenschutzes: Bodenökologie und Bodenbelastung; vorbeugende und abwehrende Schutzmaßnahmen. ecomed-Verlag Landsberg/Lech

Boatright, W.L. and Crum, A.D. (1997): Nonpolar-volatile lipids from soy protein isolates and hexan-defatted flakes: J. Am. Oil Chem. Soc. **74 (4)** 461-467

Botta, J.A., Jr (1992): 90 Day subchronic toxicity evaluation of diphenylamine in the mouse. Unpublished report No. 426E-001-034-91 from T.P.S., Inc., Mt Vernon, Indiana, USA. Submitted to WHO by the DPA Task Force, John Wise & Associates Ltd, Liberty, Missouri, USA.

Botta, J.A., Jr (1994a): 18 Month oncogenicity evaluation of diphenylamine in the mouse. Unpublished report No. 426H-002-646-91 from T.P.S., Inc., Mt Vernon, Indiana, USA. Submitted to WHO by the DPA Task Force, John Wise & Associates Ltd, Liberty, Missouri, USA.

Botta, J.A., Jr (1994b): 24 Month combined oncogenicity/toxicity evaluation of diphenylamine in rats. Unpublished report No. 426D-102-048-91 from T.P.S., Inc., Mt Vernon, Indiana, USA. Submitted to WHO by the DPA Task Force, John Wise & Associates Ltd, Liberty, Missouri, USA.

Botta, J.A., Jr (1994c): One year chronic study of diphenylamine in dogs. Unpublished report No. 426B-502-044-91 from T.P.S., Inc., Mt Vernon, Indiana, USA. Submitted to WHO by the DPA Task Force, John Wise & Associates Ltd, Liberty, Missouri, USA.

Braun, R., Schöneich, J., Ziebarth, D. (1977): In vivo formation of N-nitroso compounds and detection of their mutagenic activity in host-mediated assay. Cancer Res; **37**: 4572 - 4579

Briggs, G.G. (1981): Theoretical and experimental relationship between soil adsorption, octanol-water partition coefficients, water solubilities, bioconcentration factors, and the parachor; J. Agric. Food Chem. Soc. **29**, 1050-1059

Calnan, C.D. (1978): Diphenylamine. Contact Dermatitis; **4**: 301

Candela, M.E., Lopez, M. and Sabater, F. (1986): Effects of inhibitors on carotenoids biosynthesis in vivo in Capsicum annum fruits. Biologia Plantarum (Praha) **28**, 196-201

Centre for Chemical Substances and Preparations, Slovakia: Personal communication to BAuA (July 24, 2004)

Centre Francais du Commerce Exterieur (1997): Les residus de pesticides dans les fruits et legumes et les pommes de terre dans les pays d'Europe occidentale, 1997-04-14, p. 67

Christodoulatos, C., Koutsospyros, A.D., Brodmann, B.W. and Korfiatis, G.P. (1997): Biodegradation of diphenylamine by selected microbial cultures. J. Environ. Sci. Health, **A32**, 15-30

CITI (1992): Biodegradation and bioaccumulation data of existing chemicals based on the CSCL Japan; Chemicals Inspection & Testing Institute Japan (ed.)

Commission Directive 2000/57/EWG, 22. September 2000

Crocker, J.F.S., Brown, D.M., Borch, R.F., Vernier, R.L. (1972): Renal Cystic Disease Induced in Newborn Rats by diphenylamine Derivatives. Americ J of Pathology; **66**: 434-350

Crocker, J.F.S., Vernier, R.L. (1970): Chemically Induced Polycystic Kidney Disease in the Newborn. Pediatric Research; **4**: 448

Dahn, A. (1997): Chemical and biological processes for decomposition of nitrocellulose-based propellants in "Conversion concepts for commercial applications and disposal technologies of energetic systems"; H. Krause (ed.), Kluwer Academic Publishers, Netherlands, p. 191-212

Daniels, W. (1983): Vinyl polymers (acetate) in "Encyclopaedia of Chemical Technology"; Kirk-Othmer (ed), 3rd ed., vol. **23**, p. 826

Danielson, J.W., Oxborrow, G.S. and Placencia, A.M. (1983): Chemical Leaching of Rubber Stoppers into Parenteral Solutions. Journal of Parenteral Science and Technology. Vol **37**, No. 3, 89-92.

Danish EPA (1996): Immobilisation Test of Aniline Compounds with the Crustacean Daphnia magna. Project no. 303587

Danish Product Register (1998): Diphenylamine in Danish Product Register. National Institut of Occupational Health Copenhagen.

Danz, M., Urban, H. (1979): Carcinogens, promotors and suspicious drugs: their identical short-term effect. Exp Path; **17**: 181-184

Danz, M., Urban, H. (1980): Elevated mitotic number in the adrenal cortex as a reflection of early events in growth induction by carcinogens and promoters- a possible short-term assay. Arch Toxicol; **4**: 19-21

Deutsche RHmV (1999): - Verordnung über Höchstmengen an Rückständen von Pflanzenschutz- und Schädlingsbekämpfungsmitteln, Düngemitteln und sonstigen Mitteln in oder auf Lebensmitteln und Tabakerzeugnissen (Rückstands-Höchstmengenverordnung - RHmV); BGBl.I 49, S. 2083 - 2141

DiPaolo,J.A.; Nelson,R.L.; Donovan,P.J.; Evans,C.H. (1973): Host-mediated in vivo-in vitro assay for chemical carcinogenesis: Arch. Pathol. 95, 380-385

van Dort, H.M., Jägers, P.P., Heide, R. and van der Weerdt, J.A. (1993): Narcissus trevithian and Narcissus geranium: Analysis and synthesis of compounds: J. Agric. Food Chem. **41**, 2063-2075

Dow Chem Company (1958): Results of 90-day dietary feeding of diphenylamine to rats. Unpublished study No. 86940000831S, submitted to EPA on 05/16/94, EPA OTS0557241 with cover letter dated 05/10/94

Drzyzga, O. (1996): Transformation und Abbau von Diphenylamin und Nitro-diphenylaminen aus Rüstungsaltlasten durch anaerobe Bakterien; Vom Fachbereich 7 – Biologie –der Carl von Ossietzky-Universität Oldenburg angenommene Dissertation

Drzyzga, O. and Blotevogel, K.-H. (1997): Microbial degradation of diphenylamine under anoxic conditions; Current Microbiology **35**,343-347

Drzyzga, O. (1999): Umweltrelevanz von Diphenylamin - Ein Stoff der 3. EU-Altstoffprioritätenliste. Z. Umweltchem. Ökotox, **11** (6), 365 – 373

EC Official Journal L203 28.07.2001 pp 20-21, Document Number C(2001) 1728

ECB (1997): Doc. ECB 4/TR 2/97

ECB (2000): TGD Revision, chap. 3.5 Toxicokinetics, Draft October 10, 2000, Tgd_h_eft_dr_1_annexB_cdh.

DeEds, F. (1963): Summary of toxicological data. Chronic toxicity studies on diphenylamine. Fd Cosmet Toxicol; **1**: 331-333

Edwards, J.A., Leeming, N.M., Clark, R., Offer, J.M. (1984): Effect of diphenylamine on pregnancy of the New Zealand White rabbit. Huntingdon Research Centre, Huntingdon. Submitted by Pennwalt Corporation, USA, to WHO. Cited from FAO. Pesticide residues in food

Eknoyan, G., Weinman, E.J., Tsaparas, A., Tisher, C.C., Yarger, W.E., Suki, W.N., Martinez-Maldo-nado, M. (1976): Renal function in experimental cystik disease of the rat. J Lab Clin Med; **88**: 402-411

Elf Atochem North America Inc., Civo Institute TNO (1994a). Primary skin irritation of purified diphenylamine in albino rabbits. Document Processing Center, Office of Pollution Prevention and Toxics, EPA, OTS0557208

Elf Atochem North America Inc., Civo Institute TNO (1994b). Eye irritation of purified diphenylamine in albino rabbits. Document Processing Center, Office of Pollution Prevention and Toxics, EPA, OTS0557205

Entenmann, W., Schäcke, G. (1994): The wide-spread diffusive contamination of a former military airport from propellant contamination: Investigation and consequences for the establishment of development plan. Luxembourg International Symposium on the Rehabilitation of Former Military Sites and Demilitarization of Explosive Ordnance, pp. 405-415

Environment Agency Japan (1985): Chemicals in the Environment. Report on Environmental Survey… March 1985, p. 51

EPA (1985): Health and Environmental Effects Profile for N,N-diphenylamine. EPA/600/X-85/393, June 1985

EPA Regristration Eligibility Decision (RED) diphenylamine (1998) EPA738-R-97-010, April 1998

Epler, J.L., Larimer, F.W., Rao, T.K., Nix, C.E., Ho, T. (1978): Energy-related pollutants in the environment: Use of short-term tests for mutagenicity in the isolation and identification of biohazards. Environ Health Perspect; **27**: 11- 20

Epstein, S.S., Saporoschetz, I.B., and Hutner, S.H. (1967): J. Protozool. 14, 238-244

Epstein, W.L. (1976): cited in: Opdyke, D.L.J., diphenylamine. Fd Chem Toxicol 1978; 16, Suppl 1, 723-727

Evan, A.P., Hong, S.H., Gardner, Jr. K.D., Park, Y.S., Itagaki, R. (1978): Evolution of the collecting tubular lesion in diphenylamine-induced renal disease. Laboratory Investigation; **38** (3): 244-252

Fan, L., Vonshak, A., Gabbay, R., Hirshberg, J., Cohen, Z. and Boussiba, S. (1995): The biosynthetic pathway of astaxanthin in a green Alga Haematococcus pluvialis as indicated by inhibition with diphenylamine. Plant Cell Physiol. **36**, 1519-1524

FAO/WHO (1984): Pesticide residues in food. FAO Plant Production and Protection Paper 67: Data and recommendations of the joint meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues, Rome 24 September- 3 October 1984

Farm Chemicals Handbook (1997): R. T. Meister (ed.), Meister Publ. Comp., Vol. **83**, p. C100

Ferretti, J.J., Lu, W., Liu, M.B. (1977): Mutagenicity of benzidine and related compounds employed in the detection of hemoglobin. Amer J Clin Pathol; **67**: 626 - 527

Filmer, A.A. and Rhodes, J.C. (1985): Investigation of sprout-growth-inhibitory compounds in the volatile fraction of potato tubers. Potato Research **28**, 361-377

Fiserova-Bergerova, V., Pierce, J.T., Droz, P.O. (1990): Dermal absorption potential of industrial chemicals: criteria for skin notation. Am. J. Indust. Med. 17, 617 – 635.

Florin, I., Rutberg, L., Curval, M., Enzell, C.R. (1980): Screening of tobacco smoke constituents for mutagenicity using the Ames test. Toxicology; **18**: 219 - 232

Ford, W., Abraham, R., Rockwood, W., Golberg, L. (1972): Observations consequent upon a long-term study of diphenylamine in mice. Toxicol Appl Pharmacol; **22**: 302

Fort, D.J. et al. (1999): Progress toward identifying causes of maldevelopment induced in Xenopus by pond water and sediment extracts from Minnesota, USA. Envir. Toxicol. Chem. 18 (10), 2316-2324

Frank, R., Braun, H.E., and Ripley B.D. (1990): Residues of insecticides, and fungicides in fruit produced in Ontario, Canada, 1986-1988. Food Additives and Contaminants 7, 637-648

Gardner Jr., K.D., Solomon, S., Fitzgerrel, W.W. and Evan, A.P. (1976): Function and structure in the diphenylamine-exposed kidney. J. Clin. Invest. **57**, 796-806

Gardner, A.M., Alvarez, G.H. and Ku, Y. (1982): Microbial Degradation of ^{14}C-diphenylamine in a Laboratory Model Sewage Sludge System. Bull. Environm. Contam. Toxicol. **28**, 91-96.

GDCh (1988): Report No. 15 of the Advisory Committee on Existing Chemicals (Beratergremium für umweltrelevante Altstoffe – BUA) on "diphenylamine"

Geiger, D.L., Brooke, L.T. and Call, D.J. (1990): Acute toxicities of organic chemicals to fathead minnows (*pimephales promelas*); Center for Lake Superior environmental studies, University of Wisconsin, Vol. V, 239-240

Girardon, P., Bessiere, J.M., Baccou, J.C. and Sauvaire, Y. (1985): Volatile constituents of fenugreek seeds. Planta Medica (1985), 533-534

Gorecka-Turska, D., Mekler, U., Gorski, T. (1983): Sister chromatid exchanges in BALB/c mice bone-marrow under the influence of carcinogenic and non-carcinogenic products and half-finished products from dyestuff industry. Bromat Chem Toksykol; XVI (1): 37 - 42

Green, M. and Lillemark, L. (1994): Pesticide residues in Danish food. Publ. National Food Agency of Denmark, 1-80

Griswold, Jr. D.P., Casey, A.E., Weisburger, E.K., Weisburger, J.H., Schabel, Jr. F.M. (1966): On the carcinogenicity of a single intragastric dose of hydrocarbons, nitrosamines, aromatic amines, dyes, coumarins, and miscellaneous chemicals in female Sprague-Dawley rats. Cancer Research; **26**: 619-625

Guo, Z., Pengyan, L., Quirong, A. and Boti, J. (1995): GC-MS analysis of volatile compounds in *Eucommia ulmoides* leaves: Hebei Daue Xuebao, Ziran Kexeuban, China, **15 (3)** 36-39

Gutenmann, W.H., Lisk, D.J. (1975): A feeeding study with diphenylamine in a dairy cow. Bull Environ Contam Toxicol; **13**: 177-180

Gutenmann, W.H., Lisk, D.J. and Blanpied, G.D. (1990): Diphenylamine residues in apples and apple cider. J. Food Saf. **10**, 277-279

Guttenplan, J.B. (1987): N-nitrosamines: bacterial mutagenesis and in vitro metabolism. Mutat Res; 186:81-134

Hardy, T.L. (1974): Experimental surgery in the evaluation of drug induced renal toxicity in the rat with particular reference to diphenylamine. Proc Eur Soc Study of Drug Toxicity; **15**: 337-344

Harker, M. and Young, A.J. (1995): Inhibition of astaxanthin synthesis in the green alga, *Haematococcus pluvialis*. Eur. J. Phycol. **30**, 179-187

Hazardous substances database (HSDB)

Heidelberger, C., Freeman, A.E., Pienta, R.J., Sivak, A., Bertram, J.S., Casto, B.C., Dunkel, V.C., Francis, M.W., Kakunaga, T., Little, J.B., Schechtman, L.M. (1983): Cell transformation by chemical agents - a review and analysis of the literature. A report of the US Environmental Protection Agency gene-tox program. Mutation Research; **114**: 283-385

Hockenbury M.R. and Grady, C.P.L. (1977): Inhibition of nitrification – effects of selected organic compounds; Journal WPCF, (May), 768-777

Holmberg, B., Kronevi, T., Ackevi, S., Ekner, A. (1983): Prövning av carcinogen aktivitet hos difenylamin och gamma-butyrolakton med peroral administrering på hanmöss. Arbete och Hälsa; 34: 1-35

Hong, S.K., Szekerczes, J., Park, Y.S., Kurata, F.K. and Gardner, Jr. K.D. (1974): Effects of diphenylamine on sodium and water transport across toad skin and bladder. Toxicol. Appl. Pharmacol. **27**, 612-620

Hude von der, W., Behm, C., Gürtler, R., Basler, R. (1988): Evaluation of the SOS chromotest. Mutation Res; **203**: 81 - 94

Human lymphocytes (1996): sister chromatid exchanges and cell cycle delay. J Biol Res, Boll Soc Ital Biol Sper; Vol 72, 171-178

Intarapichet K. and Bailey, M.E. (1992): Organic volatiles from bacteria grown on beef; Thai J. Agric. Sci. **25(4)**, 299-326

JMPR (1998): Diphenylamine (addendum). First Draft prepared by A. Protzel, Environmental Protection agency, Washington DC, United States (http://www.inchem.org/documents/jmpr/jmpmono/v098pr07.htm).

Johnson, G.D., Geronimo, J. and Hughes, D.L. (1997): Diphenylamine residues in apples (*Malus domestica* Borkh.), cider, and pomace following commercial controlled atmosphere storage. J. Agric. Food Chem. **45**, 976-979

Kaiser K.L.E., Palabrica, V.S. and Ribo, J.M. (1987): QSAR of acute toxicity of mono-substituted benzene derivatives to *photobacterium phosphoreum*; QSAR in environmental toxicology – II, 153-168, ed. by Reidel Publishing Comp

KAN-DO Office and Pesticides Team (1995): Accumulated pesticide and industrial chemical findings from a ten-year study of ready-to-eat foods. Journal of AOAC International **78**, 614-631

Karawya, M.S., Khayyal, S.E., Farrag, N.M. and Ayad, M.M. (1984): Diphenylamine, an Antihyperglycemic Agent from Onion and Tea : Journal Natural Products **47 (5)**, 775-780

Karawya, M.S., Dhayyal, S.E., Farrag, N.M. and Ayad, M.M. (1986): Screening of diphenylamine as an antihyperglycemic agent in certain edible plant organs: Acta Pharmaceutica Hungarica **56**, 55-58

Kenaga, E.E. and Groing, C.A.I. (1980): Relationship between Water Solubility, Soil-Sorption, Octanol-Water Partitioning and Bioconcentration of Chemicals in Biota. Pre-publication copy of paper dated October 13, 1978, given at the American Society for Testing and Materials, Third Aquatic Toxicology Symposium, October 17-18, 1978, New Orleans, LA. (Symposium papers were published by STAM, Philadelphia, PA, as Special Technical Publication (STP) 707 in March 1980). Cited in Lyman, Reehl and Rosenblatt (1982). Handbook of Chemical Property Estimation Methods.

Kiplinger, G. (1995) Skin sensitization study of diphenylamine technical in albino guinea pigs. Final report: Lab project No. WIL-256001. Unpublished study, WIL Research Labs, Inc. Submitted to WHO by the DPA Task Force, John Wise & Associates Ltd, Liberty, Missouri, USA

Korolev, A.A., Arsenieva, M.V., Vitvitskaya, B.R., Zakharova, T.A., Kinzirsky, A.S. (1976): Experimental data for hygienic standardization of diphenylamine and diphenyldiethylurate in water bodies. Gig Sanit; **5**: 21 - 25

Krohmer, R.W. (1992a): 90 Day subchronic toxicity evaluation of diphenylamine in rats. Unpublished study No. 426C-10-034-91 from T.P.S., Inc., Mt Vernon, Indiana, USA. Submitted to WHO by the DPA Task Force, John Wise & Associates Ltd, Liberty, Missouri, USA

Krohmer, R.W. (1992b): 90 Day evaluation of diphenylamine in the dog. Unpublished study No. 426C-501-034-91 from T.P.S., Inc., Mt Vernon, Indiana, USA. Submitted to WHO by the DPA Task Force, John Wise & Associates Ltd, Liberty, Missouri, USA

Layer, R.W. (1991): Diarylamines in "Encyclopedia of Chemical Technology". Kirk-Othmer (ed), 4th ed., vol.**2**, p.452

Lenz, S.D., Carlton, W.W. (1990): Diphenylamine-induced renal papillary necrosis and necrosis of the pars recta in laboratory rodents. Vet Pathol; **27**: 171-178

Lenz, S.D., Carlton, W.W. (1991): Decreased incidence of diphenylamine-induced renal papillary necrosis in Syrian hamsters given dimethylsulphoxide. Fd Chem Toxic; **29** (6): 409-418

Lide, David R., PhD., CRC Handbook of Chemistry and Physics, 72nd Edition, 1991-1992, CRC Press, Boca Raton, Ann Arbor, Boston

Liebisch, G. (2001): Information from the Tierärztliche Hochschule Hannover, October 2001

Litton Bionetics Inc. (1977), Mutagenicity evaluation of AP 1185 final report. LBI Project NO. 2683, Litton Bionetics, Kensington, Maryland, June 1977, unpublished report submitted to BFGoodrich Chemical Company, Cleveland, Ohio 05/10/94, EPA OTS0572477 with cover letter dated 05/06/94

Lodovici, M., Casalini, C., Briani, c., Dolara, P. (1997): Oxidative liver DNA damage in rats treated with pesticide mixtures. Toxicology **117**, 55-60

Loeb, H.A. and Kelly, W.H. (1963): Acute oral toxicity of 1 496 chemicals force-fed to carp. U.S. Fish/Wildl. Service, Sp. Sci. Rep. Fish No. **471**, Washington D.C.

Lunde, G., Gether, J., Gijos, N. and Landé, M.-B.S. (1977): Organic micropollutants in precipitation in Norway. Atmos. Environment **11**, 1007-1014

Lyman, W.J., Reehl, W.F. and Rosenblatt, D.H. (1982): Handbook of Chemical Property Estimation Methods; McGraw-Hill Book Company

Malaney, G.W. (1960): Oxidative abilities of aniline-acclimated activated sludge. J. WPCF **32**, 1300-1311

Mastelic, J., Milos, M., Kustrac, D. and Radonic, A. (1998): The essential oil and glycosidically bound volatile compounds of Calamintha nepeta (L.) savi: Croatia Chemical Acta **71 (1)** 147-154

Mobile Oil Corporation (1985) An Ames Salmonella/mammalian microsome mutagenesis pre-incubation screening assay for determination of potential mutagenicity with diphenylamine. Project No. 813238, submitted to EPA on 05/02/94, EPA 0TS0572471 with cover letter dated 04/26/94

Mobile Oil Corporation (1987): Micronucleus assay of bone marrow red blood cells from rats treated via dermal administration of diphenylamine. Unpublished study No. 61956, submitted to EPA on 05/02/94, EPA 0TS0572472 with cover letter dated 04/26/94

Mobile Oil Corporation (1994): Thirteen week dermal assay of diphenylamine in rats. Unpublished study No. 61955, submitted to EPA on 05/02/94, EPA OTS0572473 with cover letter dated 04/26/94

Moio, L., Dekimpe, J., Etievant, P. and Addeo, F. (1993): Neutral volatile compounds in the raw milks from different species; Journal of Dairy Research **60**, 199-213

Moio, L., Rillo, L., Ledda, A. and Addeo, F. (1996): Odorous constituents of ovine milk in relationship to diet; J. Dairy Sci. **79** , 1322-1331

Moore, W. (1978): Diarylamines in "Encyclopedia of Chemical Technology". Kirk-Othmer (ed), 3rd ed., vol.**2**, p. 329

Muller, A., Jacobson, H., Healy, E., McMickan, S., Istace, F., Blaude, M.-N., Howden, P., Fleig, H. and Schulte, A. (2005): Hazard Assessment of chemicals inducing haemolytic anaemia: An EU Regulatory perspective. Regul. Toxicol. Pharmacol. (submitted)

Murin M., Gavora, J., Drastichová, I., Dusková, E., Madsen, T., Torslov, J., Damborg, A., Tyle, H. and Pedersen, F. (1997): Aquatic hazard and risk assessment of two selected substances produced in high volumes in the Slovak Republic; Chemosphere **34(1)**, 179-190

Murli, H. (1992a): Dose range-finding study on diphenylamine for in vivo murine micronucleus assay. Unpublished report No. 14902-0-459PO from Hazleton Washington, Inc., Rockville, Maryland, and Vienna, Virginia, USA. Submitted to WHO by the DPA Task Force, John Wise & Associates Ltd, Liberty, Missouri, USA

Murli, H. (1992b): Mutagenicity test on diphenylamine for in vivo mammalian micronucleus assay. Unpublished report No. 14902-0-455 from Hazleton Washington, Inc., Rockville,

Maryland, and Vienna, Virginia, USA. Submitted to WHO by the DPA Task Force, John Wise & Associates Ltd, Liberty, Missouri, USA.

Muszkat, L., Raucher, D., Magaritz, M., Ronen, D. and Amiel, A.J. (1993): Unsaturated Zone and Ground-Water Contamination by Organic Pollutants in a Sewage-Effluent-Irrigated Site. Ground Water **31(4),** 556-565.

Nishiuchi, Y. (1989): Toxicity of pesticides to some aquatic animals. XI. Toxicity of some pesticides to tadpoles. Seitai Kagaku **9 (4)**, 23-26

Nose, M., Nakatani, Y. and Yamanishi, T. (1971): Studies on the flavor of green tea; Agric.Biol.Chem. **35(2)**, 261-271

Oettmeier, W. (1979): Quantitative structure activity relationship of diphenylamines as inhibitors of photosynthetic electron transport and photophosphorylation. Z. Naturforsch. **34c**, 1024-1027

Opdyke, D.L.J. (1978): Diphenylamine. Fd Chem Toxicol; **16**, Suppl 1, 723-727

Orth, K. et al. (1994): Spreng und Kampfstoffe im Bereich einer Altlast: Aufgaben an Analytik und Untersuchung. In „Abfallwirtschaft in Forschung und Praxis Bank 69", published in: Militärische Lasten 1994. Ed. H. Pfaff-Schley; Erich Schmidt-Verlag Berlin

Pahren, H.R. and Melton, R.G. (1979): Mutagenic activity and trace organics in concentrates from advanced wwtp effluents. Proc. Water Reuse Symp. **3**, 2170-2186

Philbert, M., Girard-Wallon, C., Carçon, J., Ripault, J., Berry, J-P. (1978): La polykystose expérimentale du rein induite par la diphénylamine (phénylamine, anilinobenzène). Archive des maladies professionelles, de médicine du travail et de Sécurité Sociale (Paris); **40**: 685-697

Piet, G.J. and Morra, C.F. (1983): Behaviour of organic Micropollutants during Underground Passage. Cit. in EPA (1985)

Pluta, H.-J. (1990): Fischtests mit Diphenylamin. Unpublished report from Inst. Wasser-, Boden- und Lufthygiene

Probst, G.S., McMahon, R.E., Hill, L.E., Thompson, C.Z., Epp, J.K., Neal, S.B. (1981): Chemically-induced unscheduled DNA synthesis in primary rat hepatocyte cultures: A comparison with bacterial mutagenicity using 218 compounds. Environ Mutag; **3**: 11 - 32

Rheinchemie/Bayer AG (2001): business information, September 2001

Rodwell, D.E. (1992a): Range finding teratology study in rats with diphenylamine (DPA). Unpublished study No. 3255.2 prepared by Springborn Laboratories, Inc., Life Sciences Division, Spencerville, Ohio, USA. Submitted to WHO by the DPA Task Force, John Wise & Associates Ltd, Liberty, Missouri, USA.

Rodwell, D.E. (1992b): Teratology study in rats with diphenylamine (DPA). Unpublished study No. 3255.3 prepared by Springborn Laboratories, Inc., Life Sciences Division,

Spencerville, Ohio, USA. Submitted to WHO by the DPA Task Force, John Wise & Associates Ltd, Liberty, Missouri, USA.

Rodwell, D.E. (1993): Two-generation reproduction study in rats with diphenylamine (DPA). Unpublished study No. 3255.4 prepared by Springborn Laboratories, Inc., Life Sciences Division, Spencerville, Ohio, USA. Submitted to WHO by the DPA Task Force, John Wise & Associates Ltd, Liberty, Missouri, USA.

Römpp Chemie Lexikon, Version 1.0, Stuttgart/New York, Georg Thieme Verlag, 1995

Rudd, A. (2002): Marking of Fuel – The EU makes slow progress, European Refining and Marking Vol.1, Number 10, 15. November 2002

Verordnung über Höchstmengen an Rückständen von Pflanzenschutz- und Schädlingsbekämpfungsmitteln, Düngemitteln und sonstigen Mitteln in oder auf Lebensmitteln und Tabakerzeugnissen (Rückstands-Höchstmengenverordnung – RHmV, 7. Änderungs-VO) BGBl. Jahrgang 2003 Teil 1 Nr. 1, S. 11 ff. vom 14. Januar 2003 a (b)

Sander, J., Schweinsberg, F. (1972): In vivo and in vitro experiments on the formation of N-nitroso compounds from amines or amides and nitrate or nitrite. In: N-nitroso-Compounds, Analysis and Formation. Proceedings of a working Conference held at Heidelberg 1971 (Bogovski P, Preussmann R, Walker EA, Hrsg.) IARC Sci Publ; No 3, Lyon, P 97-103

Sander, J., Schweinsberg, F., Menz, H-P. (1968): Untersuchungen über die Entstehung cancerogener Nitrosamine im Magen. Hoppe-Seyler's Z Physiol Chem; **349**: 1691-1697

Schenk, H.-P. (1986): Tabellierte Einzelbestimmungen organischer Stoffe in der Umwelt. Lit.-Recherche im Auftrag des UBA Berlin. FE-Vorhaben 106 01 023/03

Schneider, K., Hassauer, M., Oltmanns, J., Schuhmacher-Wolz, U., Elmshäuser, E., Mosbach-Schulz, O. (2004): Wahrscheinlichkeitsrechnung zur Wirkungsabschätzung bei Arbeitnehmern, Endbericht des Forschungsvorhabens F1824-26 der Bundesanstalt für Arbeitsschutz und Arbeitsmedizin, in press

Schubert, D. (2000): personal communication to Bernd Scharenberg, UBA

Siglin, J.C. (1991): Repeated dose dermal toxicity: 21 Day study.

Siglin, J. (1992): 21-Day dermal toxicity study in rabbits with diphenylamine (DPA): final report: Lab Project Number: 3255. 1. Unpublished study prepared by Springborn Labs, Inc. 257p.

Slovak, A.J.M. (1980): Contact dermatitis due to benzisothiazolone in a works analytical team. Contact Dermatitis; **6**: 187-190

Sporstol, S., Urdahl, K., Drangsholt, H. and Gjoes, N. (1985): Description of a method for automated determination of organic pollutants in water. Intern. J. Environ. Anal. Chem. **21**, 129-138

Srour, R. (1994): Aromatic intermediates and derivatives, diphenylamine & derivatives, p. C.IV.II.1 – II.8, add. May 1994

Standards zur Expositionsabschätzung (1995): Bericht des Ausschusses für Umwelthygiene, Arbeitsgemeinschaft der leitenden Medizinalbeamtinnen und -beamten der Länder. Herausgeber: Behörde für Arbeit, Gesundheit und Soziales, Hamburg

Sugai, S., Murata, K., Kitagaki, T., Tomita, I. (1990): Studies on eye irritation caused by chemicals in rabbits. - 1. A quantitative structure-activity relationships approach to primary eye irritation of chemicals in rabbits. J Toxicol Sci; **15**: 245-261

Sugimura, T., Nagao, M., Wakabayashi, K. (1982): Metabolic aspects of the comutagenic action of Norharman. Adv Exp Med Bio; 136 B: 1011 - 1025

Sun Y., Mingqi L. and Youhui, Y. (1997): Studies on odorous substances in refined Konjak flour: Shipin Yu Fajiao Gongye, China; **23 (4)** 25-29

Swedish Kemikalieninspektionen

Taschenbuch für Chemiker und Physiker, vierte Auflage, Band II, organische Verbindungen, Springer Verlage, Berlin/Heidelberg/New York/Tokyo, 1983

Thomas, J.O., Cox, Jr. A.J., DeEds, F. (1957): Kidney cysts produced by diphenylamine. Stanford Med Bull; **15**: 90-93

Thomas, J.O., Ribelin, W.E., Wilson, R.H., Keppler, D.C., DeEds, F. (1967a): Chronic Toxicity of diphenylamine to Albino Rats. Toxicol Appl Pharmacol; **10**: 362-374

Thomas, J.O., Ribelin, W.E., Woodward, J.R., DeEds, F. (1967b): The chronic toxicity of diphenylamine for dogs. Toxicol Appl Pharmacol; **11**: 184-194

Tonogai, Y. Ogawa, S., ITO, Y. and Iwaida, M. (1982): Actual Survey on TLm Values of Environmental Pollutants, especially on Amines, Nitriles, Aromatic N Compounds and Artificial Dyes. J. Toxicol. Sci. 7, 193-203

UBA (1995): Bewertung der Gefährdung von Mensch und Umwelt durch ausgewählte Altstoffe; Diphenylamin, Umweltbundesamt Texte 38/95, 107-111 + 375-385

Ullmann´s Encyclopedia of Industrial Chemicals (1974): 4th Edition. Editors: Elvers, Hawkins, Schulz G. Volume 7.

Uniroyal Chemical (1999): unpublished information from 12.05.1999

US-EPA, Office of pollution provention and toxic´s use cluster scoring system

Valvis, I.I., Lischick, D., Shen, D., Sofer, S.S. (1990): In vitro synthesis of nitroxide free radicals by hog liver microsomes. Free Radical Biol Med; **9**: 345-353

Veith, G.D., De Foe, D.L., Bergstedt, V. (1979): Measuring and Estimating the Bioconcentration Factor of Chemicals in Fish. J. Fish Research Board Canada **36**, 1040-1048

Voullaire, E., Kliemt, G. (1995): Gefahrstoffe in Klein- u. Mittelbetrieben: Neue Wege überbetrieblicher Unterstützung; Fb 703, Schriftenreihe der Bundesanstalt für Arbeitsschutz und Arbeitsmedizin, Wirtschaftsverlag NW

Wickramaratne, G.A. de S. (1987): The Chernoff-Kavlock Assay: Its Validation and Application in Rats. Teratogenesis, Carcinogenesis and Mutagenesis; **7**: 73-83

Wilson, W.W., Khoobyarian, N. (1982): Potential identification of chemical carcinogens in a viral transformation system. Chem-Biol Interactions; **38** 253-259

Woodhouse, M. A., Offer, J., Darmady, E. M.: (1965) Diphenylamine induced polycystic kidneys compared with human polycystic kidney: electron microscopical observations. Nephron 2, 253-254

Wu, D. (1993): Diphenylamine: Rat metabolism study. Unpublished study XBL No. 92081, report No. RPT00131 from Xenobiotic Laboratories, Inc., Plainsboro, New Jersey, USA. Submitted to WHO by the DPA Task force, John Wise Associates Ltd, Liberty, Missouri, USA.

Yasuhara, A., Shiraishi, H., Nishikawa, M., Yamamoto, T., Uehiro, T., Nakasugi, O., Okumura, T., Kenmotsu, K., Fukui, H., Nagase, M., Ono, Y., Kawagoshi, Y., Baba, K. and Noma, Y. (1997): Determination of organic components inleachates from hazardous waste disposal sites in Japan bygas chromatography-mass spectrometry. J. Chromatogr. A, **774**, 321-332

Yoshida, J., Shimoji, N., Furuta, K., Takamura, N., Uneyama, C., Yazawa, R., Imaida, K., Hayashi, Y. (1989): Twenty-eight day repeated dose toxicity testing of diphenylamine in F344 rats. Eisei Shikensho Hokoku (Tokyo); **107**: 59-62

Zeiger, E., Anderson, B., Haworth, S., Lawor, T., Mortelmans, K. (1988): Salmonella mutagenicity tests: IV. Results from the testing of 300 chemicals. Environ Molec Mutag; 11 (supp 12): 1-158

Zhao, Y.-H., Wang, L., Gao, H. and Zhang, Z. (1993): Quantitative structure-activity relationships - Relationships between toxicity of organic chemicals to fish and to photobacterium phosphoreum. Chemosphere **26**, 1971-1979

Zhao Y.-H. and Wang, L.-S. (1995): Quantitative structure-activity relationships of hydrophobic organic chemicals; Toxicological and environmental chemistry **50**, 167-172

The report provides the comprehensive risk assessment of the substance Diphenylamine. It has been prepared by Germany in the frame of Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances, following the principles for assessment of the risks to man and the environment, laid down in Commission Regulation (EC) No. 1488/94.

The evaluation considers the emissions and the resulting exposure to the environment and the human populations in all life cycle steps. Following the exposure assessment, the environmental risk characterisation for each protection goal in the aquatic, terrestrial and atmospheric compartment has been determined.

Clarification of the production and use situation in the European Union is needed before definitive conclusions can be drawn. Also information on emissions to the environment and waste water treatment are needed. Diphenylamine is also considered in the framework of the Plant Protection Products Directive.

For human health the scenarios for occupational exposure, consumer exposure and humans exposed via the environment have been examined and the possible risks have been identified. The human health risk assessment concludes that there is no concern for any of these populations.