

# **European Union Risk Assessment Report**

## **4-METHYL-M-PHENYLENEDIAMINE**

**(TOLUENE-2,4-DIAMINE)**

### **RISK ASSESSMENT**

CAS-No.: 95-80-7

EINECS-No.: 202-453-1

28.05.2008

***FINAL APPROVED VERSION***

Information on the rapporteur

Contact point:

Bundesanstalt für Arbeitsschutz und Arbeitsmedizin

Anmeldestelle Chemikaliengesetz

Friedrich-Henkel-Weg 1-25

44149 Dortmund

e-mail: chemg@baua.bund.de

This document is the last version of the Comprehensive Risk Assessment Report **Toluene-2,4-diamine**, a substance chosen from the EU 1st Priority List in 1994.

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## 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) 793/93<sup>1</sup> 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) 1488/94<sup>2</sup>, which is supported by a technical guidance document<sup>3</sup>. 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.

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

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

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

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**0 OVERALL CONCLUSIONS/RESULTS OF THE RISK ASSESSMENT**

CAS No. 95-80-7

EINECS No. 202-453-1

IUPAC Name Toluene-2,4-diamine

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
- ( X ) 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 to the site dye1 that processes 2,4-TDA to dyes.

PEC/PNEC ratios for wastewater treatment plant, surface water and sediment are above 1 for the scenario “processing of 2,4-TDA to dyes” at site dye1. As this scenario is fully based on default values, improvement of the exposure data basis is possible. Information on TDA emission from the production of dyes at this site should be provided.

**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 to the aquatic compartment and for wastewater treatment plants for all other sites and the environmental compartments atmosphere and soil and secondary poisoning.

## Human Health

### Workers

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

There is a need for better information to adequately characterise the risks regarding the mutagenicity (germ cell mutagenicity) and developmental toxicity because the current database does not adequately cover these endpoints. The collection of additional information should, however, not delay the implementation of appropriate control measures needed to address the concern related to other endpoints (conclusion (i) on hold).

**Conclusion (iii)** There is a need for specific measures to limit the risks

There is concern for mutagenicity (somatic cell mutagenicity) and carcinogenicity as a consequence of dermal and inhalation exposure arising from all investigated occupational exposure scenarios. Extensive technical and organisational reduction measures have already led to very low levels of exposure. Carcinogenicity risk assessment was conducted with a quantitative approach. Additionally a risk evaluation for this endpoint was done by calculating with different levels of risk acceptance. The specific conclusions for the different occupational exposure scenarios critically depend on the chosen level of risk acceptance. This comparison may be helpful for risk managers in order to evaluate the necessity and priority of further risk reduction measures beyond those that has already been successfully implemented.

There is concern for skin sensitisation as a consequence of dermal exposure arising from all investigated occupational exposure scenarios. Risks of skin sensitisation are considered to be small. However, because the corresponding risk cannot be quantified or excluded, a general concern for skin sensitisation is expressed.



Consumer

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

Since a consumer exposure seems not to exist, a health risk of consumers is not expected.

Man exposed indirectly via the environment

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

There is a need for better information to adequately characterise the risks regarding the mutagenicity (germ cell mutagenicity) and developmental toxicity because the current database does not adequately cover these endpoints. The collection of additional information should, however, not delay the implementation of appropriate control measures needed to address the concern related to other endpoints (conclusion (i) on hold).

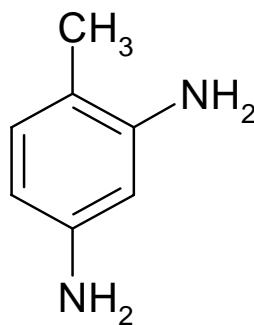
**Conclusion(iii)** There is a need for specific measures to limit the risks

The risk assessment shows that the margin of exposure could be assumed to be sufficient for mutagenicity (somatic cell mutagenicity) and carcinogenicity, but that risks cannot be excluded at any exposure, as the substance is considered as genotoxic carcinogen.

## 1 GENERAL SUBSTANCE INFORMATION

### Identification of the substance

CAS-No.:	95-80-7
EINECS-No.:	202-453-1
IUPAC Name:	1,3-diamino-4-methylbenzene
CAS Index Name:	1,3-benzenediamine, 4-methyl-
Synonyma:	2,4-TDA 2,4-toluylenediamine 4-methyl-m-phenylenediamine 2,4-diamino-1-methylbenzene toluene-2,4-diamine
Empirical formula:	C <sub>7</sub> H <sub>10</sub> N <sub>2</sub>
Molecular weight:	122.17 g/mol
Structural formula:	



Commercial TDA consists of a mixture of 2,4- and 2,6-isomers. Therefore beside 2,4-TDA the identification of 2,6-TDA is drawn up in the following:

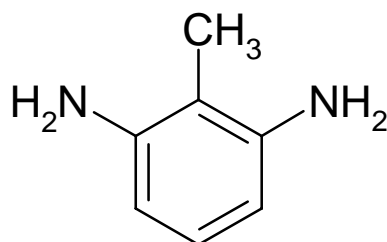
CAS No.:	823-40-5
EINECS No.:	212-513-9
IUPAC Name:	1,3-Diamino-2-methylbenzene
CAS Index Name:	1,3-benzenediamine, 2-methyl-
Synonyma:	2,6-TDA

2,6-toluylenediamine  
2-methyl-m-phenylenediamine  
2,6-diamino-1-methylbenzene  
toluene-2,6-diamine

Empirical formula:  $C_7H_{10}N_2$

Molecular weight: 122.17 g/mol

Structural formula:



Three 2,4-toluylenediamine-containing products are industrially important:

2,4/2,6-TDA (80/20) with 80 % 2,4-TDA and 20 % 2,6-TDA (CAS Nr. 25376-45-8)

2,4/2,6-TDA (65/35) with 65 % 2,4-TDA and 35 % 2,6-TDA

2,4-TDA with around 99 % 2,4-TDA.

The products 2,4/2,6-TDA (80/20) and 2,4/2,6-TDA (65/35) are produced as mixtures.

If only TDA is mentioned in the text there is no information available about the composition.

### **Purity/impurities, additives**

The 2,4-TDA may contain the following impurities:

Water < 0.1 %

2,3-Toluylenediamine < 0.2 %

Dinitrotoluenes < 0.1 %

Sum of other organic compounds  
(e.g. aniline, m-phenylenediamine) < 0.5 %

Trace amounts of 2,5-TDA, 3,4-TDA and 3,5-TDA may be present.

**Physico-chemical properties****2,4-TDA**

2,4-TDA is a clear colourless solid (at room temperature and normal pressure) with an aromatic 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 2,4 TDA

Melting point	99 °C <sup>1)</sup>	I.I.I., 2000
Boiling point	288 °C <sup>1)</sup>	I.I.I., 2000
Relative density	1.256 at 20 °C <sup>2)</sup>	I.I.I., 2000
Vapour pressure	0.017 Pa at 25 °C <sup>3)</sup>	I.I.I., 2000
Surface tension	not determined	
Water solubility	38 g/l at 25 °C <sup>4)</sup>	I.I.I., 2000
Partition coefficient	log Pow 0.074 at 25 °C <sup>5)</sup> log Pow 0.34 at 20 °C(calc)	I.I.I., 2000
Flash point	not determined	substance is a solid
Auto flammability	not flammable up to the melting point (99 °C) <sup>6)</sup>	BAM, 2003
Flammability	not flammable <sup>7)</sup>	I.I.I., 2000
Explosive properties	not explosive <sup>8)</sup>	I.I.I., 2000
Oxidizing properties	no oxidizing properties <sup>8)</sup>	I.I.I., 2000
Henry's law constant	$5.46 \cdot 10^{-5} \text{ Pa} \cdot \text{m}^3 \cdot \text{mol}^{-1}$	

<sup>1)</sup> DSC

<sup>2)</sup> Pycnometer method

<sup>3)</sup> Further values for the vapour pressure at 150 °C (14.7 hPa), 160 °C (22.7 hPa) and 180 °C (48 hPa) can be found in the literature (Milligan and Gilbert, cited in Kirk-Othmer, Encyclopaedia of chemical technology, 1978) but information about the purity of the test substance, the test method and the test conditions is missing. For the risk assessment the

value of 0.017 Pa at 25 °C is recommended. This value is derived from an experiment using the effusion method.

- 4) The values for the water solubility cited in the safety data sheets are varying between 40.7 g/l and 50 g/l at 25 °C respectively 35 g/l and 37.8 g/l at 20 °C without further information. For the risk assessment the value of 38 g/l at 25 °C is recommended. This value is derived from an experiment using the flask method.
- 5) The partition coefficient n-octanol/water was determined using the shaking flask method and resulted in a logPow value of 0.07 at 25 °C. According to Leo Hansch the logPow is calculated to be 0.34. For the risk assessment the experimental value is preferred. In the literature (Hernandez, J.W.: Phenylenediamines. Federal Register, 1982; vol. 47, no. 5) a log Pow of 0.5 is cited. Due of the lack of information about the purity of the test substance, the method and the test condition this value is not used for the risk assessment.
- 6) After general state of knowledge an auto flammability according to A.16 is not to be expected.
- 7) 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 formate in contact with water or the substance has pyrophoric properties
- 8) No test conducted because of structural reasons

**2,6-TDA**

2,6-TDA is a clear colourless solid (at room temperature and normal pressure). Data on the physical and chemical properties are given in table 1.2.

**Table 1.2** Data on the physical and chemical properties of 2,6 TDA

Melting point	103 – 105 °C <sup>1)</sup>	I.I.I., 2001
Boiling point	289 °C <sup>1)</sup>	I.I.I., 2001
Relative density	1.201 at 20 °C <sup>2)</sup>	I.I.I., 2001
Vapour pressure	0.029 Pa at 25 °C <sup>3)</sup>	I.I.I., 2001
Surface tension	no data available	
Water solubility	54 g/l at 25 °C ( pH 8.4) <sup>4)</sup>	I.I.I., 2001
Partition coefficient	log Pow -0.137 at 25 °C <sup>5)</sup> log Pow 0.34 at 20 °C(calc)	I.I.I., 2001
Flash point	not determined	substance is a solid
Auto flammability	not flammable up to the melting range (103 - 105 °C) <sup>6)</sup>	BAM, 2003
Flammability	not flammable <sup>7)</sup>	I.I.I., 2001
Explosive properties	not explosive <sup>8)</sup>	I.I.I., 2001
Oxidizing properties	no oxidizing properties <sup>8)</sup>	I.I.I., 2001
Henry's law constant	$6.55 \cdot 10^{-5} \text{ Pa} \cdot \text{m}^3 \cdot \text{mol}^{-1}$	

<sup>1)</sup> DSC

<sup>2)</sup> Pycnometer method

<sup>3)</sup> Further values for the vapour pressure at 150 °C (21.3 hPa), 160 °C (33.3 hPa) and 180 °C (76 hPa) can be found in the literature (Milligan and Gilbert, cited in Kirk-Othmer, Encyclopaedia of chemical technology, 1978) but information about the purity of the test substance, the test method and the test conditions is missing. For the risk assessment the value of 0.029 Pa at 25 °C is recommended. This value is derived from an experiment using the effusion method.

<sup>4)</sup> In the safety data sheet of the Bayer AG a value of 60 g/l at 15 °C is quoted without any information about the used method and the test conditions. For the risk assessment the value

of 54 g/l at 25 °C is recommended. This value is derived from an experiment using the flask method.

- 5) The partition coefficient n-octanol/water was determined using the shaking flask method and resulted in a logPow value of -0,137 at 25 °C. According to Leo Hansch the logPow is calculated to be 0.34. For the risk assessment the experimental value is preferred.
- 6) After general state of knowledge an auto flammability according to A.16 is not to be expected.
- 7) 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 formate in contact with water or the substance has pyrophoric properties.
- 8) No test conducted because of structural reasons.

### **2,4-/2,6-TDA(80/20)**

2,4-/2,6-TDA is a clear colourless solid (at room temperature and normal pressure). Data on the physical and chemical properties are given in table 1.3.

**Table 1.3** Data on the physical and chemical properties of 2,4-/2,6-TDA(80/20)

Melting point	80 - 90 °C	WHO, 1987
Boiling point	283 °C at 1011 hPa	Air Products, 1982
Relative density	1.2646 at 20 °C <sup>1)</sup>	Bayer, 1995a
Vapour pressure	$7.4 \cdot 10^{-3}$ Pa at 20 °C	Air Products, 1990
Surface tension	72.68 mN/m at 20 °C <sup>2)</sup>	Bayer, 1995b
Water solubility	42 g/l at 38 °C <sup>3)</sup>	Air Products, 1990
Partition coefficient	not determined	substance is a mixture
Flash point	not determined	substance is a solid
Auto flammability	not flammable up to the melting range (80 - 90 °C) <sup>4)</sup>	BAM, 2003
Flammability	not flammable <sup>5)</sup>	BAM, 2003
Explosive properties	not explosive <sup>6)</sup>	BAM, 2003
Oxidizing properties	no oxidizing properties <sup>6)</sup>	BAM, 2003

<sup>1)</sup> Pycnometer method

<sup>2)</sup> Ring method

<sup>3)</sup> In the safety data sheet of the Bayer AG a water solubility of 100 g/l at 15 °C is cited. Due to the lack of information about the purity of the test substance, the test method and the test conditions this value is not taken into account for the risk assessment.

<sup>4)</sup> After general state of knowledge an auto flammability according to A.16 is not to be expected.

<sup>5)</sup> A.10, A.12 and A.13 were not conducted. From the results of the tests with 2,4-TDA and 2,6-TDA it has to be assumed that also 2,4-/2,6-TDA is not flammable according to A.10. Due to the properties and the handling of the substance it has not to be assumed that flammable gases formate in contact with water or the substance has pyrophoric properties

<sup>6)</sup> No test conducted because of structural reasons.

The following risk assessment report is performed for 2,4-TDA in particular. Where appropriate, data for 2,4/2,6-TDA (80/20) are considered.



## Classification

### 2,4 - TDA

- **Classification according to Annex I of the directive 67/548/EEC - 26. ATP**

Category 2 carcinogen

T	Toxic
R 45	May cause cancer
R 21	Harmful in contact with skin
R 25	Toxic if swallowed
R 36	Irritating to eyes
R 43	May cause sensitization by skin contact
N	Dangerous for the environment
R 51/53	Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

- **Proposal of the rapporteur**

Classification as carcinogenic cat. 2, R 45 is confirmed on the basis of longterm studies in rats and mice. Classification as toxic, R 25 and R 21 is confirmed on the basis of acute studies in rats. Classification as sensitizing, R 43 is confirmed on the basis of animal data (Magnusson Kligmann test).

Classification as irritant, R 36 is not confirmed. Reasons for the proposed change in labelling of local effects: Draize eye irritation test demonstrated that pure toluene-2,4-diamine (2,4-TDA) causes only slight conjunctival irritation in the eyes of rabbits. Therefore the substance has not to be labelled.

### Additional classification as

Mutagenic Cat 3	
R 68	Possible risks of irreversible effects
Reprotoxic Cat 3	
R 62	Possible risk of impaired fertility
R 48/22	Danger of serious damage to health by prolonged exposure if swallowed.

is proposed on the basis of

- positive in vitro and in vivo genotoxicity data (R 68)
- severe effects on sertoli cells of male rats (fertility impairment) in feeding studies (R 62)
- serious health effects consisting of liver lesions in male and female rats, induced the development of chronic renal disease in F344 rats of both sexes (most marked in males), and premature deaths of rats due to hepatotoxicity at  $\geq 5.9$  mg/kg bw/day (long-term feeding study, NCI 1979); testicular atrophy at approximately 28 mg/kg bw/day (15 months study, Stula and Aftosmis 1976) and inhibited spermatogenesis (66% reduction) associated with a significant reduction in the weights of seminal vesicles and epididymides, morphological damage of Sertoli cells as well as with a diminished level of serum testosterone and an elevation of serum LH in male rats at doses of 15 mg/kg bw/day (Thyssen et al. 1985a, 1985b, Varma et al. 1988) (R48/25)

### 2,6-TDA

- Classification according to Annex I - 26. ATP

Category 3 mutagen

Xn	Harmful
R 68	Possible risks of irreversible effects
R 21/22	Harmful in contact with skin and if swallowed
R 43	May cause sensitization by skin contact
N	Dangerous for the environment
R 51/53	Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

### 2,4-TDA/2,6-TDA (CAS-No. 25376-45-8)

- Classification according to Annex I of the directive 67/548/EEC - 26. ATP

Category 2 carcinogen

T	Toxic
R 45	May cause cancer
R 20/21	Harmful by inhalation and in contact with skin
R 25	Toxic if swallowed

R 36	Irritating to eyes
R 43	May cause sensitization by skin contact
N	Dangerous for the environment
R 51/53	Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

- Proposal of the rapporteur

Classification as carcinogenic cat. 2, R 45 is confirmed on the basis of longterm studies in rats and mice. Classification as toxic, R 25 and R 21 is confirmed on the basis of acute studies in rats. Classification as sensitizing, R 43 is confirmed on the basis of animal data (Magnusson Kligmann test). Classification as irritant, R 36, is confirmed on the results of a test with rabbits in which the mixture caused erythema and chemosis.

Classification as Xn, R 20 "Harmful by inhalation" is not further supported on the basis of studies on rats and mice in which no mortality occurred after a 4 hour inhalation to a concentration of about 5.57 mg/l.

#### Additional classification

The proposal for classification is based on data from a well conducted carcinogenicity study in F344 rats (NCI, 1979, Cardy, 1979), and in addition from mid- and long-term diet studies using Sprague-Dawley, ChP-CD and Wistar rats comparable to guideline studies with acceptable restrictions (Ito et al. 1969, Stula and Aftosmis 1976). The critical adverse effects in rats of both sexes after long-term administration of 2,4-TDA were hepatotoxicity and chronic renal failure that contributed to a marked decrease in survival at doses of  $\geq 5.9$  mg/kg bw/day. Hepatotoxic effects were shown as fatty degeneration, cholangiofibrosis and liver cirrhosis (at  $\geq 45$  mg/kg bw/day, 36-week study; Ito 1969) as well as focal necrosis of hepatocytes to severe, diffuse, toxic degenerative lesions in the liver ( $\geq 5.9$  mg/kg bw/day, long-term study; Cardy 1979, NCI 1979). Nephrotoxicity observed at  $\geq 5.9$  mg/kg bw/day was characterised as chronic renal glomerulonephrosis with glomerular atrophy and sclerosis, interstitial inflammation and fibrosis fibrosis, and tubular degeneration and atrophy. Associated to the chronic renal disease, signs of secondary hyperparathyroidism such as bone resorption and hyperplasia of parathyroid glands were markedly expressed. Additionally, experimental data from feeding studies with rats have demonstrated that 2,4-TDA induced dose related toxic effects on the testes. Testicular atrophy was observed at 28 mg/kg bw/day (15 months study; Stula and Aftosmis 1976). Disturbed spermatogenesis and Sertoli cell damage, atrophy of accessory sex glands, reduced serum level of testosterone and reflective increase in LH levels were associated to testis toxicity occurred at doses of 15 mg/kg bw/day (10-week feeding study; Thyssen et al. 1985a, 1985b, Varma et al. 1988). Even at the dose of approximately 5 mg/kg bw/day 2,4-TDA diminished sperm reserves indicating a depression of spermatogenesis were noted (10-week feeding study; Thyssen et al. 1985b). The observed toxic effects such as mortalities and degenerative/necrotic lesions in liver, kidney and in male gonads are serious health damage according to the criteria for R48 (Annex VI of 67/548/EEC). They occurred at dosages below the R 48 guidance values, which is 50

mg/kg bw/day (90-day oral toxicity study in rodents). These effects were observed in a dose-related pattern at  $\geq 5.9$  mg/kg bw/day (liver, kidney, mortality) and 5 mg/kg bw/day (male reproductive system), respectively. Furthermore, there was clear dose-response relationship in frequency, intensity and severity for the observed effects. According to the criteria of the Directive 67/548/EEC, 2,4-TDA is proposed to be classified and to be labelled as Xn, Harmful, R48/22 (Harmful: Danger of serious damage to health by prolonged exposure if swallowed).

Classification as Mutagen category 3, R 68 (Possible risks of irreversible effects) is proposed on the basis of positive in vitro and in vivo genotoxicity data of the constituent 2,4-TDA.

Classification as Reprotoxic category 3, R 62 (Possible risk of impaired fertility) is proposed on the basis of severe effects of 2,4-TDA on sertoli cells of male rats (fertility impairment) in feeding studies. Since 2,4-TDA is the major constituent of 2,4-/2,6-TDA, also this mixture of isomers should be classified as Reprotoxic category 3, R 62.

#### Mutagen category 3

R 68 Possible risks of irreversible effects

#### Reprotoxic category 3

R 62 Possible risk of impaired fertility

R 48/22 Harmful: Danger of serious damage to health by prolonged exposure if swallowed.

N Dangerous for the environment

## **2 GENERAL INFORMATION ON EXPOSURE**

### **2.1 PRODUCTION**

The following European companies are producers or importers of TDA (in alphabetic order):

Air Products, Manchester (UK)

BASF AG, Schwarzheide (DE)

Bayer AG, Brunsbüttel (DE)

Bayer AG, Dormagen (DE)

Bayer AG, Leverkusen (DE)

Bayer Shell Isocyanates N.V., Antwerpen (BE)

DOW, Porto Marghera (IT)

Rhodia Chimie, Le Pont du Claix (FR)

Rhodia Chimie, Lille (FR) (production of TDA was stopped in 2005)

Taking into account the production volumes provided by the companies, the total amount of TDA produced in the EU is about 280,000 t/a for the year 1999/2000. Additionally, about 10,000 t/a are imported. No information is available about export volumes. Therefore, the total volume of TDA handled in the EU amounts to 290,000 t/a.

As TDA is used as precursor for the synthesis for TDI (toluylene diisocyanate), TDA volumes can be estimated on the basis of the TDI production capacities. From the reported figures of TDI capacities, the production capacity for TDA in 1993 is calculated to 303,000 t/a in Western Europe and 672,000 t/a world-wide. 76,000 t were exported from Western Europe in the same year (SRI, 1994).

### **2.2 PROCESSING AND USE**

In the EU, TDA is almost exclusively used as intermediate in the chemical industry to produce TDI (toluylene diisocyanate). Several modification reactions can be carried out at further following processing stages. According to SRI (1994) the modified or unmodified toluylene diisocyanates are processed to the following products:

about 83 %	flexible foams, esp. for upholstery within the furniture and automobile industry
about 1.5 %	semirigid foams, esp. within the automobile industry for dashboards and head restraints
about 1.5 %	rigid foams
about 14 %	non-foam application, e.g. cast and thermoplastic elastomers, microcellular polyurethanes, coatings, sealants, adhesives, resins, millable gums and fibers

In addition to the production of TDI, the processing of TDA to diethyltoluylendiamine (DETDA) and leather dyes is reported (BUA, 1995). According to information from industry, the production of DETDA in the EU has stopped several years ago (Bayer 2002).

Further processing leads to polyols (by reaction with ethylene oxide or propylene oxide) and various azo dyes. As well uses as an epoxy resin curing agent are reported (Air Products, 1983). However, industry explained that this use is only of historic interest and that there is no known commercial product containing free TDA that is used in the curing of epoxide systems. In addition, also the use of products containing free TDA isomers in the dyestuffs industry is no longer actual (Air Products, 2002).

From the information delivered by industry for this risk assessment, > 99% of the produced and imported TDA is used as intermediate for the production of TDI. The pure 2,4-TDA produced in the EU is used as intermediate for the production of dyes in the chemical industry.

### **3 ENVIRONMENT**

#### **3.1 ENVIRONMENTAL EXPOSURE**

##### **3.1.1 General discussion**

###### **3.1.1.1 Environmental releases**

TDA is produced by catalytic reduction of dinitrotoluene. As water is a by-product of this reaction, the process water will contain a certain amount of TDA.

The major part of the TDA is processed to toluylene-diisocyanate (TDI) by reaction with phosgene, generally at the same sites (no data about TDA sales were submitted). This process is performed in closed systems, equipment cleaning is done with non-aqueous solvents. Therefore significant releases are not expected. There is no information about releases during processing to non-TDI products.

TDA can be formed by hydrolysis of TDI under certain conditions. However, this reaction is dependent on the ratio of TDI/water mixing: If the pure isocyanate is spilled into water, polyurea is formed as the main product, while with small TDI amounts mixed with a great excess of water TDA will be formed (Brochhagen, 1989a). Yakabe et al. (1999) also studied the reaction of TDI with water under different conditions. They found that the conversion of TDI to TDA is a function of loading of TDI and the stirring. With good stirring of TDI at loadings below 10 mg/l the yield can be greater than 50 % while at the highest loadings used (10,000 mg/l) it was below 1 %. At the technical processes however, the application of cleaning water is avoided, so TDA releases are not expected.

Diffuse releases can occur from TDA or TDI (after hydrolysis) chemically reacted in polyurethane or epoxy matrices during use and disposal of polymer products. Trace amounts of residual monomers may be released via migration and leaching. Brown et al. (2001) tested TDI-based flexible polyurethane foams for the release of TDA under methanogenic simulated landfill conditions. A trace of free TDA was found initially in the leachate but the level returned to background levels of TDA within 200 days. No evidence of the biodegradation of the TDI-based foam was found under the conditions of these landfill simulations.

###### **3.1.1.2 Degradation**

###### **3.1.1.2.1 Aquatic degradation**

###### Biodegradation

Different tests on biodegradation showed that TDA is not readily biodegradable. In an OECD Screening Test (OECD 301 E) using effluent from a municipal sewage treatment plant as inoculum no biodegradation of 2,4-TDA was observed after 28 days. The same test was conducted with effluent from an industrial sewage treatment plant. Also in this study no

biodegradation was found after 28 days. (Bayer 1981a). In two MITI (I) tests also no biodegradation of 2,4- and 2,6-TDA was achieved (Fujiwara, 1982, CITI 1992).

The biodegradation of 2,4-TDA was examined in a test according to OECD 301F using domestic activated sludge as inoculum. For the parameters BOD, DOC and residual amount an average biodegradation of 0%, 2 % and 1.4 %, respectively, was found (Yakabe, 1995).

However, biodegradation of 2,4-TDA reached 51% of theoretical CO<sub>2</sub> yield over 36 days in the Modified Sturm Test (OECD 301B), using an unadapted inoculum (Kim 2002).

A MITI(II)-test conducted with an inoculum from a laboratory sewage treatment plant fed predominantly with municipal sewage resulted in a degradation of 4 % after 28 days for 2,4/2,6 -TDA (80/20) (Caspers et al.1986). A Zahn-Wellens-Test conducted with sludge from an industrial sewage treatment plant as inoculum that is assumed to be adapted to TDA showed a DOC elimination of 100 % after 6 days for 2,4-TDA and of 89 % after 28 days for 2,6-TDA (BASF 1993). Biodegradation related to COD was 93 % for 2,4-TDA and 85 % for 2,6-TDA.

With an electrolytic respirometer the primary degradation of 2,4-TDA and 2,6-TDA at concentrations of 50 and 100 mg/l as well as the inhibition of nitrification was examined (Snyder 1987). Industrial waste water that was adapted to aromatic amines was used as inoculum in a concentration of 500 mg/l volatile suspended solid. After 26 days of incubation at 35 °C, 2,4-TDA in both concentrations was degraded to 100 %. Nitrification was not inhibited at 50 mg/l 2,4-TDA, but proceeded only to NO<sub>2</sub>-. However, at 100 mg/l 2,4-TDA nitrification was almost completely inhibited. 2,6-TDA in concentrations of 50 and 100 mg/l was only degraded to 23 % respectively to 12 % after 26 days. At both concentrations nitrification was almost completely inhibited.

Asakura and Okazaki (1995) examined the biodegradation of 2,4-TDA by activated sludge that was acclimated by different methods. First, the activated sludge that was cultured with synthetic waste water (corn steep liquor) was acclimated to aniline (100 mg/l) for about one month. The sludge was then exposed to the acclimation processes with aniline and TDA for 100 and 200 days. In addition, sludge was also acclimated to corn steep liquor and TDA for 200 days. To the so acclimated sludge 2,4-TDA was added and the respiration rate was measured. It was found, that the respiration rate did not increase in the sludge acclimated for 100 days with aniline and 2,4-TDA. However, the sludge that was acclimated for 200 days showed a considerable increase in respiration rate after addition of 2,4-TDA. On the other hand, the sludge that was acclimated for 200 days with TDA and corn steep liquor showed no increase in respiration rate.

With a fill-and draw type unit Matsui et al. (1975) examined the biodegradability of 2,4-TDA. Activated sludge from an industrial waste water treatment plant was used as inoculum. As this plant serviced several chemical manufacturers, the sludge was considered to be well acclimated to a variety of chemicals, including TDA. Biodegradation was measured as decrease in COD and TOC over time. After 4 hours COD fell by 34 % from 105 mg/l to 69 mg/l. TOC decreased within 2 hours by 24 % from 58 mg/l to 44 mg/l and within 4 hours by 45 % from 58 mg/l to 32 mg/l.



Sandridge (1984) found that due to different degradation behaviour the ratio of the two isomers in the effluent of a sewage treatment plant may change in favour of 2,6-TDA.

The above cited tests show that 2,4-TDA and 2,6-TDA are only biodegradable by adapted inocula.

From a Zahn-Wellens test conducted with industrial sewage sludge as inoculum it can be concluded that both isomers are inherently biodegradable in industrial sewage treatment plants. As 2,4-TDA was completely degraded within 6 days a rate constant of  $0.1 \text{ h}^{-1}$  can be derived according to the TGD for industrial sewage treatment plants. For 2,6-TDA a rate constant of  $0 \text{ h}^{-1}$  is deduced from the result of the Zahn-Wellens test according to the TGD as the special criteria necessary for a rate constant of  $0.1 \text{ h}^{-1}$  are not fulfilled. The different degradation behaviour of the two isomers is also confirmed by Snyder (1987) and Sandridge (1984).

By measuring the influent and effluent concentration of 2,4- and 2,6-TDA from an industrial sewage treatment plant an elimination of  $> 90 \%$  for each isomer is indicated (Snyder 1987). Because it is unclear whether these data are representative for industrial wwtps, this value for wwtp elimination is not used in the further exposure assessment.

Results from biodegradation simulation tests in surface water are not available. Therefore the rate constant have to be determined according to the TGD. As TDA is not biodegradable by non-adapted microorganisms a rate constant of  $0 \text{ d}^{-1}$  for both isomers is assumed for this compartment.

### Photolysis

The UV-spectra ( $\lambda_{\text{max}}$  at 295 nm for 2,4- and 289 nm for 2,6-TDA; Bayer 1992b) indicate that direct photolysis in water may occur. In a test on photolytic degradation in aqueous solution, a quantum yield of 0.0027 for direct photodegradation in polychromatic light was determined and half-lives were calculated (Bayer, 1997). According to the GC-SOLAR program, the half-lives are 28.6 d in summer and  $> 1$  year in winter (marginal conditions: pure water from close to the surface, 10th degree of longitude, 50th degree of latitude, clear sky, typical ozone concentrations in the atmosphere). According to the Frank & Klöpffer program, the mean values of the half-lives range from 44 d in June to  $> 1$  year in the winter months (marginal conditions: pure water from close to the surface, stagnant water, geographic and climatic conditions of 50th degree of latitude, no contribution of another mono- or bimolecular elimination process). The estimated environmental photolysis half-lives obtained with the different models are well comparable. However, dullness and adsorption of surface waters are not considered. Because of these effects, the photolytical active zone is only close to the surface of real surface waters. Considering the total water body, the real environmental half-lives should be at least one order of range higher than the calculated. Therefore, for the exposure assessment degradation by direct photolysis is not considered.

### Hydrolysis

Based on the molecular structure, hydrolysis is not to be expected under environmental conditions.

### 3.1.1.2.2 Degradation in soil

The microbial degradation of 2,4-TDA and 2,6-TDA in soil was investigated under aerobic and anaerobic conditions using  $^{14}\text{C}$  labeled TDA (Cowen et al., 1996). The results show that biodegradation started immediately after mixing with the aerobic soil. With the binding of the amines to soil the degradation rate decreased later. The test indicates biodegradation for 2,4-TDA and 2,6-TDA of 1.9 % and 2.7 % after 3 days, 4 % and 6.2 % after 7 days, 7.9 % and 11.1 % after 14 days, 10.8 % and 14.2 % after 28 days and 14.8 % and 18.1 % after 56 days, respectively. During the latter period of the incubation some of the  $^{14}\text{CO}_2$  was lost, so the results for 210 and 365 days must be rejected. The degradation rates indicate that biodegradation slowed down after TDA had formed covalent bounds to humic substances (cf. 3.1.1.3.2). It is not possible to calculate a half-life for biodegradation of TDA in soil from this test, but it can be assumed that TDA covalently bound to organic matter is degraded almost similar to the humic acids themselves. Analogously to the investigations for 3,4-dichloroaniline, a mean half-life of 1000 d can be assumed (cf. 3.1.1.3.2). Under anaerobic methanogenic conditions no  $^{14}\text{CH}_4$  or  $^{14}\text{CO}_2$  was recovered after 73 days of incubation.

The biodegradation of 2,4- and 2,6-TDA was studied in anaerobic soils under a wide range of redox conditions (West et al., 2002). Approximately 2 mg/kg dw of  $^{14}\text{C}$ -labeled TDA were added to anaerobic laboratory batch microcosms containing 25 g soil and 10 ml of an aqueous mineral medium. The laboratory microcosms were prepared using either a sand soil which had a history of contact with leachates from a municipal solid waste landfill or a loamy sand soil which has a history of contamination by various aromatic amine compounds. Using these two soils and a wide range of redox conditions, the effects of soil texture and redox potential on the biodegradation of TDA under anaerobic conditions were examined. Very limited biodegradation of  $^{14}\text{C}$ -TDA was observed in the sand soil under anaerobic, denitrifying conditions. After approx. one year microcosms containing the  $^{14}\text{C}$ -2,4-TDA produced maximum average  $^{14}\text{CO}_2$  yields equivalent to 1.3 % mineralisation. Microcosms containing the  $^{14}\text{C}$ -2,6-TDA produced maximum average  $^{14}\text{CO}_2$  yields equivalent to only 0.3 % mineralisation within the same time period. The addition of a co-substrate, 2-aminobenzoic acid (6 mg/kg), to the reaction mixtures appeared to enhance the mineralisation of TDA under denitrifying conditions to 2.1 %. In the loamy sand soil under anaerobic, denitrifying conditions yields of  $^{14}\text{CO}_2$  from the combined 2,4/2,6-TDA isomers reached maximum levels equivalent to 1 % of the applied radioactivity. Yields of  $^{14}\text{CO}_2$  under Fe(III) reducing, sulfate reducing and methanogenic conditions remained < 1 % of the applied radioactivity after 374 d.

### 3.1.1.2.3 Degradation in sediments

There are no data available on biodegradation of TDA in sediments. For the oxic sediment layer, the same reaction half-life (1000 d) as for soils is used. As according to the TGD 10 % of the sediment compartment is considered to be aerobic, in the following exposure calculations a half-life of 10,000 days is assumed for the sediment compartment.

### 3.1.1.2.4 Atmospheric degradation

In a test on photochemical-oxidative degradation in the atmosphere the reaction constant with OH-radicals was determined (Becker et al, 1988). From this half-lives of 2 h for 2,4-TDA and  $\leq 3.8$  h for 2,6-TDA can be calculated ( $C_{OH} = 5 \cdot 10^5$  molec/cm<sup>3</sup>).

### 3.1.1.3 Distribution

(for calculations cf. appendix A1)

#### 3.1.1.3.1 Volatilisation

With a Henry's law-constant of  $5.46 \cdot 10^{-5}$  Pa·m<sup>3</sup>·mol<sup>-1</sup> for 2,4-TDA and of  $6.55 \cdot 10^{-5}$  Pa·m<sup>3</sup>·mol<sup>-1</sup> for 2,6-TDA no significant volatilization from water is expected.

#### 3.1.1.3.2 Adsorption

Experiments with radiolabelled 2,4-TDA and 2,6-TDA revealed that the substances form covalent bonds with the organic fraction in soil. Two soils, a sandy loam and a silt loam were used for the experiments. Under both aerobic and anaerobic conditions sorption of 2,4-TDA was shown to proceed in two steps, an initial rapid sorption followed by a slower sorption that was not even completed after 7 days of contact. Because the behaviour for both soils was very similar, the values for both have been averaged. The K<sub>d</sub>-values for 2,4-TDA were determined to 19.4 l·kg<sup>-1</sup> after 8 hours and 142 l·kg<sup>-1</sup> after 7 days for aerobic conditions. The values for anaerobic conditions are 12.6 l·kg<sup>-1</sup> and 63 l/kg after 8 h and 7 d, respectively. The sorption curves for 2,6-TDA are similar to that of 2,4-TDA. The K<sub>d</sub> values for 2,6-TDA were determined to 10.9 l·kg<sup>-1</sup> after 8 hours and 110 l·kg<sup>-1</sup> after 7 days for aerobic conditions. The values for anaerobic conditions are 8.3 l·kg<sup>-1</sup> and 37 l·kg<sup>-1</sup> after 8 h and 7 d, respectively (Cowen et a., 1996). As TDA sorption to soil proceeds via chemisorption to the organic fraction, these K<sub>d</sub>-values were normalised to the organic carbon content of the soil. The following K<sub>oc</sub>-values are given by the authors:

	Aerobic		Anaerobic	
	8 h	7 d	8 h	7 d
2,4-TDA	1339	9,763	884	4,454
2,6-TDA	757	7,805	567	2,571

It should be kept in mind that the term "K<sub>oc</sub>" generally describes the distribution of a substance between the pore water and the organic matter when the substance is physically bound; if chemisorption occurs the use of this term is not quite correct.

The sorption of TDA in anaerobic soils under a wide range of redox conditions was studied by West et al. (2002) (cf. 3.1.1.2.2). Approximately 2 mg/kg dw of <sup>14</sup>C-labeled TDA were added to anaerobic laboratory batch microcosms containing 25 g soil and 10 ml of an aqueous mineral medium. The laboratory microcosms were prepared using either a sand soil which had a history of contact with leachates from a municipal solid waste landfill or a loamy sand soil which has a history of contamination by various aromatic amine compounds. Using these two soils and a wide range of redox conditions, the effects of soil texture and redox potential on the covalent binding with organic matter and particle-associated adsorption processes of TDA were examined. The covalent binding of TDA in soils followed first order reaction kinetics, and the rate and extent of this process was highly dependent upon both soil texture and redox potential. In laboratory microcosms containing sand soil and denitrifying microbial activity, the 2,4-TDA isomer was more rapidly removed from the water phase than 2,6-TDA, with half-lives of 37 days and 65 days, respectively. Addition of the combined TDA isomers appeared to increase the sorption of 2,4-TDA ( $t_{1/2}$  of 13 days) while initially slowing the rate of 2,6-TDA sorption ( $t_{1/2}$  of 75 days). In the loamy sand soil sorption was biphasic. In the first phase half-lives for removal of each TDA isomer from the water phase were < 0.1 days. In the second phase a half-life of 60 days was estimated.

The redox potential of the sand soil was lowered by adding an easily metabolisable carbon source to the microcosms containing denitrifying, Fe(III)- and sulfate-reducing and methanogenic populations. The binding of TDA to soils decreased in parallel with the reduction of the soil redox. Half-lives for removal of TDA from the water phase were estimated to 248 d (2,4-TDA) and 347 d (2,6-TDA) in the sand soil under stimulated denitrifying conditions. In the microcosms under the other stimulated redox conditions, no removal of TDA from the water phase through sorption occurred within one year. However, introduction of oxygen to the systems after 216 days of incubation resulted in an immediate removal of aqueous-phase radioactivity with half-lives in the range of 9 to 15 days.

From this study it can be concluded that microbially-mediated reduction of quinone functionalities of natural organic matter is likely responsible for suppressing the irreversible binding of TDA to soil poised under Fe(III) reducing, sulfate reducing and methanogenic conditions. Therefore, TDA has the potential to remain unbound if releases to highly reduced soil or sediment environments.

The chemical binding effects are already well-known as a property of MDA and 3,4-dichloroaniline and are described in detail in the respective environmental risk assessment reports in the scope of the first EU priority list.

With a “Koc” of 9,763 l·kg<sup>-1</sup> for 2,4-TDA and a “Koc”-value of 7,805 l·kg<sup>-1</sup> for 2,6-TDA, the following distribution constants are calculated in accordance to the TGD models:

Table 3.1: Distribution constants for 2,4-TDA:

$K_{p_{soil}}$	195 l·kg <sup>-1</sup>	$K_{soil-water}$	293 m <sup>3</sup> ·m <sup>-3</sup>
$K_{p_{susp}}$	976 l·kg <sup>-1</sup>	$K_{susp-water}$	245 m <sup>3</sup> ·m <sup>-3</sup>
$K_{p_{sed}}$	488 l·kg <sup>-1</sup>	$K_{sed-water}$	245 m <sup>3</sup> ·m <sup>-3</sup>

Table 3.2: Distribution constants for 2,6-TDA:

$K_{p_{soil}}$	156 l kg <sup>-1</sup>	$K_{soil-water}$	234 m <sup>3</sup> ·m <sup>-3</sup>
$K_{p_{susp}}$	780 l kg <sup>-1</sup>	$K_{susp-water}$	196 m <sup>3</sup> ·m <sup>-3</sup>
$K_{p_{sed}}$	390 l kg <sup>-1</sup>	$K_{sed-water}$	196 m <sup>3</sup> ·m <sup>-3</sup>

### 3.1.1.3.3 Distribution according to Mackay level I

According to a level I fugacity model the following distribution between the different environmental compartments can be estimated for 2,4-TDA

Table 3.3: Results of Mackay I calculation for 2,4-TDA

Compartment	2,4-TDA	2,6-TDA
air	< 0.01 %	< 0.01 %
water	4	5
sediments	32	32
soil	64	63

As input parameter the physical-chemical data described in chapter 1 were used except for the log K<sub>ow</sub>. This value was recalculated from the experimentally determined “K<sub>oc</sub>” of 9,763 l/kg (2,4-TDA) resp. 7,805 (2,6-TDA) according to the equation given in the TGD (log K<sub>oc</sub> = 0.62 \* log K<sub>ow</sub> + 0.85) to consider the binding of TDA to the organic fraction of soil and sediment. The resulting log K<sub>ow</sub> of 5 and 4.9 was used for the fugacity model .

### 3.1.1.3.4 Distribution in waste water treatment plants

According to the model SIMPLETREAT, the fate of the TDA isomers in wastewater treatment plants (wwtps) is predicted as follows:

Table 3.4: Fate of the TDA isomeres in industrial wwtps

%	2,4-TDA	2,6-TDA
to air	0	0
to water	59	100
to sludge	0	0
degraded	41	0
removal	41	0

Although both isomers show a high binding potential to soil and sediments (Koc values 9,763 l/kg and 7,805 l/kg), no adsorption onto sewage sludge is assumed as for sewage sludge only physical adsorption due to lipophilicity has to be considered, not chemical binding like in soil and sediment (formation of covalent bindings to humic acids). This assumption is based both on the absence of humic acid in the sewage sludge and on the short retention time of the substance in the sewage treatment plant.

### 3.1.1.3.5 Accumulation

For 2,4-TDA a log Pow of 0.074 was measured. With this value a BCF can be determined according to the TGD using the following equation:

$$\log \text{BCF}_{\text{fish}} = 0.85 \cdot \log \text{Pow} - 0.7$$

This leads to a BCF of 0.23 l·kg<sup>-1</sup>.

For 2,6-TDA a log Pow of -0.137 was measured, leading to a BCF of 0.15 l·kg<sup>-1</sup>.

Experimental results on bioaccumulation are available for 2,4-TDA and for TDA (not specified which isomer was used). At concentrations of 0.3 mg/l and 0.03 mg/l 2,4-TDA a BCF of < 5 respectively < 50 l·kg<sup>-1</sup> was determined for *Cyprinus carpio* (CITI 1992). Veith et al. (1979) determined a BCF of 91 l·kg<sup>-1</sup> for *Pimephales promelas* using a concentration of TDA (unspecified isomer) in water of 1 µg/l. This value is difficult to interpret as it does not fit into a correlation between measured BCF and log Kow for other aromatic amines (e.g. MDA, aniline, 3,4-DCA). In addition, the analytical method employed to determine the water concentration seems not adequate for TDA (extraction with hexane). By this method probably the water concentration was underestimated and thus the BCF overestimated. Although the authors state that the accuracy of the analytical method was examined by determining the

recovery of known amount of chemical in water and tissue and that at least 90 % of the added chemical was recovered, it is not clear whether this was really examined for every single of the 30 tested chemicals, which are mainly unpolar chlorinated compounds for which the extraction method is fully applicable. To consider these uncertainties, the BCF value of 91 is not used for the risk assessment. Instead, a BCF value of 5 determined by CITI is used. This value correlated very well with the values for other aromatic amines.

The bioavailability of the reaction product of TDA with humic acids was not examined. Sediment studies performed with other aromatic amines having the same binding behaviour to humic acids as TDA do not provide an unequivocal answer as to whether the bound substances are bioavailable for benthic organisms.

### 3.1.2 Aquatic compartment (incl. Sediment)

#### 3.1.2.1 Predicted environmental concentrations in water

##### Estimation of PEC<sub>local</sub> / Generic approach

In the *TGD*, a generic exposure scenario for the release of intermediates during production and processing into surface water is proposed. As > 99 % of the EU TDA volume is processed to TDI, and the release factor during processing is assumed to be negligible, for production and processing a total release factor of 0.3% into the sewage and subsequent purification in a wwtp is assumed.

Using the highest single production capacity of 100,000 t/a (2,4-/2,6-TDA (80/20)), an emission duration of 300 d/a, an elimination in a wwtp of 41 % for 2,4-TDA and of 0 % for 2,6-TDA, a wwtp flow of 10,000 m<sup>3</sup>/d and a dilution factor of 40, a **PEC<sub>local</sub>** of **1.68 mg/l** can be estimated according to the TGD model.

##### Estimation of PEC<sub>local</sub> / Site-specific approach

For the calculation of the PEC<sub>local</sub>, site-specific data were used as far as they were available. In the absence of site-specific data default values from the TGD were used.

In almost all cases, 2,4-/2,6-TDA mixtures are produced. In the following exposure calculations it is assumed that the TDA load in the raw sewage has the same composition as the technical product (80:20), although this is only a rough estimate because of differences in water solubility of the two isomers, but there are no specific emission data available.

Because there are different wwtp elimination factors for both isomers, the ratio between the two isomers in the effluent of the wwtp changes in favour of 2,6-TDA. For all but 1 sites the effluent concentration of total TDA is given. To calculate from this value the influent concentration and, based on this, the total releases into the waste water, the different elimination of the two isomers in wwtp has to be taken into account. It can be calculated that

the effluent concentration of total TDA is 67.2 % of the influent concentration according to the following equation:

$$C_{\text{eff}} = 0.8 \cdot 0.59 \cdot C_{\text{infl}} + 0.2 \cdot C_{\text{infl}} = (0.8 \cdot 0.59 + 0.2) \cdot C_{\text{infl}} = (0.672) \cdot C_{\text{infl}}$$

With: 0.8: proportion of 2,4-TDA of total TDA in influent

0.2: proportion of 2,6-TDA of total TDA in influent

0.59: percentage of 2,4-TDA directed to water after treatment in wwtp

The  $C_{\text{local}}$  is calculated from the  $C_{\text{eff}}$  according to the following equation:

$$C_{\text{local}} = C_{\text{eff}} / D \cdot (1 + K_{\text{p}_{\text{susp}}} \cdot \text{SUSP}_{\text{water}} \cdot 10^{-6})$$

$$D = \text{EFFLUENT}_{\text{stp}} + \text{FLOW}_{\text{river}} / \text{EFFLUENT}_{\text{stp}}$$

A maximal dilution factor of 1000 is used for emission into a river.



Table 3.5 Estimated PECs and underlying data:

Site	Speciation of product	life stage	site-specific data	default values	C <sub>emf</sub> [µg/l]	C <sub>local</sub> [µg/l]	Release factor [t/t]  (emission into wwtp)
A	80/20	prod. + proc. to TDI	effluent conc. for TDA, sewage flow	Dilution 1:100	< 10	< 0.0026	1.66·10 <sup>-7</sup>
B	80/20	prod. + proc. to TDI	effluent conc. for TDA , sewage and river flow; (a max. dilution factor of 1000 is used)	-	< 70	< 0.07	5.28·10 <sup>-5</sup>
C	2,4-TDA	prod.	effluent conc. for TDA, number of days, sewage and river flow		< 20	< 0.03	0.001
D	80/20	prod. + proc. to TDI	effluent conc. for TDA, sewage and river flow	-	9.4	0.58	1.2·10 <sup>-6</sup>
E	80/20	prod. + proc. to TDI	effluent conc. for TDA, sewage and river flow	-	35.2	0.13	2.58·10 <sup>-6</sup>
F	80/20	prod. + proc. to TDI	effluent conc. for TDA, sewage and river flow, no biological wwtp	-	300	0.3	3·10 <sup>-6</sup>
G**	80/20	prod. + proc. to TDI	effluent conc. for TDA, sewage and river flow*, no biological wwtp	-	<3000 140	< 23.4 0.87	4.23·10 <sup>-5</sup> 1.54·10 <sup>-6</sup>
H	80/20	prod. + proc. to TDI	conc. of TDA in process water, dilution in wwtp,	elimination in wwtp, dilution 1:100	< 6.6l	< 0.065	2.5·10 <sup>-6</sup>

\* No exact river flow rate for the site is available. The used value was interpolated from a measured mean flow rate 10 km upstream and a measured mean flow rate 4 km downstreams. 1/3 of the resulting value was used as low flow.

\*\* This site stopped the production of TDA in 2005.

For site G 2 Clocal were calculated. The first calculation is based on the information, that the concentration of TDA in the effluent is below the detection limit of 3 mg/l. With this a Clocal of 23.4 µg/l is estimated. Later on the TDA concentration was analysed for 1 day in the effluent using a lower detection limit (25 µg/l). 9 measurements were performed, the concentration ranged from 25 µg/l to 140 µg/l. As the measurements represent only one production/processing day, as a worst-case approach the maximum value of 140 µg/l was used to calculate the Clocal. This gives a value of 0.87 µg/l. The max. measured value of 140 µg/l was also used for the derivation of the total releases and the release factor.

There is one river which receives the effluents from 2 sites. This can lead to environmental concentrations which are higher than the Clocal figures calculated from single site emissions. The sites B and C are in close vicinity, so the Clocal figures are added as a worst case approach:

Sites	Σ Clocal [µg/l]
B,C	< 0.1

A total release of 2,286 kg/a to wastewater treatment plants and 220 kg/a directly to surface water can be estimated from the available data. These figures are used as input for the calculation of the regional PECs.

#### Release during processing to TDI

The imported volume of 10,000 t/a TDA is processed at unknown sites to TDI. No emissions of TDA from this life-cycle step are to be expected (see chapter 3.1.1.1).

#### Release during processing to other products than TDI

The pure 2,4-TDA produced in the EU is used as intermediate for dye production in the chemical industry. About 50 % of the 2,4-TDA produced in the EU is exported, the other 50 % are processed within the EU to dyes by one site. No information about releases into the environment from this site is available. Therefore, a generic scenario using the default values according to the TGD is calculated:

volume of 2,4-TDA processed to dyes: about 20 t/a  
 emission factor: 0.02 (table A3.3, T < 1000 t/a)  
 number of days: 20 d/a (table B3.2)  
 elimination in wwtp: 0.41 (default)  
 flow rate of wwtp: 10,000 m<sup>3</sup>/d (default)  
 dilution factor: 40 (default)

A C<sub>eff</sub> of 1.18 mg/l and a C<sub>local</sub> of 30 µg/l is estimated for this site (dye1).

In addition, there is information from another site producing dyes from 2,4-TDA in the EU. For this site, site-specific information is available. 3.61 t/a of 2,4-TDA is processed to dyes by

this site. The following generic and site-specific information is used for the calculation of the  $C_{local}$ :

emission factor: 0.02 (table A3.3, T < 1000 t/a)  
number of days: 20 d/a  
elimination in wwtp: 0.41 (default)  
flow rate of wwtp: 16000 m<sup>3</sup>/d (site-specific)  
flow rate of receiving river: 70 m<sup>3</sup>/s (site-specific)  
dilution factor: 380

A C<sub>eff</sub> of 0.13 mg/l and a C<sub>local</sub> of 0.35 µg/l can be estimated for this site (dye2).

A total release to wwtp of 472 kg/a 2,4-TDA is estimated for this life-cycle-step.

#### Releases during the use of TDA subsequent products

The degradation of <sup>14</sup>C-labelled polyester and polyether based polyurethane foams in different media was investigated by Martens & Domsch (1981). When the foams were exposed to a leachate from a refuse tip, no TDA was detected in the water phase after 3 months at 22°C. Only at a higher temperature (50°C), traces of TDA (corresponding maximum 0.25% of 2,4- and 0.38% of 2,6-TDA content) were found.

A significant environmental exposure from these sources is not expected.

#### Sediments

Because of the binding properties of TDA onto humic substances, an accumulation of TDA derivatives in sediments cannot be excluded. As both isomers have different partitioning coefficients, the calculation of the  $PEC_{local, sed}$  has to be performed for both isomers separately and has then to be added. Due to the different elimination in wwtp, the ratio of the two isomers in the effluent changes in favor of 2,6-TDA. As explained above, the effluent concentration of total TDA can be calculated to 67.2 % of the influent concentration for the 80/20 isomer mixture. The proportion of 2,4-TDA to 2,6-TDA in the effluent can be calculated to:

$$2,4\text{-TDA} : 2,6\text{-TDA} = (0.8 \cdot 0.59) : (0.2 \cdot 1) = 0.47 : 0.2$$

Therefore, the  $C_{local, water}$  is composed of the two isomers in the ratio 70 % : 30 % (2,4-TDA : 2,6-TDA).

The  $PEC_{sediment}$  is calculated according to the following formula:

$$PEC_{sed} = \frac{K_{susp-water}}{RHO_{susp}} \cdot PEC_{aqua} \cdot 1000 \text{ l/m}^3$$

Using a  $K_{\text{susp-water}}$  of  $245 \text{ m}^3 \cdot \text{m}^{-3}$  and  $196 \text{ m}^3 \cdot \text{m}^{-3}$  for 2,4- and 2,6-TDA, resp., and the  $C_{\text{local-water}}$  values for each isomer, the  $PEC_{\text{local-sed}}$  are calculated from the water concentrations as follows:

$$PEC_{\text{sed}} = \frac{245}{1150} \cdot (C_{\text{local-aqua}} \cdot 0.7) \cdot 1000 \text{ l/m}^3 \\ + \frac{196}{1150} \cdot (C_{\text{local-aqua}} \cdot 0.3) \cdot 1000 \text{ l/m}^3$$

Table 3.6: Estimated PECsediment

<b>Company</b>	<b>Clocal<sub>water</sub> [µg/l]</b>	<b>PEClocal<sub>sed</sub> [µg/kg ww]</b>
A	< 0.0026	< 0.52
B	< 0.07	< 14
C*	< 0.03	< 6.4
D	0.58	116
E	0.13	26
F	0.3	60
G*	< 23.4 0.87	< 4,686 174
H	< 0.065	< 13
Σ B,C	< 0.1	< 20.4
Processing to dyes *: Dye1	30	6,390
Dye2	0.35	74.5

\* This site stopped the production of TDA in 2005.  
\*\* only 2,4-TDA is emitted

### 3.1.2.2 Monitoring

There are no monitoring data for the hydrosphere available.

### 3.1.3 Atmosphere

No significant releases of TDA into the atmosphere during production and processing to TDI are expected. For a German site, an emission of 10 kg/a is stated, at further sites the exhaust gases are incinerated. The TGD proposes a default value of 0 as release factor (appendix I, table A1.1).

A study on the gas phase reaction of TDI with moist air revealed that no TDA was formed (Holdren et al., 1984). It can be concluded that a relevant TDA exposure does not occur from TDI emissions.

### **3.1.4 Terrestrial compartment**

During production and processing of TDA, neither direct nor indirect releases into the soil are expected to occur in a significant amount.

The degradation of <sup>14</sup>C-labelled polyester and polyether based polyurethane foams in composted municipal waste and parabrownish earth was investigated by Martens & Domsch (1981). After 3 months at 22°C, no TDA was detected in both media. This is also confirmed by the study of Brown et al. (2001) (cf. 3.1.1.1).

It can be assumed that no or only trace amounts of TDA are discharged during deposition of polyurethane wastes on landfills.

### **3.1.5 Non compartment specific exposure relevant to the food chain**

Because of the low accumulation of TDA in fish via water, the exposure route fish - fish eating bird or mammal is likely to be not relevant. However, the reaction product of TDA with sediment organics accumulates in sediments and is probably bioavailable. A biomagnification via the route sediment - sediment dwelling worm – worm eating fish - fish eating mammal or bird can not be excluded.

Due to missing experimental data on bioaccumulation with sediment dwelling organisms, a quantitative assessment of secondary poisoning via this route cannot be performed for TDA

### **3.1.6 Regional Exposure**

According to the *Technical Guidance Document*, generally the regional and the local PECs have to be added to calculate the total PEC which is relevant for the environmental risk assessment. This method is not appropriate for TDA, because of the following reason:

Point sources which are scattered over a large region cause the major releases into the hydrosphere. The substance is only emitted into surface waters, and it is unlikely that the emission of one site will reach a second source. Thus, it cannot be assumed that the sites are emitting into a pre-polluted environment. Therefore, only local PECs are taken for the aquatic risk assessment and the aqueous  $PEC_{local}$  are equated with the  $C_{local}$ .

However, regional PECs should be calculated as input parameters for the indirect exposure of man via the environment. For the estimation of the regional background concentration the total release amounts into the environment from production of TDA are used.

The total emissions were estimated to 2,978 kg/a, consisting of 546 kg/a 2,4-TDA and 2,432 kg/a 80/20-TDA. From the 80/20 TDA 220 kg/a are released directly to surface waters. As the two isomers have partly different physico-chemical properties and environmental behaviour, the  $PEC_{regional}$  is calculated separately for the two isomers. The total releases for 2,4-TDA are 2,492 kg/a and for 2,6-TDA 486 kg/a, from which 176kg/a 2,4-TDA and 44 kg/a 2,6-TDA

are released directly to surface waters. These emissions are separated between the continent and the region in the ratio 90:10:

Table 3.7: Environmental emissions of 2,4-TDA and 2,6-TDA

	<b>Continental releases (90 %)</b>	<b>Regional releases (10 %)</b>
<b>2,4-TDA</b>	2,243 kg/a (2,084 kg/a via wwtp, 158 kg/a directly)	249 kg/a (232 kg/a via wwtp, 18 kg/a directly)
<b>2,6-TDA</b>	437 kg/a (397 kg/a via wwtp, 40 kg/a directly)	49 kg/a (45 kg/a via wwtp, 4 kg/a directly)

The following results were obtained (for the calculation see Appendix A2):

Table 3.8: Continental and regional PECs for 2,4-TDA, 2,6-TDA and total TDA:

<b>Compartment</b>	<b>2,4-TDA</b>		<b>2,6-TDA</b>		<b>Total TDA</b>	
	<b>Continental conc.</b>	<b>Regional conc.</b>	<b>Continental conc.</b>	<b>Regional conc.</b>	<b>Continental conc.</b>	<b>Regional conc.</b>
Surface water (dissolved) [µg/l]	$1.58 \cdot 10^{-3}$	$5.46 \cdot 10^{-3}$	$5.2 \cdot 10^{-4}$	$1.77 \cdot 10^{-3}$	$2.1 \cdot 10^{-3}$	$7.2 \cdot 10^{-3}$
Sediment [µg/kg ww]	0.56	1.89	0.15	0.48	0.71	2.4
Atmosphere [µg/m <sup>3</sup> ]	$1.58 \cdot 10^{-12}$	$5.4 \cdot 10^{-12}$	$7.47 \cdot 10^{-13}$	$2.51 \cdot 10^{-12}$	$2.3 \cdot 10^{-12}$	$7.9 \cdot 10^{-12}$
Agric. Soil [µg/kg ww]	$5.46 \cdot 10^{-7}$	$1.87 \cdot 10^{-6}$	$2.14 \cdot 10^{-7}$	$7.19 \cdot 10^{-7}$	$7.6 \cdot 10^{-7}$	$2.6 \cdot 10^{-6}$
Agr. soil, porewater [µg/l]	$3.17 \cdot 10^{-9}$	$1.1 \cdot 10^{-8}$	$1.55 \cdot 10^{-9}$	$5.22 \cdot 10^{-9}$	$4.7 \cdot 10^{-9}$	$1.6 \cdot 10^{-8}$
Industr. Soil [µg/kg ww]	$2.04 \cdot 10^{-6}$	$7 \cdot 10^{-6}$	$7.87 \cdot 10^{-7}$	$2.64 \cdot 10^{-6}$	$2.8 \cdot 10^{-6}$	$9.6 \cdot 10^{-6}$
Nat. Soil [µg/kg ww]	$2.04 \cdot 10^{-6}$	$7 \cdot 10^{-6}$	$7.87 \cdot 10^{-7}$	$2.64 \cdot 10^{-6}$	$2.8 \cdot 10^{-6}$	$9.6 \cdot 10^{-6}$

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

#### 3.2.1 Aquatic compartment

##### Available effect data

The following results from acute and long-term toxicity tests with aquatic organisms are available:

Table 3.9: Short-term toxicity to fish

Species	duration [h]	LC50 [mg/l]	test system	test substance	reference
<i>Pimephales promelas</i>	96	1420 (m)	flow-through; analytical monitoring	2,4-TDA	Geiger et al. 1990
<i>Oryzias latipes</i>	96	912 (m)	flow-through, analytical monitoring	2,4-TDA	Holcombe et al. 1995
<i>Oryzias latipes</i>	96	219 (n)	semistatic	TDA 80/20	Tadokoro et al. 1991a/ 1992 (test result from previous unpublished study of the authors cited in these references)
<i>Brachydanio rerio</i>	96	392 (n)	static	TDA 80/20	Caspers et al. 1986
<i>Pagrus major</i> (marine) (ca. 1 g)	48 72 96	1.06 (n) 0.264 (n) 0.161 (n)	semi-static	TDA 80/20	Tadokoro et al. 1991b
<i>Pagrus major</i> (marine) (ca. 1 g)	96	0.221 (n)	static	TDA 80/20	Tadokoro et al. 1991a / 1992 (test result from previous unpublished study of the authors cited in these references)
<i>Pagrus major</i> (marine) (ca. 1 g)	96	0.2-0.4 (n)	semi-static	2,4-TDA	Tadokoro 1991b
<i>Pagrus major</i>	96	> 1.6 (n)	semi-static	2,6-TDA	Tadokoro 1991b



(marine) (ca. 1 g)					
<i>Pagrus major</i> (marine) (ca. 10 g)	48 72 96	> 5 (n) 2.42 (n) 0.73 (n)	semi-static	2,4-TDA	Tadokoro 1994
<i>Pagrus major</i> (marine) ca. 10 g)	48 72 96	> 400 (n) > 400 (n) 283 (n)	semi-static	2,6-TDA	Tadokoro 1994
<i>Pagrus major</i> (marine) (ca. 20 g)	48 72 96	> 10 (n) 5.88 (n) 0.83 (n)	semi-static	2,4-TDA	Tadokoro 1994
<i>Pagrus major</i> (marine) (ca. 24 g)	96	2.1 (n)		2,4-TDA	Tadokoro 1994 (value cited from Japan Frozen Food Inspection Co)
<i>Pagrus major</i> (marine) (ca. 24 g)	48 72 96	> 500 (n) > 500 (n) 354 (n)	semi-static	2,6-TDA	Tadokoro 1994
<i>Pagrus major</i> (marine) (ca. 24 g)	96	> 280 (n)		2,6-TDA	Tadokoro 1994 (value cited from Japan Frozen Food Inspection Co)
<i>Scophthalmus maximus*</i> (marine)	96	> 100 (n)	semi-static	TDA 80/20	Morris 1998

m: measured concentration; n: nominal concentration

The test performed with the turbot *Scophthalmus maximus* (turbot) is regarded as not valid for the following reasons: 1) only 5 fish per concentration were tested, 2) the test substance was added directly to 10 l volumes of seawater and the authors report undissolved test substance throughout the whole test period, 3) no clear dose-effect curve was obtained: while at the concentrations 0.1, 1 and 100 mg/l no fish died, at 10 mg/l 40 % of the fish were found to be dead.

Among the tested fish species, the marine species *Pagrus major* (red sea bream) was most sensitive to TDA. The 96h-effect values found with this species for 2,4-TDA and 80/20-TDA are more than a factor of 1000 lower than the corresponding effect values available for other

fish species. Different tests have been performed by the same authors to examine the toxicity of TDA to *Pagrus major*. The first studies were performed in a static, the latter in a semi-static system. In the studies reported in Tadokoro et al. (1994) aeration was additionally employed during the exposure. However, in these tests not only the exposure and aeration system but also the size of the test organisms was changed (from 1 g to 10 and 20 g). In the studies available for *Pagrus major*, the toxicity was shown to increase remarkably within the 96h exposure period.

The lowest effect value of 0.161 mg/l was found in a study performed in a semi-static system with fish of 1 g size. Although in this test the oxygen concentration at test end was below the value of 60 % saturation prescribed by the OECD guideline, the study is not regarded as invalid. The oxygen content at test start was about 80 % of saturation. At test end it was between 54 % and 58 % of saturation (related to oxygen saturation concentration in freshwater). The lowest oxygen content was found in the control where no mortalities occurred. For the other concentrations the oxygen content at test end was only slightly below the value prescribed in the OECD guideline. It has also to be kept in mind that the test solution was renewed after 48 hours and that it can therefore be assumed that the oxygen concentration in the test solutions was not at the level measured at test end throughout the whole exposure period. Therefore, it is not likely that the small deviation from the guideline in relation to the oxygen content has affected the test result. In addition, it should also be considered that the test was performed in natural seawater for which the oxygen content at saturation is lower than for freshwater. Regarding this it can be assumed that the oxygen content in the test vessels was not below 60 % of saturation for seawater and the validity criterion of the OECD guideline may be fulfilled for this test if it is transferred to seawater.

The LC<sub>50</sub> values reported by Tadokoro et al. (1994) for 2,4- and 2,6-TDA are significantly higher than the effect values described in the former publications of the same authors. As two different parameters were varied (oxygen concentration and size of the test organisms), no clear conclusion on the reasons for this variation can be drawn. On the one hand one can conclude from these data that the sensitivity of *Pagrus major* to TDA is dependent on size/weight of the used fish: Fish with a weight of about 1 g were most sensitive. However, even for fish with a weight of about 10 g and about 20 g effect values for 2,4-TDA were clearly below 1 mg/l (the oxygen content in the test vessels was above 60 % saturation during the whole exposure period). Only with fish of 24 g weight the effect value for 2,4-TDA was above 1 mg/l (2.1 mg/l). OECD and EU test guidelines recommend juvenile fish in the range of 2 to 6 cm length (dependent on the used species, weight not given). As the tested specimens of *Pagrus major* were in the range of 8.6 – 11.6 cm (10-24 g) it can be concluded that the tests with the 1 g juveniles are most relevant for the effect assessment. On the other hand it cannot be completely excluded that the different oxygen content in the different studies has probably influenced the test results. In addition, the authors mentioned a colour change in the test solutions: in the test with 2,4-TDA test solutions were transparent at test start and became violet at test end. In the 2,6-TDA study test solutions were transparent and brown at the preparation and turbid at the end of the test. No further information is given by the authors. *Pagrus major* is native in coastal waters surrounding Japan and China. It cannot be found in European coastal waters. However, for effects assessment the principle of “representative organism” is used, i.e. it does not play a role whether the studied species is native in the region for which the assessment is performed. Therefore, the effect values found for *Pagrus major* are not rejected for the European risk assessment. The only possible explanation for the great differences in the available toxicity data for fish is a species-specific toxicity of TDA to *Pagrus major*. However, the cause for this species-specific toxicity is

unclear. It cannot be stated that TDA is in general more toxic to marine than to freshwater organisms as this was not the case for the tested invertebrate species.

Only with *Pagrus major* both substances, 2,4-TDA and 2,6-TDA were tested. Therefore, there cannot be made a clear statement concerning the relative toxicity of 2,4-TDA and 2,6-TDA to other fish species. The high sensitivity of *Pagrus major* is restricted to the 2,4-TDA and the TDA 80/20. Pure 2,6-TDA is much less toxic to *Pagrus major* than 2,4-TDA. The effect values obtained are in the range of the effect values found for TDA 80/20 with the other tested species.

In addition to the toxicity tests with TDA tests with TDI 80/20 are available for several fish species (Table 3.10). TDI is not stable in water and hydrolyses to TDA and oligourea. The extent of TDA formed during hydrolysis of TDI is dependent on the stirring conditions and the ratio TDI and water (c.f. 3.1.1.1). As the TDA concentration was measured in these tests, the reported effect values related to TDA can be used as indication on the toxicity of TDA. With the assumption that only TDA was responsible for the toxicity observed in the TDI studies and the toxicity was not influenced by formed oligourea, by any other reaction product of TDI or by TDI itself, the following LC50 values related to TDA can be estimated:

Table 3.10: Short-term toxicity of TDI 80/20 to fish

Species	Exposure period	LC50 TDI [mg/l]	LC50 TDA [mg/l]	Test conditions	reference
<i>Oryzias latipes</i>	96	6,050	2.56-4.43	Static preparation, static test	Tadokoro 1990
<i>Oryzias latipes</i>	96	4,170	15.0-16.3	Stirring preparation, static test	Tadokoro 1990
<i>Oncorhynchus mykiss</i>	96	133	6.16	Stirring preparation, static test	Tadokoro 1991b
<i>Mugil cephalus</i> (estuarian)	96	4,100 (not valid as oxygen content fell < 60 % of saturation)	10.9	Stirring preparation, static test	Tadokoro 1991b
<i>Paralychtis olivaceus</i> (marine)	96	45.8 (not valid as oxygen content fell < 60 % of saturation)	3.9	Stirring preparation, static test	Tadokoro 1991b
<i>Pagrus major</i> (marine)	96	0.36 (not valid as oxygen content fell < 60 % of saturation)	about 0.2	Stirring preparation, static test	Tadokoro 1991b

For *Pagrus major* the LC50 values obtained in the studies using TDA as test substance are in the same order with the LC50 related to the measured TDA concentration from the TDI studies. For *Oryzias latipes* the effect values related to TDA are much lower than obtained in the above cited studies performed with pure TDA. The other species tested with TDI have not been tested with pure TDA. The effect values related to TDA (about 4 to 11 mg/l) show that these species are in deed less sensitive than *Pagrus major*, however only by a factor of 10 to 100. It has to be noted that the ratio of 2,4-TDA and 2,6-TDA in the test solution was much different from the ratio of isomers in the added TDI and that the TDA concentration in the test solution did not correlate to the concentrations of TDI.

### Long-term toxicity to fish

Holcombe et al. (1995) tested the long-term toxicity of 2,4-TDA in a larval test with *Oryzias latipes*. Larvae between 0 and 3 days old were exposed in a flow-through system to the chemical for 28 days. Fish were fed live brine shrimps twice a day. Analytical monitoring of the test solution was performed twice a week. At the lowest tested concentration of 40.3 mg/l growth of the larvae was significantly reduced, while survival was significantly reduced at a concentration of 68.8 mg/l. No NOEC can be derived from this test. As at the LOEC of 40.3 mg/l the effect on growth was more than 20 % it is not possible to derive a NOEC from the LOEC according to the TGD.

The toxicity of 80/20 TDA to embryos and sac-fry stages of *Danio rerio* was investigated according to OECD guideline 212 (Bruns, 2002a). Fertilized eggs (8 to 64 cell stage) were exposed under flow-through conditions to the test substance for 10 days. The guideline demands the termination of the test just before the yolk sac of any of the larvae in any of the test chambers has been completely absorbed. Therefore, the evaluation of the test results is based on the findings after 9 days of exposure, as the yolk of the larvae has been completely absorbed at this time. Tested endpoints were hatching, mortality, behaviour and body abnormalities and length of the larvae. Substance concentration was verified by GC analysis and was found to be in the range of 92.7 to 144 % of the nominal concentration. Therefore, the effect values were related to nominal concentrations. The most sensitive endpoint was behaviour abnormality. A NOEC of 3.16 mg/l and a LOEC of 10 mg/l was found for this parameter. For both hatching and mortality a NOEC of 10 mg/l and a LOEC of 31.6 mg/l was derived. A comparison of the NOEC of 3.16 mg/l obtained in this study with the short-term LC50 of 392 mg/l found for the same species indicates an acute/chronic ratio of more than 2 orders of magnitude.

Table 3.11: Short-term toxicity to invertebrates

Species	duration [h]	EC50/LC50 [mg/l]	test method	test substance	reference
<i>Daphnia magna</i>	24	12 (m)	OECD 202; semistatic, analytical monitoring	2,4-TDA	Danish EPA, 1998
<i>Daphnia magna</i>	48	1.6 (m)	OECD 202; semistatic, analytical monitoring	2,4-TDA	Danish EPA, 1998
<i>Daphnia magna</i>	24	27.6 (pH 6) (m) > 40 (pH 7.5 + 9) (m)	static, analytical monitoring	TDA 80/20	Tadokoro 1991a
<i>Daphnia magna</i>	48	2.02 (pH 6) (m) 4.26 (pH 7.5) (m) 4.85 (pH 9) (m)	static, analytical monitoring	TDA 80/20	Tadokoro 1991a
<i>Daphnia magna</i>	48	7.85 (n)		TDA 80/20	Tadokoro 1990
<i>Mysidopsis bahia</i> (marine)	96	4.32 (n)		TDA 80/20	Tadokoro 1991a / 1992 (test result from previous study of the authors cited in these references)
<i>Moina macrocopa</i>	24	12.5 (n)	static	2,4-TDA	Fujiwara 1982
<i>Moina macrocopa</i>	24	14 (n)	static	2,6-TDA	Fujiwara 1982

m: measured concentration; n: nominal concentration

The lowest effect value was found for *Daphnia magna* with an 48h-EC<sub>50</sub> of 1.6 mg/l. As with 2,4-TDA the 24h-EC<sub>50</sub>-values for *Daphnia magna* and *Moina macrocopa* are in the same order, it can be assumed that both species are similar sensitive. Only 1 test was conducted with 2,6-TDA using *Moina macrocopa* as test organisms. The effect value from this test is in the same order with the value for 2,4-TDA for the same species. Therefore, it can be

concluded that the toxicity of 2,4-TDA and 2,6-TDA is almost the same for all daphnids. From the available test with the marine species *Mysidopsis bahia* it can be concluded that freshwater and marine invertebrates are of similar sensitivity to TDA.

In addition to the toxicity tests with TDA tests with TDI 80/20 are available for *Daphnia magna* and *Mysidopsis bahia*:

Table 3.12: Short-term toxicity of TDI 80/20 to invertebrates

Species	exposure period	EC50/LC50 TDI [mg/l]	EC50/LC50 TDA [mg/l]	test conditions	reference
<i>Daphnia magna</i>	48	12.5	ca. 5	Stirring preparation, static test	Tadokoro 1991a
<i>Mysidopsis bahia</i>	96	14	ca. 6	Stirring preparation, static test, feeding during test	Tadokoro 1991a

Although these data are not used for a quantitative effect assessment of TDA they show that the effect values related to the TDA concentration is for both organisms within the range of the effect values determined with the pure TDA.

Table 3.13: Long-term toxicity to invertebrates

Species	duration	NOEC [mg/l]	test method	test substance	Reference
<i>Daphnia magna</i>	21 d	0.282 (m)	OECD 211, semistatic, analytical monitoring	TDA 80/20	Bruns 2002b
<i>Moina macrocopa</i>	14 d	0.8 (n)	semistatic	2,4-TDA	Fujiwara 1982
<i>Moina macrocopa</i>	14 d	0.9 (n)	semistatic	2,6-TDA	Fujiwara 1982

m: measured concentration; n: nominal concentration

The lowest effect value was found for *Daphnia magna* with a 21d-NOEC of 0.282 mg/l. For *Moina macrocopa*, tests with 2,4-TDA and 2,6-TDA were performed indicating similar toxicity of the two isomers to invertebrates.

Table 3.14: Toxicity to aquatic plants

Species	duration [h]	effect value [mg/l]	test substance	reference
<i>Scenedesmus subspicatus</i>	72	EbC50 = 126 (n) EbC12 = 10 (n)	2,4-TDA	Bayer AG, 1988
<i>Selenastrum capricornutum</i>	96	EbC50 = 9.54 (?) EbC20 = 7.7 (?)	2,4-TDA	Dodard et al., 1999
<i>Selenastrum capricornutum</i>	96	EbC50 = 57 (?) EbC20 = 55 (?)	2,6-TDA	Dodard et al., 1999

n: nominal concentration; (?): not clear, whether nominal or measured concentration

Tests were performed with two algae species. For *Scenedesmus subspicatus* a test with 2,4-TDA is available while with *Selenastrum capricornutum* both isomers were tested. From these tests it can be concluded that the 2,4-TDA isomer may be slightly more toxic than the 2,6-TDA. In all three tests biomass was used as test endpoint. Although growth rate is more preferred as test parameter in algae tests, a recalculation of the effect values is not regarded as necessary, as algae are not the most sensitive species to TDA and therefore the available biomass data will not influence the PNECaqua.

In addition to the toxicity tests with TDA tests with TDI are available for green algae:

Table 3.15: Toxicity of TDI 80/20 to aquatic plants

Species	exposure period [h]	EC50 TDI [mg/l]	EC50 TDA [mg/l]	test conditions	reference
<i>Chlorella vulgaris</i>	72	4,000 (biomass)	ca. 8 (only roughly estimated)	Stirring preparation, static test	Tadokoro 1990
<i>Skeletonema costatum</i>	72	4,230 (biomass)	ca. 8 (only roughly estimated)	Stirring preparation, static test	Tadokoro 1991a

Again, these values are not used for a quantitative effect assessment.

### Microorganisms

Only 1 test with microorganisms is available that can be used for the derivation of the PNECmicroorganism. In a respiration inhibition test with activated sludge a 3h-EC50 > 100 mg/l was found for TDA 80/20. The effect value is related to nominal concentration (Caspers et al. 1986).



### Determination of PNEC<sub>aqua</sub>

The marine fish species *Pagrus major* showed the highest sensitivity to 2,4-TDA and TDA 80/20 (96 h-LC<sub>50</sub> of 0.161 mg/l). Pure 2,6-TDA was clearly less toxic to this species. The reason for the great difference in toxicity of 2,4-TDA and TDA 80/20 between *Pagrus major* and the other fish species is unclear. Nevertheless, as the results are regarded as valid, the effect values found with *Pagrus major* are used for the derivation of the PNEC<sub>aqua</sub>. Although pure 2,6-TDA is much less toxic to this species than 2,4-TDA and TDA 80/20, only 1 PNEC on the basis of the effect value found for TDA 80/20 with *Pagrus major* is derived. At nearly all sites TDA 80/20 is produced and released via the wastewater into the environment. By passing a biological wwtp, the ration of 2,4- and 2,6-TDA is slightly changed in favour of 2,6-TDA to 70:30 due to different degradation behaviour of the 2 isomers. Nevertheless, as a pragmatic approach the PNEC<sub>aqua</sub> based on the effect value for 80/20 TDA is used for the risk assessment. Long-term tests with species from three trophic levels are available. The lowest NOEC found was a 21d-NOEC of 0.282 mg/l (80/20-TDA) for *Daphnia magna*. As this NOEC is slightly higher than the lowest 96h-LC<sub>50</sub> for *Pagrus major* (0.161 mg/l), the PNEC is derived by application of an assessment factor of 100 to the acute value according to the TGD.

$$\text{Therefore } \text{PNEC}_{\text{aqua}} = 0.161 \text{ mg/l} / 100 = 1.6 \text{ } \mu\text{g/l (PNECaqua}_1)$$

Although the study is regarded as valid, the Technical Meeting decided to derive alternatively a second PNEC from the *Daphnia* long-term study due to the above discussed uncertainties with the interpretation of the study with *Pagrus major* (influence of oxygen content on test results). An assessment factor of 50 is applied to the NOEC of 0.282 mg/l according to the TGD as no long-term test with the most sensitive species in short-term tests (*Pagrus major*) is available.

$$\text{Therefore: } \text{PNECaqua} = 0.282 \text{ mg/l} / 50 = 5.64 \text{ } \mu\text{g/l (PNECaqua}_2)$$

Both PNECs are used for the risk characterisation.

### Determination of PNEC<sub>microorganism</sub>

The only relevant test result is the 3 h-EC<sub>50</sub>-value of > 100 mg/l for activated sludge. For the determination of the PNEC<sub>microorganism</sub> an EC<sub>50</sub>-value of 100 mg/l is used and an assessment factor of 100 has to be applied to this value.

$$\text{Therefore: } \text{PNEC}_{\text{microorganism}} = 100 \text{ mg/l} / 100 = 1 \text{ mg/l}$$

### Sediment

Two tests with sediment dwelling organisms are available.

Species	duration	NOEC [mg/kg dw]	test substance	reference
<i>Chironomus riparius</i>	28 d	500	TDA 80/20	I.I.I. 2004
<i>Lumbriculus variegatus</i>	28 d	12.3	TDA 80/20	I.I.I. 2005

The report (I.I.I. 2005) for the test with *Lumbriculus variegatus* gave a NOEC 333 mg/kg dw. A recalculation with the Williams-test shows a significant effect by 37 mg/kg dw. So the NOEC is 12.3 mg/kg dw.

With this NOEC (12.3 mg/kg) and an assessment factor of 50 according to the TGD.

$$\text{the } \text{PNEC}_{\text{sed}} = 12.3 \text{ mg/l} / 50 = 0.24 \text{ mg/kg dw (PNECaqua}_1)$$

### 3.2.2 Atmosphere

No data available.

### 3.2.3 Terrestrial compartment

#### Available data

#### Soil dwelling organisms

##### *Eisenia fetida*

(2,4/2,6-TDA (80/20), nominal concentration, artificial soil, TNO 1992a)

14 d-LC<sub>50</sub> > 1000 mg/kg dw

14 d-LC<sub>0</sub> = 464 mg/kg dw

(effect: weight) 14 d-NOEC = 215 mg/kg dw

#### Terrestrial plants

##### *Avena sativa*

(2,4/2,6-TDA (80/20), nominal conc., semi-natural soil, organic carbon content: 1.1 %, TNO 1992b)

(effect: emergence)	17 d-NOEC = 320 mg/kg dw
	17 d-EC <sub>50</sub> = 904 mg/kg dw
(effect: growth)	14 d-NOEC = 320 mg/kg dw
	14 d-EC <sub>50</sub> = 320 - 1000 mg/kg dw
(effect: survival)	14 d-NOEC > 1000 mg/kg dw

### *Lactuca sativa*

(2,4/2,6-TDA (80/20), nominal conc., semi-natural soil, organic carbon content: 1.1 %, TNO 1992b)

(effect: emergence)	17 d-NOEC = 100 mg/kg dw
(effect: growth)	14 d-NOEC = 100 mg/kg dw
	14 d-EC <sub>50</sub> = 320 - 1000 mg/kg dw
(effect: survival)	14 d-NOEC = 320 mg/kg dw

The tests with *Eisenia fetida* and the terrestrial plants are regarded as short-term tests. The lowest LC<sub>50</sub>- or EC<sub>50</sub>-value is used for calculating the PNEC<sub>soil</sub>. Both *Lactuca sativa* and *Avena sativa* show an EC<sub>50</sub>-value for growth between 320 and 1000 mg/kg dw. For reasons of precaution an EC<sub>50</sub>-value of 320 mg/kg dw is used for the determination of the PNEC<sub>soil</sub>. As there are only test results from short-term tests with species from two trophic levels available, an assessment factor of 1000 has to be applied.

Therefore: **PNEC<sub>soil</sub> = 320 mg/kg dw / 1000 = 0.32 mg/kg dw**

### 3.2.4 Non compartment specific effects relevant to the food chain

A biomagnification via food chain is not expected via the route water - fish. Due to possible bioaccumulation for sediment organisms, biomagnification cannot be excluded for the route sediment - sediment dwelling worm - worm-eating fish –fish eating mammal or bird.

On the basis of mammalian toxicity data, 2,4-TDA is classified as toxic. According to the TGD 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 these studies have to be converted into a food concentration by using the ratio between body weight and daily food intake as conversion factor. In the TGD conversion factors for several laboratory test species (rats, mice...) are given.

In a 2 year study with rats that were exposed to 2,4-TDA via the food, a LOAEL of 5.9 mg/kg bw/d was found. As no NOAEL is available from this study, the PNEC<sub>soil</sub> is derived from

the LOAEL This LOAEL have to be converted into a food concentration by using a conversion factor of 20. With this a LOEC of 118 mg/kg food can be derived. According to the TGD, for the calculation of the PNEC<sub>oral</sub> an assessment factor of 30 has normally to be applied to this value a NOAEL found in a chronic study. However, as no NOAEL but only a LOAEL is available for 2,4-TDA, it is proposed to increase the assessment factor by a factor of 2 to 60. Therefore, a PNEC<sub>oral</sub> of 1.97 mg/kg food is calculated.

It has to be kept in mind that 2,4-TDA as a genotoxic carcinogen may affect individual top predators of species with long life-cycles at concentrations below the PNEC<sub>oral</sub>. Especially for endangered species where individuals may need to be protected to support the survival of the species this may be a problem. However, it is assumed that the risk assessment for man indirectly exposed via the environment is also protective for individual top predators.

### **3.3 RISK CHARACTERISATION**

#### **3.3.1 Aquatic compartment**

The result of the aquatic effects assessment are a PNEC<sub>microorganism</sub> of 1000 µg/l, a PNEC<sub>aquatic</sub> of 1.6 µg/l resp. 5.64 µg/l and a PNEC<sub>sed</sub> of 12.3 mg/kg dw. In the following table, the risk characterisation ratios for all known TDA production and processing sites as well as the 2 known sites that process 2,4-TDA to dyes together with the underlying databasis for the PEC calculations are presented.

Table 3.16: PEC/PNEC ratios for surface water and WWTP

Site	life stage	site-specific data	default values	Ceffl. [µg/l]	Ceffl. / PNECmicro.	PEClocal [µg/l]	PEClocal / PNECaqua <sub>1</sub>	PEClocal / PNECaqua <sub>2</sub>
A	prod. + proc. to TDI	effluent conc. for TDA, sewage flow	Dilution 1:100	< 10	< 0.01	< 0.0026	< 1.6 · 10 <sup>-3</sup>	< 4.6 · 10 <sup>-4</sup>
B	prod. + proc. to TDI	effluent conc. for TDA , sewage and river flow	-	< 70	< 0.07	< 0.07	< 4.3 · 10 <sup>-2</sup>	< 1.2 · 10 <sup>-2</sup>
C	prod.	effluent conc. for TDA, number of days, sewage and river flow		< 20	<0.02	< 0.03	<1.8 · 10 <sup>-2</sup>	< 5.3 · 10 <sup>-3</sup>
D	prod. + proc. to TDI	effluent conc. for TDA, sewage and river flow	-	9.4	0.009	0.58	0.36	0.1
E	prod. + proc. to TDI	effluent conc. for TDA, sewage and river flow	-	35.2	0.035	0.13	8.1 · 10 <sup>-2</sup>	2.3 · 10 <sup>-2</sup>
F	Prod. + proc. to TDI	effluent conc. for TDA, sewage and river flow, no biological wwtp	-	300	-	0.3	0.19	0.05
G*	Prod. + proc. to TDI	effluent conc. for TDA, sewage and river flow, no biological wwtp	-	<3000 140	-	< 23.4 0.87	< 14.6 0.54	< 4.15 0.15
H	Prod. + proc. to TDI	conc. of TDA in process water, dilution in wwtp,	dilution 1:100	< 6.6	< 0.0066	< 0.065	< 4 · 10 <sup>-2</sup>	< 1.1 · 10 <sup>-2</sup>
Σ B,C						< 0.1	< 8.1 · 10 <sup>-2</sup>	< 1.8 · 10 <sup>-2</sup>

Site	life stage	site-specific data	default values	Ceffl. [µg/l]	Ceffl. / PNECmicro.	PEClocal [µg/l]	PEClocal / PNECaqua <sub>1</sub>	PEClocal / PNECaqua <sub>2</sub>
Dye1	Processing to dyes		emission factor: 2 %; number of days: 20, wwtp flow rate + elimination, dilution factor	1,180	1.18	30	18.7	5.3
Dye2	Processing to dyes	Sewage and river flow, number of days	emission factor: 2 %; elimination in wwtp	0.13	0.00013	0.35	0.2	6.2·10 <sup>-2</sup>

\* This site stopped the production of TDA in 2005.

### Waste-water treatment plant

For the scenario “processing of 2,4-TDA to dyes” at site dye1 the wwtp effluent concentration is above the PNECmicorganisms. As this scenario is fully based on default values, improvement of the data basis is possible. Information on TDA emission from this site should be provided.

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

The WWTP effluent concentrations are below the PNECmicroorganisms for all known production and processing sites.

- 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

### Surface water

PEC/PNEC ratios are below 1 for all sites except the scenario “processing of 2,4-TDA to dyes” at site dye1. As this scenario is fully based on default values, improvement of the data basis is possible. Information on TDA emission from this site should be provided.

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

For all other sites the PEC/PNEC ratios are below 1.

- 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

The same conclusion of the risk characterisation is obtained for both values of the PNEC<sub>auqa</sub>.

### Sediment

The following PEC/PNEC ratios for the sediment compartment were obtained:

Table 3.17: PEC/PNEC ratios for the sediment compartment

	PEC <sub>local</sub> [µg/kg ww]	PEC/PNEC <sub>sed</sub>	
A	< 0.52	< 2.1 · 10 <sup>-3</sup>	
B	< 14	< 5.7 · 10 <sup>-2</sup>	
C	< 6.4	< 2.6 · 10 <sup>-2</sup>	
D	116	0.47	
E	26	0.11	
F	60	0.24	
G*	< 4,686 174	1.9 · 10 <sup>-2</sup> 0.71	
H	< 13	5.3 · 10 <sup>-2</sup>	
Σ B,C	< 20.4	8.3 · 10 <sup>-2</sup>	
Processing to dyes:			
Dye1	6,390	26	
Dye2	74.5	0.30	

\* This site stopped the production of TDA in 2005.

The PEC/PNEC ratio for the site Dye 1 that process 2,4-TDA to dyes is above 1.

The exposure estimation for the site dye1 is fully based on default values, therefore, improvement of the exposure data basis is possible.

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

For all other sites the PEC/PNEC ratios are below 1.

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

### 3.3.2 Atmosphere

As no significant exposure is expected, a quantitative risk assessment for this compartment is not necessary.

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



### 3.3.3 Terrestrial compartment

As no significant exposure is expected, a quantitative risk assessment for this compartment is not necessary.

- 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

### 3.3.4 Non compartment specific effects relevant to the food chain

Because of the low accumulation of TDA in fish via water, the exposure route fish - fish eating bird is likely to be not relevant. However, the reaction product of TDA with sediment organics accumulates in sediments and cannot be excluded to be bioavailable based on the limited existing data. A biomagnification via the route sediment - sediment dwelling organisms – (worm eating fish) –fish eating mammal or bird can not be excluded. The toxicity tests with *Lumbriculus variegatus* and *Chironomus reparius* provide no indices for a strong accumulation in sediment dwelling organism.

A PNEC<sub>oral</sub> of 1.97 mg/kg food was derived from a LOAEL from a 2 year study in rats. However, a PNEC<sub>oral</sub> cannot be estimated on the basis of QSAR from the log K<sub>ow</sub> because TDA forms covalent bounds to organic material in sediments that may be bioavailable to sediment dwellers. Therefore, a qualitative assessment of this endpoint is performed. The highest PNEC<sub>water</sub> based on site-specific exposure data is 0.87 µg/l (site G). With the assumption that 50% of the diet of a predator organism comes from this local area and 50 % from the regional area results in a PNEC<sub>mean</sub> of  $(0.87 + 7.6 \cdot 10^{-3})/2 = 0.44 \mu\text{g/l}$ . The PNEC<sub>oral</sub> is 1.97 mg/kg food. This means that a bioaccumulation factor of TDA of more than 4400 l/kg is needed to give a concern for secondary poisoning. Such a high bioaccumulation factor is however not expected for TDA. E.g. for 3,4-DCA a max. BAF of 570 l/kg was obtained for the sediment worm *Lumbriculus variegatus* in a water-sediment microcosm. Considering in addition the rapid biotransformation of TDA in higher organisms it can be concluded that TDA represents no risk for the food chain.

- 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

## 4 HUMAN HEALTH

### 4.1 HUMAN HEALTH (TOXICITY)

#### 4.1.1 Exposure assessment

##### 4.1.1.1 General discussion

##### 4.1.1.2 General discussion

Toluene-2,4-diamine (2,4-TDA) is predominantly produced and further processed as a mixture with Toluene-2,6-diamine (2,6-TDA): TDA 80/20 (80 % 2,4-TDA, 20 % 2,6-TDA). A 65:35 mixture of these isomers and pure 2,4-TDA are also commercially available but not widely used. The technical-grade products contain 99 % 2,4-TDA or 2,4-/2,6-TDA mixtures, respectively.

For production volumes see section 2.

2,4-TDA is predominantly used as an intermediate in the chemical industry. The major part (98 -99 %) is processed as a 2,4-/2,6-TDA 80/20 mixture to toluylene diisocyanate (TDI). A minor amount is used as a starting product for the production of acridine, sulphur and azo dyes (Römpp, 1990/91).

Direct uses of 2,4-TDA in the EU are unknown, some applications are mentioned in the American literature (see chapt. 4.1.1.2).

For workers the inhalative and dermal exposure routes are the most likely.

##### 4.1.1.3 Occupational exposure

For 2,4-TDA occupational exposure limits (OEL) and short term limits (STEL) are established in the EU (cf. Table 4.1.1.2.).

Table 4.1.1.2: Occupational exposure levels (ARIEL, 2000)

Country	OEL	STEL
Germany *	0.1 mg/m <sup>3</sup>	0.4 mg/m <sup>3</sup> **
Austria	0.1 mg/m <sup>3</sup>	0.4 mg/m <sup>3</sup>
Netherlands	0.1 mg/m <sup>3</sup>	-

\* TRK-value, technical based occupational exposure limit obligation to minimize exposure

\*\* The average concentration shall never exceed 4 times limit value (15 minute average – Excess Factor 4)

### General

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<sup>th</sup> or 95<sup>th</sup> percentiles are derived as representing reasonable worst case situations. For the purpose of exposure assessment only data measured later than 1990, if available, are taken. If quantitative exposure data are not available, model estimates are taken. Scenarios are clustered as far as possible to make the description transparent.

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.

There is an agreement between the EU-memberstates, within the framework of existing substance, 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, 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 criteria. For most down stream 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 often quantitative information on dermal exposure is not available, the EASE model is mostly used for assessing dermal exposure.

Industrial activities using 2,4-TDA and its mixtures with 2,6-TDA present opportunities for exposure. Exposure ranges depend on the particular operation and the risk reduction measures in use.

Most of the produced pure 2,4-TDA and 2,4-/2,6-TDA (80/20) is handled in closed systems. On account of the high melting temperature (99°C), 2,4-/2,6-TDA (80/20) and most of the pure 2,4-TDA are transferred in a molten state via heated pipelines and tanks to the next processing stage. About 1000 t are produced as pure TDA, therefrom < 100 t/a in form of pastilles. A minor part is used for the production of a dye. Other uses of TDA-pastilles are not known. During the handling of the cooled substance or mixtures (2,4-/2,6-TDA (80/20)) as well as during the handling of 2,4-TDA pastilles, on account of the low vapour pressure, inhalative exposure to vapour is expected to be low. Inhalation exposure to dust is possible if pastilles are produced or used.

Occupational exposure may occur in the:

- production and further processing as a chemical intermediate (scenario 1)

- production of 2,4-TDA pastilles (scenario 2)
- use of 2,4-TDA pastilles for the production of dyes (scenario 3).

A few commercial uses for 2,4-TDA (in part both isomers) per se have been found in the US-literature (Kirk-Othmer, 1991). In epoxy curing applications, 2,4-TDA has been used as a component of a mixture with aliphatic glycidal ether resins as well as by itself. 2,4-TDA is also used as amine curatives (Lee, Nevill, 1982; May, 1988). 2,4-TDA is cited to be a monomer in aromatic polymers, e.g. various polyimides. The importance of these products for the EU is not known. Since no further information on these use is available, they are not considered within the framework of this assessment.

Another potential source of exposure may be the thermal degradation of polyurethane products, whereby TDI may be formed and react to 2,4-TDA by hydrolysis (Brochhagen, 1989). At present, the formation of 2,4-TDA outside the life cycle of the product is not considered in this exposure assessment.

#### **4.1.1.3.1 Scenario 1: Production and further processing as a chemical intermediate**

2,4-TDA is manufactured predominantly as a mixture of the two isomers 2,4-TDA and 2,6-TDA (80/20) in a continuous process by nitration of toluene with subsequent catalytic hydrogenation in closed systems. The final product is purified by distillation.

According to information provided by one manufacturer, approx. 1000 t/year pure 2,4-TDA are produced, therefrom 100 t/year as pastilles (see scenario 2).

Approx. 98 - 99 % of the EU-wide produced TDA (mainly 2,4/2,6-TDA 80/20) is converted to TDI in closed systems using phosgene. In the EEC, the production of DETDA by alkylation of 2,4-/2,6-TDA 80/20 has been stopped (Bayer 2002). Since pure 2,4-TDA and 2,4-/2,6-TDA 80/20 are solid at ambient temperature (melting point of pure 2,4-TDA: 99 °C), transfer occurs via heated pipelines.

Most of the manufactured 2,4-TDA is fed as a chemical intermediate directly to the next processing stage. Small amounts are transferred and filled in a molten form via heated pipelines in tanks using gas displacement devices or at workplaces equipped with local exhaust ventilation (filling drums). One company produces 2,4-TDA which is transferred via rail or street to the location of further processing. In this, works at the filling stations are performed throughout the shift.

Sampling is performed several times per day at different locations. The containers used to collect the samples are tightly coupled to the closed reaction system with the sampling arrangement being placed in an encapsulated housing.

Within 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, coupling and decoupling of transfer lines, cleaning, maintenance, repair works and the taking of process samples. Inhalative exposure in other areas is usually minimised by technical equipment (e.g. special designed filling stations, local exhaust ventilation). Depending on the conditions in each case, such processes are carried out under extraction systems. In addition, the units are

rinsed and the workers wear protective clothing (gloves and eye protection) and respiratory protection (Bayer, 1995).

## Inhalation exposure

### *Workplace measurements*

Table 4.1.1.2.1: 2,4-TDA exposures at workplaces in the large-scale chemical industry during production and further processing

Job category / activities	Year of measurements	Number of samples	Range of measurement data [mg/m <sup>3</sup> ]	50 <sup>th</sup> -percentile [mg/m <sup>3</sup> ]	95 <sup>th</sup> -percentile [mg/m <sup>3</sup> ]
<b>8-h time weighted average</b>					
<b>2,4-TDA and TDI production, all measurements</b>	1998 – 2002	63	-	< 0.002	< 0.01
<b>2,4-TDA production</b>	1998 – 2002	37	-	< 0.0025	< 0.01
<b>TDI production</b>	1998 – 2002	26	-	< 0.002	< 0.00875

For the purpose of determining 2,4-TDA in the air at the workplace, the substance is adsorbed to impregnated silicagel and then desorbed and determined high-liquid-chromatographically by HPLC. The selection of specific conditions allows the simultaneous collection of dust and vapour.

Due to the measurement method and the measurement strategy which were applied, the currently available measurement results (Table 4.1.1.2.1) are regarded as valid.

The measurement data show, that TDA was not detected in any sample. Limits of detection were between 0.001 mg/m<sup>3</sup> and 0.01 mg/m<sup>3</sup>, depending on the purpose of the measurement. There is a sub-collective of measurements available which has been taken with the lower detection limit (0.0015 mg/m<sup>3</sup> and below). This sub-collective was neither taken at a special time interval nor at selected working conditions. Companies belonging to the Association ISOPA (European Diisocyanate & Polyol Producers Association) confirmed in a detailed manner, that the processes and activities relevant for inhalation exposure are almost the same for all companies of ISOPA involved. It is assumed, that this is also valid for companies not represented by this association. Based on the limit of detection of 0.0015 mg/m<sup>3</sup>, the inhalation exposure yields in 0.00075 mg/m<sup>3</sup> (half of the limit of detection).

Additional confidential measurement results were provided by 5 companies. All measurement results (n > 100) were below the detection limit, which is in all but one cases 0.01 mg/m<sup>3</sup> (exception 0.5 mg/m<sup>3</sup>). The information on the results is limited, in part, stationary sampling was performed. In part, both TDA-isomers were detected.

In the scope of the implementation of TRK-values for 2,4-TDA (TRGS 901, 1999), results of workplace measurements were published, too. 27 shift averages taken during the production of 2,4-TDA and its further processing to TDI were  $< 0.02 \text{ mg/m}^3$  with one exception of  $0.07 \text{ mg/m}^3$  2,4-TDA (year of measurement unknown). At four other workplaces 23 shift averages  $< 0.02 \text{ mg/m}^3$  and 19 short term values  $< 0.04 \text{ mg/m}^3$  were obtained. No further details are given.

According to information provided by two manufacturer, 265 male workers are exposed in the area of production of 2,4-TDA. In the area of further processing, about 55 male employees are exposed continuously and 35 occasionally (Bayer, 1994).

### **Conclusions**

Inhalative exposure has to be assessed for the production and the further processing of 2,4-TDA in the large-scale chemical industry. Exposure is possible during transfer and drumming, coupling and decoupling of transfer lines, cleaning, maintenance, repair works and the taking of process samples.

For the assessment of risks of daily inhalative exposure to 2,4-TDA during the production and further processing in areas with high levels of protection belonging to the large-scale chemical industry  $0.00075 \text{ mg/m}^3$  (half of the limit of detection of a representative sub-collective) should be taken.

This exposure assessment is based on data from 1998-2002. Taking all data and information available into account, the exposure assessment is judged to be based on sufficient, representative and reliable data set.

It is to be assumed that the substance is processed daily. Consequently, the duration and the frequency of exposure to 2,4-TDA are assumed to be daily and for the entire length of the shift.

### **Dermal exposure**

For the purpose of assessing dermal exposure, a company was visited. At the production plant, the exposure relevant activities were observed in detail. The following conclusion can be drawn:

- workers are well instructed and trained in using technical and personal protective equipment (gloves, glasses, respiratory protection),
- suitable gloves are used,
- exposure relevant is filling (coupling / decoupling of transfer lines) and, to a less extent, sampling,
- only potential dermal exposure was observed.

Immediate dermal contacts are avoided due to closed system technology and the proper use of suitable gloves. Furthermore, worker would avoid any direct dermal contact to hot TDA. In this, the protection provided by the suitable gloves becomes an important parameter in risk

assessment. The protection of the gloves is considered in a default value of 90%. However, the protection efficiency might be higher, but quantification is not possible.

In the following, the observations are briefly described in the light of (potential) dermal exposure:

#### Sampling:

Sampling is performed several times per day and lasts approx. 5 minutes. The sampling device runs largely automatically. The exposure relevant activity is taking the open sampling glass filled with ca. 100 ml TDA (140 °C) and closing it with a screw top. This task lasts about 10 seconds. A worker explained, that potential dermal exposure at the outside of the gloves occurs seldom (once per week). A skin area of 1 cm<sup>2</sup> is typically exposed. Workers wear protective gloves and glasses, and respiratory protection.

#### Tank Filling

A worker performs tank filling about 3 times per shift. The exposure relevant activity is coupling and decoupling of transfer lines and especially cleaning the flange from residual liquid TDA using a cloth. During coupling activities, potential dermal exposure (TDA on the outside of the protective gloves) occurs once a day with an exposed area of 15 cm<sup>2</sup> (2 fingers). Potential dermal exposure is higher when transfer lines are decoupled: exposed area of 420 cm<sup>2</sup>, 3 times per shift. The exposed area is relatively high because the contaminated cloth was used to clean the gloves. The rapporteur was informed procedures were changed that after the visit in a way that the exposed area during decoupling is reduced to 15 cm<sup>2</sup>. Workers were instructed to probe the gloves for contaminations (black colour) and exchange contaminated gloves immediately after the working step.

It has to be noted that the exposure relevant activities were performed very carefully. It could be seen, that workers are well instructed and trained in using technical and personal protective equipment.

#### Assessment of potential dermal exposure:

Taking into account that dermal contact occurs only incidentally, the scenario could be described:

Input parameters:	Non dispersive use, direct handling, incidental
Exposure level:	0 – 0.1 mg/cm <sup>2</sup> /day.

Taking into account the exposed area of 15 cm<sup>2</sup>, potential dermal exposure is assessed to 1.5 mg/person/day. As a rough estimation, a protection of 90 % is assumed for the use of suitable gloves thus leading to a dermal exposure of 0 – 0.15 mg/person/day. The upper value is regarded to represent the worst case situation. The suitability of the used gloves under real working conditions is considered in the assumption of 90%-protection. However, the protection efficiency might be higher.

At TM I/04, different protection factors were discussed. The observations made at the companies show, that potential dermal exposure occurs on a daily scale and that protection factors accounting a lower exposure frequency are not appropriate.

Other activities (sampling, cleaning and maintenance) will lead to lower dermal exposure: For sampling, a worker stated, that potential dermal exposure occurs once a week. For maintenance activities, which are typically performed not more than once per month, parts of the plant are emptied with pressurised nitrogen and cooled down before opening. Residual solid, non-dusty 2,4-TDA may still stick to the parts and, hence, these parts are rinsed with water. The concentration of 2,4-TDA in the washing water is typically 500 – 2000 ppm. Contact to the washing water will lead to lower dermal exposure level than assessed for filling.

Hand-eye-contacts can be excluded to a large extent.

### ***Conclusion***

For the assessment of daily dermal exposure, a plant was visited. Observations revealed that potential dermal exposure occurs daily. Based on these observations and in application of the EASE model, 0.15 mg/person/day is assessed as the reasonable worst case exposure. In this, the protective effect of suitable gloves is considered. It has to be noted that the exposure relevant activities were performed very carefully. It could be seen that workers are well instructed and trained in using technical and personal protective equipment. The observations made in the plant are regarded to be representative for European plants. The suitability of the used gloves under real working conditions is considered in the assumption of 90%-protection. However, die protection efficiency might be higher.

#### **4.1.1.3.2 Scenario 2: Production of 2,4-TDA pastilles**

According to information provided by one manufacturer, < 100 t/year 2,4-TDA are produced as pastilles.

The 2,4-TDA pastilles are formed in encapsulated compression belts and are filled in drums equipped with exhaust ventilation. Further confidential information is available showing that different technical measures are in place to minimise exposure. Exposure is restricted to a single campaign per year lasting approx. 4 weeks.

The diameter of the pastilles ranges between 3 and 5 mm. No information is available on the potency of 2,4-TDA pastilles to develop dusts during handling (e.g. filling).

### ***Inhalation exposure***

Table 4.1.1.2.2: 2,4-TDA exposures at workplaces during production of 2,4-TDA pastilles



Job category / activities	Year of measurements	Number of samples	Range of measurement data [mg/m <sup>3</sup> ]	Geometric mean [mg/m <sup>3</sup> ]	95 <sup>th</sup> -percentile [mg/m <sup>3</sup> ]
<b>8-h time weighted average</b>					
Pastillation – filling/drumming	1990 - 1994	4	< 0.005 - < 0.01	-	-
	1990 - 1994	10	< 0.01 - < 0.05	< 0.016	-

### *Conclusions*

Inhalative exposure has to be assessed for the production of 2,4-TDA pastilles in the large-scale chemical industry. Exposure is possible during drumming, cleaning, maintenance, repair works and the taking of process samples.

For the assessment of risks of daily inhalative exposure to 2,4-TDA during the production of pastilles in areas with high levels of protection belonging to the large-scale chemical industry 0.025 mg/m<sup>3</sup> (half of the highest limit of detection) should be taken.

### *Dermal exposure*

Dermal exposure in the chemical industry is estimated considering that the substance is manufactured and further processed primarily in closed systems and that the use of gloves and eye protection is highly accepted. In case of 2,4-TDA pastilles, filling is performed automatically. For handling the solid substance, gloves tested according to EN 374 are used.

As a rule, for the use of suitable gloves, 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 under real working conditions (e.g. mechanical fail).

Taking into account that dermal contact occurs incidentally, a worst case situation could be described by the scenario:

Input parameters: Non dispersive use, direct handling, incidental

Exposure level: 0 – 0.1 mg/cm<sup>2</sup>/day.

The consideration of an exposed area of 420 cm<sup>2</sup> leads to exposure levels of 0 - 42 mg/person/day.

As a rough estimation, a protection of 90 % is assumed for the use of suitable gloves thus leading to a dermal exposure of 0 - 4.2 mg/person/day. The upper value is regarded to represent the worst case. Since the material is handled in form of pastilles, dermal exposure is assumed to be below the upper value. No information on the abrasion properties of the pastilles is available. Nevertheless, it is quite reasonable, that exposure in case of pastilles is lower than exposure would be if a dusty material is handled. As a rough estimation, a factor of ten below the upper level should be taken as representing the reasonable worst case situation.

Hand-eye-contacts can be excluded to a large extent.

### ***Conclusion***

For the assessment of daily dermal exposure during the production of 2,4-TDA and its further processing to TDI, 0.42 mg/person/day should be taken as representing the reasonable worst case situation.

#### **4.1.1.3.3 Scenario 3: Use of 2,4-TDA pastilles for the production of dyes**

The 2,4-TDA pastilles (approx. 100 t / year) are predominantly used for the production of dyes.

There is only one company known that applies 2,4-TDA pastilles in the production of dyes. According to information provided by this company, 3,6 t /year are used. According to this company, the process is a non-aqueous batch process, in which 2,4-TDA is heated in a closed vessel under reflux together with other components. The resulting product is washed and finished.

Worker were protective clothing and gloves, and respiratory protection when filling 2,4-TDA into the reaction vessel.

A limited number of batches is performed per year, so that exposure is assessed to be not daily. Exposure is possible when the bags are opened and emptied. According to information from industry, exposure is not daily.

### ***Inhalation exposure***

The company provided one measurement result of 0.05 mg/m<sup>3</sup> (limit of detection, shift average, 1996). From 1990 - 1994, measurement values ranging from 0.005 – 0.014 mg/m<sup>3</sup> (shift average) were available.

### **Conclusion**

0.025 mg/m<sup>3</sup> should be taken as representing the reasonable worst case situation (not daily exposure, half of the highest detection limit).

### ***Dermal exposure***

The dye is produced in only one company which described that worker wear suitable gloves (for handling solid materials, gloves are assumed to suitable for protection against penetration). Dermal exposure might occur during opening the bags and filling 2,4-TDA pastilles into the reaction vessel.

Input parameters: Non dispersive use, direct handling, incidental.

Exposure level: 0 – 0.1 mg/cm<sup>2</sup>/day.

In consideration of an exposed area of 420 cm<sup>2</sup> and a protection efficiency of 90 % achieved by the gloves, dermal exposure is assessed as 4.2 mg/person/day on a non-daily basis. No information on the abrasion properties of the pastilles is available. Nevertheless, it is quite reasonable, that exposure in case of pastilles is lower than exposure would be if a dusty material is handled. As a rough estimation, a factor of ten below the upper level should be taken as representing the reasonable worst case situation.

### **Conclusion**

For the assessment of the risks from non-daily dermal exposure, a level of 0.42 mg/person/day should be taken.

#### **4.1.1.3.4 Summary**

2,4-TDA is predominantly processed in form of mixtures with 2,4-/2,6-TDA (80/20) which are used as an intermediate in the chemical industry. The major part (98 – 99 %) is processed to toluylene diisocyanate (TDI), a starting product for the production of polyurethane products. A small quantity of pure 2,4-TDA is produced as pastilles (< 100 t/a, scenario 2), which are further processed to dyes (3.6 t/a, scenario 3). The use of pastilles for the production of dyes is the only known use.

Based on the information from industry, it is concluded that 2,4 TDA is manufactured and processed at a very high level of protection. For all scenarios, only limits of detection are given as measures for inhalation exposure. These limits of detection are often set at 1/10 of the OEL (0.1 mg/m<sup>3</sup>).

Relevant inhalation and dermal exposure levels are given in table 4.1.1.2.2 A and 4.1.1.2.2 B, respectively. In case of inhalation exposure, for scenario 1 a sufficient number of measurement values (limits of detection) with detailed descriptions of the processes is available. There is a sufficient subscenario of measurement results with limits of detection below 0.0015 mg/m<sup>3</sup>. Half of this level is taken forward to the risk characterisation.

For the assessment of dermal exposure in scenario 1, a plant was visited. The observations revealed that potential dermal exposure on the outside of the gloves occurs daily. The following conclusion can be drawn:

- workers are well instructed and trained in using technical and personal protective equipment (gloves, glasses, respiratory protection),
- suitable gloves are used,
- exposure relevant is filling (coupling / decoupling of transfer lines) and, to a less extent, sampling,
- only potential dermal exposure was observed.

Immediate dermal contacts are avoided due to closed system technology and the proper use of suitable gloves. In this, the protection provided by the suitable gloves becomes an important parameter in risk assessment. The protection of the gloves is considered in a default value of 90%. However, the protection efficiency might be higher, but quantification is not possible.

For scenario 2 and 3, less information is available, but the assessed exposure levels are to be regarded as representative (half of the highest levels of detection, 95<sup>th</sup> percentiles could not be derived based on the available information). In both cases, only one company realises the scenario.

Dermal exposure of scenario 2 and 3 is assessed using the EASE model. Since the dermal part of the EASE model was developed based on liquids, it is to be assumed, that the actual dermal exposure is lower than the assessed one. No information on the abrasion properties of the pastilles is available. Nevertheless, it is quite reasonable, that exposure in case of pastilles is lower than exposure would be if a dusty material is handled. As a rough estimation, a factor of ten below the upper level should be taken as representing the reasonable worst case situation.

Table 4.1.1.2.2 A: Summary of inhalation exposure data (reasonable worst case) of 2,4-TDA which are relevant for occupational risk assessment

Inhalation exposure								
Area of production and use	Form of exposure	Activity	Duration [hs/day]	Frequency [days/year]see	Shift average [mg/m <sup>3</sup> ]	Method	Short-term exposure[mg/m <sup>3</sup> ]	Method
<b>Production and further processing as a chemical intermediate</b>								
1) Production and further processing as a chemical intermediate	dust	filling, decoupling of transfer lines, sampling, cleaning, repair, maintenance	8 h / day	daily	0.00075	half of the detection limit of a representative sub-collective	-	-
2) Production 2,4-TDA pastilles	dust	drumming, sampling, cleaning, repair, maintenance	2 hours / day (assumed)	1 campaign per year (4 weeks)	0.025	half of the highest limit of detection	-	-
3) Use of 2,4-TDA pastilles for the production of dyes	dust	filling, repair, maintenance	2 hours per day (assumed)	not daily	0.025	half of the highest limit of detection	-	-

Table 4.1.1.2.2 B: Summary of dermal exposure data (reasonable worst case) of 2,4-TDA which are relevant for occupational risk assessment

Dermal exposure								
Area of production and use	Form of exposure	Activity	Frequency [days/year]	Contact level <sup>1)</sup>	Level of exposure [mg/cm <sup>2</sup> /day]	Exposed area [cm <sup>2</sup> ]	Shift average [mg/p/day]	Method (use of gloves)
<b>Production and further processing as a chemical intermediate</b>								
1) Production and further processing as a chemical intermediate	cooled substance	cleaning, repair, maintenance, decoupling of transfer lines	daily	incidental	0 - 0.1	15	0.15 <sup>2)</sup>	EASE (use of suitable gloves)
2) Production 2,4-TDA pastilles	pastilles, dust	drumming, sampling, cleaning, repair, maintenance	1 campaign per year (4 weeks)	incidental	0 – 0.1	420	0.42 <sup>3)</sup> (worst case, exposure might be considerably lower)	EASE (use of suitable gloves)
3) Use of 2,4-TDA pastilles for the production of dyes	pastilles, dust	filling, repair, maintenance	not daily	incidental	0 – 0.1	420	0.42 <sup>3)</sup>	EASE (use of suitable gloves)

<sup>1)</sup> Contact level according to the EASE model

<sup>2)</sup> Protection efficiency of suitable gloves is assumed to be 90%

<sup>3)</sup> Assessment based on the EASE model. Taking into account that TDA is handled in form of pastilles, dermal exposure is assessed to be a factor of 10 below the model estimate.

#### **4.1.1.4 Consumer exposure**

2,4-TDA is used in Germany almost exclusively as an intermediate in the manufacture of 2,4-toluylene diisocyanate, a starting product for polyurethane products, and very occasionally in the manufacture of colorants.

There are no indications of any direct application of 2,4-TDA by the consumer (ISOPA, 2003).

Under special chemical conditions (high temperature, high pressure, presence of solvolytic alcohols) TDA may be liberated from polyurethane products. However, under normal use conditions it can be expected that a liberation of TDA would not occur. Continuous monitoring of product quality has revealed that the 2,4-TDA content in flexible foam PUR consumer products is below detectable limits (ca. 0.1 ppm) (EUROPUR, 2003). Therefore, a formation of TDA outside the life cycle of polyurethane products is not considered in this exposure assessment.

#### **4.1.1.5 Indirect exposure via the environment**

Based on the environmental concentrations in the different compartments, the indirect exposure to humans via the environment through food, drinking water and air is estimated.

On the local scale, the human intake is calculated on the basis of the exposure in the vicinity of the biggest point source (based on site-specific information) (site G, cf. 3.1.2.1). On the regional scale, the average intake due to exposure via the regional background concentration for each isomer (cf. 3.1.6) is estimated.

As the two isomers have different physico chemical properties, the calculation for 2,4-TDA and 2,6-TDA is performed separately. The results are then added to give the human intake for TDA isomer mixture. It is assumed that the  $C_{local,water}$  composed of the two isomers in the ratio 70 % : 30 %.

The following input data are used for the calculation:

Table 4.1.1.4A: Input data for the calculation of indirect exposure for the local scenario for site F:

	<b>Total TDA</b>	<b>2,4-TDA</b>	<b>2,6-TDA</b>
PEC <sub>water</sub> [µg/l]	0.87	0.61	0.26
PEC <sub>water</sub> _annual [µg/l]	0.71	0.5	0.21

For the regional scenario the PEC<sub>regional</sub> for the two isomers were used as input data:

Table 4.1.1.4 B: Input data for the indirect exposure calculation for the regional scenario

	<b>2,4-TDA</b>	<b>2,6-TDA</b>
<b>Compartment</b>	<b>Regional conc.</b>	<b>Regional conc.</b>
Surface water (dissolved) [µg/l]	5.46 · 10 <sup>-3</sup>	1.77 · 10 <sup>-3</sup>
Sediment [µg/kg ww]	1.89	0.48
Atmosphere [µg/m <sup>3</sup> ]	5.4 · 10 <sup>-12</sup>	2.51 · 10 <sup>-12</sup>
Agric. Soil [µg/kg ww]	1.87 · 10 <sup>-6</sup>	7.19 · 10 <sup>-7</sup>
Agr. soil, porewater [µg/l]	1.1 · 10 <sup>-8</sup>	5.22 · 10 <sup>-9</sup>

The following results were obtained:

Table 4.1.1.4.C: Resulting Dose<sub>tot</sub> from the local scenario for site G:

	2,4-TDA	2,6-TDA	Total TDA (Σ 2,4, 2,6)
Dose <sub>tot</sub>	0.018 µg/kg bw d	0.008 µg/kg bw d	0.026 µg/kg bw d



Table 4.1.1.4D: Resulting Dose<sub>tot</sub> from the regional scenario:

	2,4-TDA	2,6-TDA	Total TDA (Σ 2,4, 2,6)
Dose <sub>tot</sub>	0.0002 µg/kg bw d	0.00006 µg/kg bw d	0.00026 µg/kg bw d

The input data for the model calculation are presented in detail in the appendix A 3.

The main contributions to the intake at both local and regional exposure are the DOSE<sub>drw</sub> and the DOSE<sub>fish</sub> with fractions of about 78 and 22%, respectively, to the total daily dose.

#### 4.1.1.6 (Combined exposure)

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

#### 4.1.2.1 Toxicokinetics, metabolism and distribution

##### Animal data:

##### *Absorption*

A good absorption via the gastro-intestinal tract can be assumed according to the quantity of excretion via the urine and the faeces in animal experiments (see also chapter elimination). 48 hours after oral gavage of 60 and 3 mg/kg bw 2,4-<sup>14</sup>C-TDA to male Fischer F344 rats, 65 and 64 % of the administered radioactivity were excreted in the urine, indicating that at least this amount has been absorbed (Sauerhoff et al. 1977; Timchalk et al. 1994).

2,4-TDA can also penetrate the skin. The penetration of 54% in monkeys is more complete than the penetration of 24% in humans (adult rhesus monkeys and human volunteers, 4 µg/cm<sup>2</sup> <sup>14</sup>C ring-labelled TDA-dihydrochloride, vehicle acetone, uncovered abdominal skin in monkeys, uncovered ventral forearm skin in humans, contact area from 3 to 15 cm<sup>2</sup>, exposure period 24 hr, urine was collected over a 5-day period and was analysed for the radiolabel) (Marzulli et al. 1981).

##### *Tissue distribution*

In rats the highest tissue concentrations of radioactivity were measured in liver and kidney (7 to 12 µg/g wet weight after an interperitoneal dose) 4 to 24 h after oral (DuPont 1974) or intra-peritoneal administration of 300 mg/kg bw and 77 mg/kg bw <sup>14</sup>C ring-labelled 2,4-TDA, respectively (Grantham et al. 1979). Levels of radioactivity in heart, lungs, spleen, and testes were significantly lower (2 to 6 µg/g wet wt). The concentration in blood was maximal after i.p. injection in rats after 1 to 8 h. It decreased within 6 to 24 h to a low residual level (Grantham et al. 1979). The peak blood levels after oral dosing were reached within 2 hours (DuPont 1974). There are no species-related differences in tissue distribution in mice and rats

(Unger et al. 1980; Unger et al.1978; Grantham et al. 1979). In mice and rats biphasic progressions of the tissue elimination were observed (Sauerhoff et al. 1977; Unger et al. 1980; Unger et al.1978).

### *Biotransformation*

After oral administration of 2,4-TDA to rats, rabbits and guinea pigs (50 or 60 mg/kg bw), only 0.1 to 3 % of the dose was excreted as unchanged compound in the 12 and 24 hr urines (Timchalk et al.1994; Waring and Pheasant 1976). These results from GC-MS and TLC-MS analysis, respectively, suggest a nearly total biotransformation. Additional to the elimination of the unchanged parent compound many metabolites and conjugates could be identified in the urine with differences in rats and mice. The major urinary metabolites in the rat were 4-acetylamino-2-aminotoluene, 2,4-diacetylamino-2-aminotoluene, and 4-acetylamino-2-aminobenzoic acid. In the mouse they were 4-acetylamino-2-aminobenzoic acid, 4-acetylamino-2-aminobenzoic acid, and 2,4-diacetylamino-2-aminobenzoic acid. As further metabolites, alpha-hydroxy-2,4-diacetylamino-2-aminotoluene and alpha-hydroxy-2,4-diacetylamino-2-aminotoluene have been identified. Urinary excretion was faster in the mouse and in addition, the amounts of metabolites excreted as glucuronic acid conjugates in mice were about twice of that in rats.

2,4-TDA is metabolized to a high extent in mammalian species. It has been found to undergo oxidation on the aromatic ring as well as the benzylic methyl group to form phenolic and benzoic acid metabolites (see figure 4.1.2.1). 2,4-TDA and its metabolites are also excreted as acid-labile conjugates. Rats, rabbits, and guinea pigs will metabolize 2,4-TDA to aminophenols as a major pathway (about 40% of an oral dose) (Waring & Pheasant 1976). Grantham et al. (1979) identified using HPLC-MS-analyses acetyl-derivates of toluenediamine and diaminobenzoic acid as metabolism products in rats and in mice (see figure 4.1.2.1). The acetylation pathway represents about 15% of an intraperitoneal dose. Mono- as well as di-acetyl-derivates of 2,4-TDA were observed in different quantities in the urine of rats (15% of the dose), rabbits (8%), guinea pigs (8%), and dogs (about < 1% mono acetyl derivatives were detected using HPLC in 24 h-urine samples of two dogs after oral doses of 100 mg 2,4-TDA/animal daily for eight days; no further details were given) (Waring & Pheasant 1976; Burroughs 1975). Grantham et al. (1979) found additional to the acetyl-derivatives an elimination of sulfate conjugates in 10 % of the dose in the 24-hour urine in rats and mice, whereas glucuronic acid conjugates occurred at a higher level often in mice (17.4% of the dose) than in rats (7.5% of the dose). An additional 30 to 35% of the dose were unidentified water stable metabolites in this study.

In vitro studies in the presence of cytosols of different organs and species show that the N-acetylation giving 4-mono-acetyl- and 2,4-diacetyl-derivates principally occur in the liver. There are differences evident in species and sex (Glinsukon et al. 1975; Glinsukon 1976). In comparing the species the N-acetylation in hamsters was the strongest followed by guinea pigs, rabbits, mice, and rats. With human liver cytosol only traces of N-acetyl-derivates were observed, and with cytosol of dogs nothing could be found. The acetylation with liver cytosol of female mice or male rats was greater than in each case in the other sex (Glinsukon et al. 1975). By virtue of the lower acetylation in humans and dogs one can assume different metabolic pathways in rodents.

N-deacetylation was examined by detecting the products formed during the incubation of 4-acetylamino-2-aminotoluene and 2,4-diacetylamino-2-aminotoluene with the liver cytosol fraction

from male rats. The cytosol catalyzed the deacetylations to produce 2,4-TDA and 2-acetylamino-4-aminotoluene (Sayama et al. 2002).

During incubation of 2,4-TDA with rat liver-S9-Mix from Aroclor-pretreated animals a dimethyl-diamino-dihydro-hydroxyphenazine was observed as the main metabolite (no data on the concentration used) (Cunningham et al. 1988).

#### *Binding to macromolecules*

After a single oral dose of 0.25 mmol/kg (30.5 mg/kg bw) or 10 multiple doses of 0.1 mmol/kg over a time range of 19 days (12 mg/kg bw) in rats hemoglobin adducts were not detectable whereas for 2,6-TDA a hemoglobin binding index of 0.2 mmole/mole Hb/dose (single oral dose) and 0.4 mmole/mole Hb/dose (multiple oral dose) were measured, respectively (Neumann et al. 1993).

A dose-dependent formation of hemoglobin adducts for 2,4-TDA and 2,6-TDA was reported by Wilson et al. (1996) for doses from 0.5 to 250 mg/kg bw. The maximum Hb adduct content amounted to 0.36 nmol/g Hb for both isomers after i.p. application of 250 mg/kg bw to Fischer F344 rats.

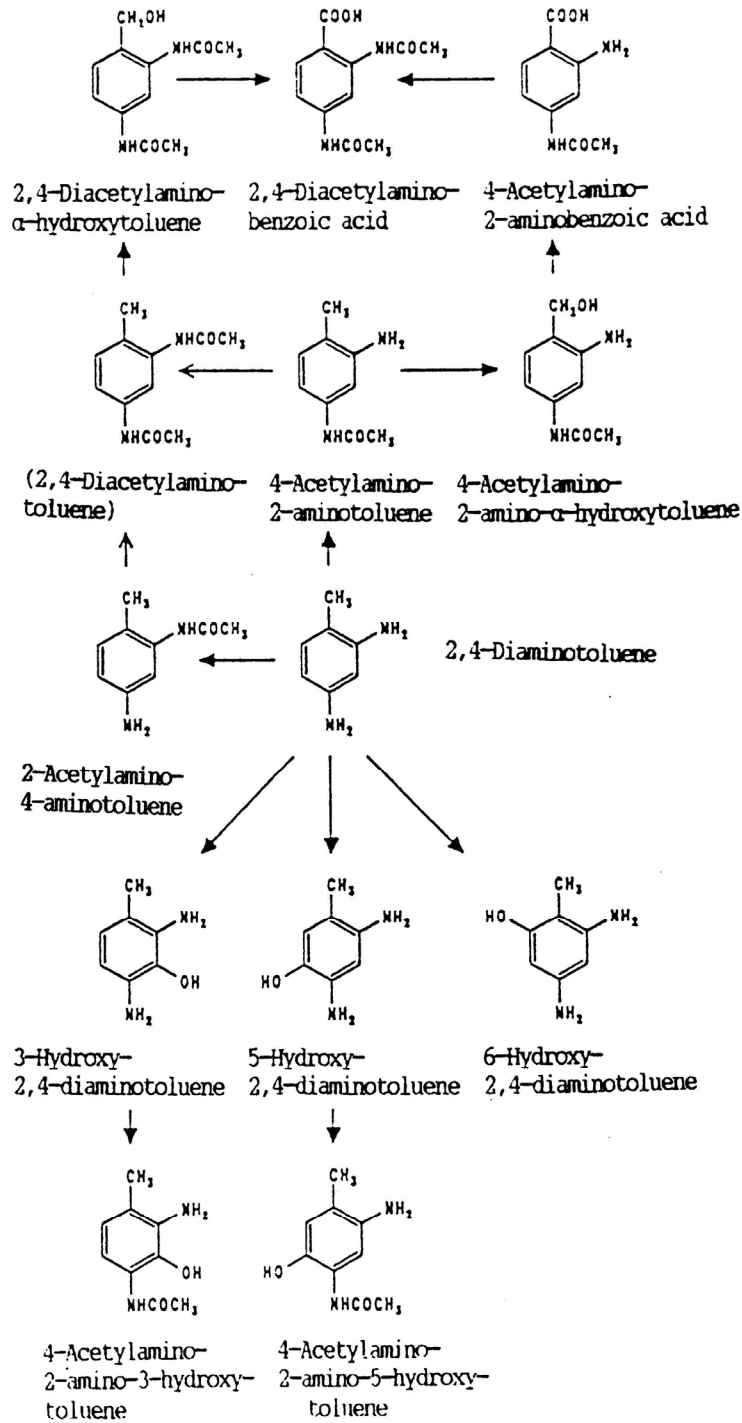
#### *Elimination*

The excretion of the 2,4-TDA metabolites predominantly occurs via urine in rats and mice (Grantham et al. 1979; Timchalk et al. 1994; Sauerhoff et al. 1977). The renal elimination after i.p. injection in mice is more rapid and more complete than in rats and is essentially completed in both species after 48 h. Rats excrete up to 74% of the activity via urine within 48 h whereas 92% of the dose was excreted in mice after 48 h (Grantham et al. 1979). After a single oral dose (0.5, 50 or 60 mg <sup>14</sup>C-TDA/kg bw) the rat eliminated 60 to 65% of the dose within 48 hours in the urine (Sauerhoff et al. 1977; Timchalk et al. 1994). The excretion of the activity in the feces of rats after i.v. or i.p. injection was 20 - 30% after 2 to 5 days. Since these studies show very similar fecal excretion rates it can be assumed that the absorption of 2,4-TDA in rats after oral dosing is almost complete and the fecal elimination of orally administered 2,4-TDA occurs via bile. Within 2 days mice excreted only 3% of the total radioactivity in the feces after i.p. injection (Grantham et al. 1979).

A study in rats with oral administration or i.v. injection of 3 mg/kg bw resulted in an elimination half-life time in urine of 4.6 h. An oral dose of 60 mg/kg bw increased the elimination half-life to 8 hours (Timchalk et al., 1994).

**Figure 4.1.2.1:** Metabolic scheme of 2,4-TDA

(according to Grantham et al. 1979; Waring & Pheasant 1976; Timchalk 1994; Bartels et al. 1993)



The subchronic pretreatment with 2,4-TDA in mice increased the excretion in urine within 6 h only insignificantly from 45% up to 54% of the dose (Unger et al. 1979) with a decrease of elimination with the feces.

#### Human data:

2,4-TDA can penetrate the skin. The penetration of 24% over an exposure time of 24 h in adult male humans ( $4 \mu\text{g}/\text{cm}^2$  TDA dihydrochloride, vehicle acetone, skin contact area from 3 to  $15 \text{ cm}^2$ ) was shown (Marzulli et al. 1981).

In an in-vitro-study with human liver only traces of N-acetylated 2,4-TDA were observed, less than 1% of the level of hamster liver cytosol (Glinsukon et al. 1975).

#### Summary

2,4-TDA is almost completely absorbed via the gastrointestinal tract in animals, and well absorbed via the skin (in man 24% over an exposure time of 24 h). No data are available on inhalation. Thus, for risk assessment purposes a systemic availability of 100% will be applied (worst case assumption).

In rats the highest tissue concentrations were measured in liver and kidney after oral or intraperitoneal administration. Concentrations in heart, lungs, spleen, and testes were significantly lower. The concentration in blood was maximal after i. p. injection in rats after 1 to 8 h. There are no species-related differences in tissue distribution in mice and rats.

In rats, rabbits, and guinea pigs unchanged 2,4-TDA was excreted via urine from 0.1 to 3%. 2,4-TDA is hydroxylated on a major pathway at the ring under formation of aminophenols and N-acetylation takes place. Mono- as well di-acetyl-derivates were observed in different quantities in the urine of rats, mice, rabbits, guinea pigs, and dogs. However, only very small amounts of the monoacetyl derivative were detected in dogs. It was found an elimination of sulfate conjugates in the 24-hour urine in rats and mice, whereas glucuronic acid conjugates occurred at a higher level often in mice than in rats.

The excretion of the 2,4-TDA metabolites predominantly occurs via urine in rats and mice. A study in rats with oral administration or i.v. injection of 3 mg/kg bw resulted in an urinary elimination half-life of 4.6 h. An oral dose of 60 mg/kg bw increased the elimination half-life to 8 hours.

#### 4.1.2.2 Acute toxicity

##### 2,4-TDA

##### Animal data:

##### *Oral*

Pure 2,4-TDA has proven to be toxic in tests with rats and mice, resulting in oral LD50 values between 73 and 350 mg/kg body weight:

In a test performed similar to current EU and OECD guidelines, an oral LD50 value of 73 mg/kg bw was determined for female rats and a value of 136 mg/kg bw for male rats: m-TDA (vehicle lutrol) was administered orally to groups of 10 male rats each, using doses of 200, 180, 150, 100 and 50 mg/kg bw. A dose of 50 mg/kg did not cause mortalities or clinical signs. 2/10 male rats died after application of 100 mg/kg within 5 days; 5/10 rats died after application of 150 mg/kg within 4 days; 8/10 died after 180 mg/kg and 10/10 after 200 mg/kg. In a second study, the substance was orally applied to groups of 10 female rats each, using doses of 500, 100, 70, 50 and 10 mg/kg bw. A dose of 10 mg/kg did not cause mortalities or clinical signs in this test. 1/10 female rats died after application of 50 mg/kg at day 3; 5/10 died after application of 70 mg/kg within 4 days; 8/10 died after 100 mg/kg and 10/10 after application of 500 mg/kg. Clinical signs observed in males at doses > 50 mg/kg and in females at doses > 10 mg/kg included bad general appearance, enhanced diuresis, sedation, diarrhea and loss of body weight. At necropsy, no macroscopically visible changes were detected (Bayer 1981, unpublished report).

In a test with 10 rats per dose using a „standardized method“ (no further information) an oral LD50 value of 270 mg/kg bw was determined. Clinical signs included apathy; deaths occurred within a few days (days of deaths not stated). At necropsy, slight hyperaemia of the lungs, discoloration of lungs and livers and inflammation in the gastro-intestinal tract were detected. Methaemoglobin was slightly enhanced at the LD50 dose 48 hours after substance application (but even after i.p. application only MetHb levels of 8% were observed). Maximum values of methaemoglobin were found after 48 hours following oral exposure: in mice 2,08 and 2,97% were measured after dosing with 300 and 350 mg/kg, respectively. In rats, values between 2.06 and 3.18% were observed after dosing between 240-540 mg/kg bw. Heinz bodies could not be detected (Weisbrod and Stephan 1983). In a similar test with 10 mice per dose using this „standardized method“ (no further information) an oral LD50 value of 350 mg/kg bw was determined. As in the test with rats, clinical signs included apathy and deaths occurred within a few days (days of deaths not stated). At necropsy, slight hyperaemia of the lungs, discoloration of lungs and livers and inflammation in the gastro-intestinal tract were detected. Methaemoglobin was slightly enhanced at the LD50 dose 48 hours after substance application, Heinz bodies could not be detected (Weisbrod and Stephan 1983).

##### *Inhalation*

There are no data available on acute inhalation toxicity of pure 2,4-TDA. However, taking into account the fact that a mixture of 80% 2,4-TDA and 20% 2,6-TDA has a similar acute

toxicity profile as pure 2,4-TDA, results of tests with that 80/20 mixture are considered sufficient to assess the acute inhalation toxicity of the pure 2,4-TDA.

Rationale:

Oral exposure: in rats, the LD50 for pure 2,4-TDA was found to range between 73 and 350 mg/kg bw, whereas for the 20/80% isomeric mixture of 2,4/2,6 TDA an LD50 between 150-179 mg/kg bw was observed. Dermal exposure: for rats, LD50 values of 1200 and 463 mg/kg bw, respectively, were observed for pure 2,4-TDA and the 20/80% isomeric mixture of 2,4/2,6 TDA. Since the isomeric mixture showed similar toxicity via the oral and greater toxicity via the dermal route, it was considered appropriate to refer to an inhalation study with the isomeric mixture, since no data were available for pure 2,4-TDA.

Inhalation toxicity of the 80/20 mixture of 2,4/2,6-TDA seems to be of no concern as judged on the basis of tests with rats, mice and rabbits. These tests are not in compliance with current test guidelines. Nevertheless, they can be used for risk assessment.

### *Dermal*

A dermal LD50 value of 1200 mg/kg bw was determined for rats using „a standardized method“ with 10 rats per dose (24 hours application with water as vehicle, no further information). Clinical signs observed included apathy, deaths occurred within a few days (days of deaths not stated). At necropsy, slight hyperaemia of the lungs, discoloration of lungs and livers and inflammation in the gastro-intestinal tract were detected. Methaemoglobin was slightly enhanced at the LD50 dose 6 hours after substance application. Maximum values of methaemoglobin were found after 6 hours following dermal exposure in rats, ranging between 1,22-3,39% after application of 900 and 1200 mg/kg bw. Heinz bodies could not be detected (Weisbrod and Stephan 1983).

### Human data:

No data available.

### Isomeric mixture 2,4/2,6-TDA (80/20)

Animal data:

### *Oral*

The 80/20 mixture of 2,4/2,6-TDA was toxic to harmful in tests with rats, mice, rabbits and cats, resulting in oral LD50 values between 50 and 500 mg/kg body weight. None of the tests was performed according to current test guidelines:

In two tests with female rats, oral LD50 values were detected for the 80/20 mixture of 2,4/2,6 TDA using the vehicles lutrol (LD50 = 150 mg/kg body weight) in the first and arachis oil (LD50 = 164 mg/kg bw) in the second test: Five to six doses ranging from 80 to 220 mg/kg were used. At the highest dose of 200 or 220 mg/kg 9/10 or 10/10 of the rats died. Death

occurred within 1 to 9 days. Clinical signs included sedation and bad general appearance (Bayer 1974, unpublished report).

For male rats (15 animals/dose group) different LD50 values for oral application of TDA with vehicle dimethyl sulfoxide were calculated after a 2 days (LD50 = 927 + 138 mg/kg bw) and after a 14 days (LD50 = 502 + 205 mg/kg bw) observation period in order to demonstrate that delayed deaths were observed (this observation is supposed to show a difference between early deaths caused by depression of the central nervous system, and delayed deaths caused by visceral toxicity). Deaths occurred within 1 to 4 days. Major clinical signs were central nervous system depression manifested by a decrease in spontaneous activity. Tremors and twitches preceding motor incoordination and loss of righting reflex were observed. At necropsy, light brown patches and dark spots in liver were detected (Zalchari, 1978).

An oral LD50 value of 179 mg/kg bw was calculated with a study with 15 male rats per dose using 8 different doses ranging from 25 to 500 mg/kg. Deaths occurred within 6 days, the dose without clinical signs was 25 mg/kg bw. No clinical signs were reported (Bayer 1971, unpublished report).

An oral LD50 value of 380 mg/kg b.w. was calculated within a study with 15 male mice per dose using 7 different doses ranging from 50 to 1000 mg/kg bw. Deaths occurred within 4 days, the dose without clinical signs was 50 mg/kg bw. No clinical signs were reported (Bayer 1971, unpublished report).

An LD50 value of approximately 500 mg/kg was calculated in a study with 3 male rabbits per dose using doses of 50, 250 and 500 mg/kg bw. Doses of 50 and 250 mg/kg bw did not cause any mortality nor clinical signs. After oral application of 500 mg/kg bw 2/3 rabbits died within 2 days; and 3/3 animals exhibited clinical signs including bad general appearance, laboured respiration and cyanosis (Bayer 1971, unpublished report).

In a study on formation of methaemoglobin after a single oral dose, 1/2 female cats died after oral application of 50 mg/kg within 48 hours: two cats per dose group were orally treated with doses of 0.5 and 2.5 mg/kg bw substance (males) or 10 and 50 mg/kg bw (females). At all doses enhanced methaemoglobin formation was detected ranging from a 70% increase after a 50 mg dose and a 5.4% increase after a 0.5 mg dose. General appearance was not changed after application of the doses 0.5 and 2.5 mg/kg bw. At higher dose levels of 10 and 50 mg/kg bw, reduced well-being was observed (Bayer 1971, unpublished report).

### *Inhalation*

Inhalation toxicity of the 80/20 mixture of 2,4/2,6-TDA is considered to be low as judged on the basis of tests with rats, mice and rabbits. These tests are not in compliance with current guidelines. Nevertheless, they can be used for risk assessment.

In a study with rats and mice LC50 values of > 5.57 mg/l/4 hours were calculated. This test used calculated concentrations of vapour-dust-mixtures with a high amount of particles and thus, the effective concentration was probably lower because of deposition of compound: Ten rats and 20 mice were exposed for 4 hours (whole-body exposure, sex of the animals not reported) to concentrations of approximately 5.57 mg / l (generated by dispersing fumes of 80°C heated substance by means of a propeller). No mortalities occurred within an observation period of 14 days, but all animals demonstrated bad general appearance and laboured respiration. A similar study with rats and mice using concentrations of approx. 1.835



mg/l (generated by means of 100°C heated substance) revealed no mortalities and no clinical signs (Bayer 1971, unpublished report).

In a second study, two rabbits (whole-body exposure, sex of the animals not reported) were exposed for 4 hours to concentrations of approximately 17.16 mg / l (generated by dispersing fumes of 140°C heated substance by means of a propeller). One animal died within an observation period of 14 days. Clinical signs included bad general appearance and laboured respiration. No signs of nasal irritation were detected. A similar study using two rabbits and 9.487 mg/l substance concentration (generated by means of 100°C heated substance) revealed no mortality, clinical signs were similar to those in the first study (Bayer 1971, unpublished report).

### *Dermal*

In a study performed similar to the current OECD test guideline, a dermal LD50 value of 463 (326-658) mg/kg body weight was detected for female rats: The 80/20 mixture of 2,4/2,6-TDA was dermally applied to 5 or 10 animals per dose (24 hours occlusive application, vehicle arachis oil) with 6 different doses ranging from 50 to 1000 mg/kg. Clinical signs observed included bad general appearance, disturbance of respiration and convulsions starting at about 1 hour after application and lasting till the end of the study at day 7. At higher doses, paralysis of hind legs and cyanosis were observed. The animals dosed at 50 mg/kg did not demonstrate any macroscopically visible changes at necropsy. However, at higher doses blue-black discolouration of skin and subdermis, sub-serous and petechial haemorrhages in stomach, ulcera in the gastro-intestinal tract, discoloured kidneys, changes in the liver and enlarged adrenals were detected at necropsy (Bayer 1974, unpublished report).

### Human data:

no data available

### Conclusion:

Human data on acute toxicity of 2,4-TDA are not available. In animal tests the substance was toxic after oral (with LD50 values between 73 and 350 mg/kg bw) and harmful after dermal application (LD50 value of 1200 mg/kg bw). Based on these test results, the substance is to be classified as „toxic“ and labeled with R 21 (harmful in contact with skin) and R 25 (toxic if swallowed). Formation of methaemoglobin is a characteristic effect of TDA isomers observed in animals. According to Bayer (1971, unpublished report) cats are very sensitive for this effect, however, humans are assumed to be less sensitive.(cf. Blom, 2001).

No human nor animal data are available on acute inhalation toxicity of pure 2,4-TDA. However, taking into account the fact that a mixture of 80% 2,4-TDA and 20% 2,6-TDA has a similar acute toxicity profile as pure 2,4-TDA, results of tests with that 80/20 mixture are considered sufficient to assess the acute inhalation toxicity of the pure 2,4-TDA. Assessment of acute inhalation toxicity of the mixture is based on results of tests lacking reliable detection of concentrations inhaled. No mortality occurred after a 4 hour inhalation to concentrations of appr. 5.57 mg/l, but all animals appeared in a bad health state. No effects were seen at 1.8 mg/l, therefore this concentration will be used as NOAEC for risk characterisation.

### 4.1.2.3 Irritation

#### 2,4-TDA

##### Animal data:

No skin irritation was observed in a Draize test according to OECD Guideline 404 with 3 rabbits: 500 mg of the substance (moistened with water) was occlusively applicated to the skin for 4 hours. None of the rabbits exhibited any signs of irritation (Bayer 1982, unpublished report).

Slight conjunctival redness was observed in a Draize eye irritation test using 3 rabbits: 100 mg of the substance was instilled into one eye of each of the animals. Only slight conjunctival redness (grade 1) was detected in 1/3 animals over a period of 72 hours (Draize scores for conjunctival redness for 1, 24, 48, 72 hours and 7 days were 0/0, 1/0, 1/0, 1, and 0/0, values for treated/ untreated eye). Neither corneal nor iridial lesions were observed. After an observation time of 7 days, no effects were present. (Bayer 1982, unpublished report).

Animal data on respiratory irritation are not available from acute inhalation studies.

##### Human data:

No data available.

#### Isomeric mixture 2,4-/2,6-TDA (80/20)

##### Animal data:

„Moderate dermal irritation“ was observed after a 24 hours exposure time within a Draize skin test with rabbits: No edema but erythema grade 1 were detected in 3 rabbits 24 hours after exposure (no data on method used). Erythema enhanced within 3 days and thus, all 3 animals demonstrated erythema grade 2 at the observation time of 72 hours (end of the study). Reversibility of these enhancing skin irritation was not examined (Dunn 1978a) The study was not considered as appropriate for risk assessment.

In a Draize eye test with 6 rabbits the substance demonstrated severe corrosive properties when instilled to the eye: Corneal opacity (grade 4), severe iritis (grade 2), severe hyperemia (grade 4) and necrosis of the conjunctiva, marked chemosis and discharge were observed within a three-days observation period (Dunn 1978b). The severe eye lesions would lead to classification as „irritant“ and labelling with R 41 (risk of serious damage to eyes) according to EU classification criteria. However, the test item used in this study is described as a brownish-black crystalline solid; its purity was not given. As pure TDA is a colourless solid, the tested substance was probably severely contaminated with oxidation products exhibiting different toxicity. Hence, this study should be regarded as invalid. Taking into account the result of a former study with rabbits, which noted pronounced erythema and chemosis after application of 50 mg 2,4-/2,6-TDA (80/20), reversible within 4 days (Bayer 1971, unpublished report, no details reported), lead to the conclusion that the existing classification

with R36 should be warranted. No effects were observed in another Draize test, when 0.1 mL of a 5% solution of 2,4-/2,6-TDA (80/20) was used (Blades, 1976).

Animal data on respiratory irritation were not reported from acute inhalation studies.

Human data:

No data available.

Conclusion:

Human data on local irritation/corrosion due to 2,4-TDA are not available. In Draize tests with rabbits, the substance did not cause skin irritation and demonstrated only slight conjunctival redness after instillation to the eye. Thus, labelling of the pure 2,4-TDA with R 36 (irritating to eye) according to current EU regulations is not appropriate. Studies on eye irritation of 2,4-/2,6-TDA (80/20) lead to the conclusion, that the existing classification of the isomeric mixture with R 36 should be warranted. No reliable studies on skin irritation for 2,4-/2,6-TDA (80/20) have been performed. Data on respiratory irritation are not available from acute inhalation studies.

**4.1.2.4 Corrosivity**

Results from Draize tests reported in 4.1.2.3 clearly demonstrate that 2,4-TDA is not a corrosive substance.

**4.1.2.5 Sensitisation**

2,4-TDA

Animal data:

In a Magnusson Kligman test 10/10 guinea pigs showed a positive response after the first challenge of a 25% test concentration. After a second challenge with a 5% test concentration 5/10 animals reacted positively (i.d. concentration: 0.5%; topical induction concentration; 50%; Kynoch and Elliot 1977). In a modified Draize test none of the 7 guinea pigs showed a positive response after treatment with a 0.1% test concentration in DMSO (Allied Chemical Corporation 1978c). The first test was conducted in compliance with OECD Test Guideline 406, but not the second test.

Animal data on respiratory sensitisation is not available.

Human data:

Patch tests were performed on 40 patients with various kinds of dermatitis and eczema. All patients were hypersensitive to p-phenylenediamine. Cross sensitivity to m-toluene diamine was detected in 67.5% of the patients. The test concentration was 2% in yellow paraffin (Kleniewska 1975). These data show an extremely high cross sensitization rate.

Data on respiratory sensitisation of humans is not available.

### **Conclusion:**

Based on a sensitization rate of up to 100% in a Magnusson Kligman test the substance is labelled as R 43 (may cause sensitization by skin contact). In addition, in humans cross sensitivity to p-phenylene diamine has to be considered. Animal or human data on respiratory sensitisation is not available.

#### **4.1.2.6 Repeated dose toxicity**

##### 2,4-TDA

##### Animal studies

Results of the most reliable repeated dose toxicity studies using the oral route (feeding and gavage) of exposure to 2,4-TDA identified the liver as the main target organ and documented that 2,4-TDA induced toxic effects on the male reproductive system. In a series studies these effects of 2,4-TDA on reproduction, especially on the fertility of male rats was examined. Further information for that is described in section 4.1.2.9. However, these studies did not follow the present repeated dose toxicity testing protocols (only adult male rats were studied). Most of them have been focused on selected parameters.

Several experimental investigations are long-term studies designed for examination of carcinogenicity. Information on nonneoplastic findings of 2,4-TDA in experimental animals is also available from these studies, described in detail in section 4.1.2.8. Therefore, for long-term studies see also: 4.1.2.8.

##### *Oral*

- Gavage studies

##### 8-day study (mouse)

The effects of repeated oral exposure of 2,4-TDA over a period of 8 days have been investigated in mice, in order to provide suitable exposure levels for subsequent reprotoxicity studies. In the study, 10 female CD-1 mice/dose group were administered orally by gavage with 0, 150, 175, 200, 225, and 250 mg/kg bw/day 2,4-TDA (purity commercial grade) suspended in corn oil for up to 8 days following a post-dosing period of 8 days. There were no haematological and/or biochemical parameters, and no histopathology available. With 175

mg/kg bw/day or more there was retardation of body weight gain (10%) and mortality from the fifth dosing day to the fifth post-dosing day. During the exposure period animals showed rough coat, and hunched posture. The maximum tolerated dose (MTD) was 150 mg/kg bw/day (Smith 1983).

#### 14-day study (mouse)

This study was carried out to determine the toxic and immunotoxic potential of 2,4-TDA. Female B6C3F1 mice (no data on number/group, a total of 864 mice were used) were administered by gavage for 14 consecutive days to vehicle (distilled water) or 2,4-TDA (purity commercial grade) at doses of 25, 50, and 100 mg/kg bw/day. On day 15, one day after the last exposure, the animals were evaluated for immunologic parameters. There was a slight decrease in body weight gain (not significant) during the first week in mice exposed to 100 mg/kg bw/day 2,4-TDA. After two weeks of treatment the body weight gain was comparable to that of controls. In all dosed mice, there were no changes in erythrocytes parameters including erythrocyte number, hemoglobin, or hematocrit. At  $\geq 25$  mg/kg bw/day, leucocyte number increased dose dependently up to 60% above the level of control group. In addition, an increase of proportion of lymphocytes in the blood was seen. Alanine aminotransferase activity was increased and urea nitrogen levels were depressed at  $\geq 50$  mg/kg bw/day. At necropsy, there were no treatment-related gross lesions in all dosed mice. An increase in absolute and relative liver weight was seen at 100 mg/kg bw/day (42%) and a decrease in spleen weight was also noted. Mild to moderate centrilobular necrosis in the liver was observed at 50 and 100 mg/kg bw/day. Microscopic examination of other organs (lung, thymus, spleen, kidney or mesenteric lymph node) revealed no effects. At  $\geq 25$  mg/kg bw/day, the following immune parameters were suppressed: splenic macrophage phagocytosis (45%), IgM (46%) and IgG (56%) responses in sheep erythrocytes, serum C3 production, host resistance to bacteria infections and NK cell activity (39%). A number of immune parameters are enhanced including number of blood leucocytes, proportion of lymphocytes in the blood, proportion of T and B lymphocytes in the spleen, the delayed hypersensitivity response (123%) and hepatic reticuloendothelial activity. The host resistance to either of two tumor models was unaffected by 2,4-TDA exposure.

The presented data of the study indicated that the liver is an important target organ for 2,4-TDA exposure in mice. Evidence for liver damage includes increased liver weight, increased ALAT and depressed urea nitrogen at  $\geq 50$  mg/kg bw/day. Mild to moderate centrilobular necrosis in the liver was also observed at 50 mg/kg bw/d. Alterations in some immune parameters were described in mice at dosage of  $\geq 25$  mg/kg bw/day. An overall NOAEL was not demonstrated (White 1989 et al.; Burns 1994 et al.).

- Diet studies

#### 3- to 10-week studies (rat)

In experiments to determine the effect of 2,4-TDA on spermatogenic tissues, groups of male Sprague-Dawley rats were fed a diet containing 0, 0.03, or 0.06% (approx. 0, 15, or 30 mg/kg bw/day) 2,4-TDA (purity 98%) for up to 10 weeks. Effects on androgen-binding protein

(rABP) production, on ultrastructural changes in seminiferous tubules, and on testicular and epididymal weights were evaluated and in addition, early effects of 2,4-TDA administration on spermatogenesis and testicular morphology were determined. Testes and epididymides were examined. Blood was collected for determining serum rABP or testosterone concentrations. No other tissues or organs were examined. Further information is described in section 4.1.2.9.

In the first experiment, 9 rats/group were fed a 0, or 0.03% (0, and approx. 15 mg/kg bw/d) 2,4-TDA diet for 10 weeks. Treated rats showed a 3.8- and 8.9-fold increase in serum rABP content and in testicular cytosol concentration, respectively; media of cultured seminiferous tubules contained 7 times as much rABP levels as those of control tubules, whereas a 67% decrease in epididymal rABP levels was determined. Examination of testicular tissue by electron microscopy showed variable degenerative changes in Sertoli cells. Affected cells exhibited differing degrees of cytoplasmic swelling, membrane disruption, and vacuolization. These findings were patchy in distribution. Spermatocytes and spermatids showed no ultrastructural abnormalities.

In the second experiment, groups of 5 animals were fed 0, or 0.03% (0, or approx. 15 mg/kg bw/d) 2,4-TDA containing diet. After 4, 6, 8, and 10 weeks, one experimental and one control animal were sacrificed and testes and epididymides were examined. Intake of 2,4-TDA for 4, 6, or 8 weeks resulted in significantly decreased body weight gain ( $p < 0.01$ ) in the 2,4-TDA treated animals when compared to controls and in a doubling of testes/body weight ratios (significant in week 6,  $p < 0.05$ ; and in week 8,  $p < 0.01$ ) and a highly correlated 2.5- to 2.9-fold increase in seminiferous tubule fluid volume. After 10 weeks of treatment to 0.03% 2,4-TDA, testicular weight was the same as in controls but seminiferous tubule fluid volume was still elevated. Epididymal weights were significantly increased after 4 weeks ( $p < 0.02$ ) and significantly decreased after 10 weeks ( $p < 0.05$ ) on 2,4-TDA feeding. No effect on epididymal sperm counts was seen after 4 weeks of 2,4-TDA intake; the count then decreased to 37-57% of control rats when feeding 2,4-TDA was extended to 6, 8, or 10 weeks.

In the third experiment, groups of 9 rats were fed a diet containing 0, or 0.06% (0, or approx. 30 mg/kg bw/d) 2,4-TDA for 1 or 3 weeks. The administration of 0.06% 2,4-TDA in the rat for one week led to a significantly decrease in body weight gain, epididymal sperm content (25%), and in epididymal weight, without gross testes effects. Some Sertoli cells contained a few small cytoplasmic vacuoles, but the alterations in treated animals were limited and most Sertoli cells had normal ultrastructural appearance. After 3 weeks of 0.06% 2,4-TDA feeding, body weight gain was significantly lower in comparison to controls (Doses 0/0.06%: +20.8/+4.4%) and sperm counts were further reduced (40%), and were accompanied by a dramatic increase in testes weight (more than doubled). At necropsy, the color and consistency of the seminiferous tubules appeared normal. Examination of testicular tissue in electron microscopy showed thickening of the peritubular tissue and topographical irregularities in the tubular basal lamina. No significant changes in serum testosterone levels were noted in the 2,4-TDA treated rats for one or three weeks. The results of this third experiment demonstrate toxicity on testicular spermatogenesis within 3 weeks of 2,4-TDA feeding, and suggest that the early inhibition of spermatogenesis by 2,4-TDA is mediated through Sertoli cell damage.

Overall, treatment of rats to 0.03% (approx. 15 mg/kg bw/d) 2,4-TDA for 10 weeks demonstrated changes in tissue rABP, and changes in Sertoli cell cytostructure. Treatment with 0.03% (approx. 15 mg/kg bw/d) 2,4-TDA for 4, 6, and 8 weeks led to reduced body

weight gain and increase in testes weights (nearly doubled testes/body weight ratios). Even feeding of 0.06% (approx. 30 mg/kg bw/d) 2,4-TDA for one week resulted in significantly decreased body growth, smaller epididymidis, a 25% decrease in epididymal sperm content, without gross testes effects or major Sertoli cell alterations. After 3 weeks of treatment of 0.06% (approx. 30 mg/kg bw/d) 2,4-TDA, rats showed reduced epididymal sperm reserves, and increased alterations in Sertoli cell ultrastructure. These data demonstrated toxic effects on sperm production within one week of 0.06% (approx. 30 mg/kg bw/d) 2,4-TDA feeding. The sperm counts and ultrastructural changes seen after three weeks of dietary TDA-treatment differed from those seen at 10 weeks of 2,4-TDA feeding only in their severity. A NOAEL for effects on the male reproductive system could not be derived in this study (Varma et al. 1988).

#### 7-week studies (rat and mouse)

The effects of repeated oral exposure of 2,4-TDA over a period of 7 weeks have been investigated in rats and mice, in order to provide suitable exposure levels for subsequent carcinogenicity studies. As such, no clinical chemistry and haematological parameters were measured. Groups of 5 F344 rats and 5 B6C3F1 mice of each sex were fed diets containing 2,4-TDA at one of several doses for 7 weeks, followed by one week post observation. Rats received doses of 0, 250, 500, 1000, 2000, or 3000 ppm (0, approx. 18, 36, 72, 144, or 216 mg/kg bw/d) 2,4-TDA (purity 99.9%). Mice were given doses of 0, 100, 200, 300, 500, 700, or 1000 ppm (0, approx. 15, 30, 45, 75, 105, 150 mg/kg bw/d).

In rats mortality was noted at  $\geq 2000$  ppm ( $\geq 144$  mg/kg bw/d). At doses of  $\geq 500$  ppm ( $\geq 36$  mg/kg bw/d), a dose-dependent decrease of body weight gain was induced.

Dose [ppm]	mean weight at week 7 as % of control: male/female:
250	96/91
500	82/93
1000	59/80

In rats receiving 1000 ppm (approx. 72 mg/kg bw/d), slight increases in heamatopoiesis in the liver and cytoplasmatic vacuolation of hepatocytes were observed in both sexes. In addition, small amounts of bile duct hyperplasia occurred in males. In the rat, no adverse effects were shown at 250 ppm (approx. 18 mg/kg bw/day).

In mice mortality was noted from 1000 ppm (approx. 150 mg/kg bw/d). No clinical or histopathological findings were reported for the male mice at 700 ppm (approx. 105 mg/kg bw/d) or for the females at 1000 ppm (approx. 150 mg/kg bw/d). At 200 ppm (approx. 30 mg/kg bw/d), female mice showed a depression in body weight gain of 10% and at 1000 ppm of 25%, respectively.

The overall NOAEL was 250 ppm (approx. 18 mg/kg bw/d) for rats and 100 ppm (approx. 15 mg/kg bw/d) for mice (NCI 1979).

#### 10-week studies (rat)

In another feeding study, effects of 2,4-TDA on the male reproductive system were evaluated. Adult male Sprague-Dawley rats in groups of 8-10 received 0, 0.01, or 0.03% (0, approx. 5, or 15 mg/kg bw/d) 2,4-TDA (purity 98%) in the basis diet for 10 weeks and were killed 4 weeks after treatment. Testes and epididymides were examined by light microscopy. Further information is described in section 4.1.2.9.

No effects relating to mortality and body weight gain were found in animals fed 0.01% (approx. 5 mg/kg bw/d) 2,4-TDA for 10 weeks. At 0.03% (approx. 15 mg/kg bw/d) 2,4-TDA, animals showed a decrease in body weight gain after 4 weeks of treatment, such that the total weight gain was 27% less than of control rats. Light microscopic examination of the testes revealed reduced numbers of sperm in the seminiferous tubules. There were focal (occasional) or diffuse hypospermatocytogenesis (more than half of seminiferous tubules affected). No evidence of interstitial cell degeneration was found. Some tubules in affected testes were characterized by exfoliation of cells into the tubular lumen. In addition, a decreased numbers of sperm in cauda epididymides were noted. Numerous seminiferous tubules from affected testes and from epididymal tubules were devoid of sperm. Spermatogonia in affected tubules were generally absent. No such signs of toxicity were found in animals fed 0.01% 2,4-TDA (approx. 5 mg/kg bw/d) for 10 weeks. Results presented in this study indicate that 2,4-TDA is capable of exerting an inhibitory or toxic effect on spermatogenesis in the rat (Thyssen et al. 1985a).

A further experiment was undertaken to evaluate the endocrinologic and spermatogenic effects of 2,4-TDA in the rat. Adult male rats were fed 2,4-TDA (purity 98%) at dose levels of 0, 0.01, and 0.03% (0, approx. 5, and 15 mg/kg bw/d) for 10 weeks followed by normal feeding for 11 weeks. At the end of week 10 and at 11 weeks post 2,4-TDA treatment, the animals were killed, and cauda epididymal sperm counts and reproductive organ (testes, epididymidis, seminal vesicle, ventral prostate) weights were measured. Blood samples were obtained for analyses of testosterone and gonadotropins. At 0.03% (approx. 15 mg/kg bw/d) 2,4-TDA for 10 weeks, animals gained 36% less body weight than the control animals. In addition, there was a significant decrease in seminal vesicles ( $p < 0.05$ ) and mean epididymal weights ( $p < 0.05$ ). The mean testes and prostate weights of 2,4-TDA-treated rats were similar to those from controls. However, diminished sperm reserves were found in animals at the dose of 0.01% (approx. 5 mg/kg bw/d) 2,4-TDA. Males treated with 0.03% (approx. 15 mg/kg bw/d) 2,4-TDA showed a significant decrease ( $p < 0.05$ ) in sperm count. The serum testosterone levels were significant reduced ( $p < 0.05$ ) in these males. In accordance with that elevated LH concentrations were noted. No significant treatment-related effects were seen in analyses of FSH.

After treatment-free period of 11 weeks, the sperm count in animals treated with 0.03% (approx. 15 mg/kg bw/d) 2,4-TDA remained significantly depressed ( $p < 0.001$ ). Sperm concentrations correlated well with the testes and epididymidis weight. There was a significant decrease in mean testis weight ( $p < 0.001$ ) and a significant decrease in mean epididymidis weight ( $p < 0.001$ ) among these males. No significant changes in mean seminal vesicle and ventral prostate weights compared to controls were noted. The serum testosterone levels were lowered and the LH concentrations were elevated, both non-significantly, whereas FSH levels were not significantly changed by treatment.



Overall, the results indicate that 2,4-TDA exerts a dose-related toxic effect on spermatogenesis due to an adverse effect on Sertoli cells. First indications of damage to male gonads of the rat were get from sperm evaluation. Even at the dose of 0.01% (approx. 5 mg/kg bw/d) 2,4-TDA diminished sperm reserves were noted (for further details cf. 4.1.2.9). Thus a NOAEL for effects on the male reproductive system cannot be established (Thysen et al. 1985b).

#### 36-week study (rat)

Two groups, each of 12 male Wistar rats, were fed diets containing 600 or 1000 ppm (approx. 45 or 75 mg/kg bw/d) 2,4-TDA (purity commercial grade) for 30-36 weeks. A third group (6 male rats) was kept as control on the basis diet. Testing procedures and documented results were comparable to testing efforts according to the current regulatory test protocols. However, this study is reliable with restrictions. The study has been focused on selected parameters and was not performed according to GLP, but was nevertheless well documented and scientifically acceptable. For details see also Chapter 4.1.2.8.

In the 600 ppm (approx. 45 mg/kg bw/d) feeding group, 11 rats survived for 35 weeks, and in the group fed 1000 ppm (approx. 75 mg/kg bw/d), 9 animals survived for 35 weeks. After 4-8 treatment weeks animals showed a decrease in body weight gain, followed by weight loss (mean weight as % of control: 0.1%: 51%; 0.06%: 57%). The relative liver weight was 300% or more of the controls. Liver carcinomas exhibited invasion and showed metastases in male Wistar rats, given dose levels of 600 ppm (approx. 45 mg/kg bw/d) or more. In the tumorfree portion of the liver, there was extensive proliferation of oval cells with differentiation to bile duct epithelium. Numerous areas of nodular hyperplasia were observed in the liver. In addition, areas of fatty degeneration, cholangiofibrosis and cirrhosis were present. An overall NOAEL was not demonstrated (Ito 1969 et al.).

#### 15-month study (rat)

This study was designed to evaluate the long-term toxicity of a chlorotoluene diamine mixture, 2,4-TDA was used as a positive control. 36 male and 36 female ChP-CD rats were fed a diet containing 1000 ppm (approx. 75 mg/kg bw) 2,4-TDA (purity commercial grade) for two weeks, 500 ppm (approx. 38 mg/kg bw) for 5.5 months and 250 ppm (approx. 19 mg/kg bw) for 9 months. Calculated average daily intake was 367 ppm (approx. 28 mg/kg bw). Control group consisted of 12 untreated male and 10 female rats.

In the 2,4-TDA treated groups, both sexes exhibited an increase in mortality rate, a marked reduction in body weight gain (mean weight as % of control: 33%), slight anemia and leucocytosis. Microscopically, hemosiderin was found in various tissues in amounts greater than found in controls. An increase in serum ALP, GPT, and bilirubin levels were noted in 2,4-TDA treated animals compared with controls. These findings are consistent with those found histopathologically in the liver: focal necrosis of hepatocytes, cystic bile ducts, cholangitis, cholangiofibrosis, hematopoiesis and hemosiderin. Severe atrophy of the spleen was seen in both sexes. Males developed proteinuria and glucosuria after 9 months on test. At microscopic examination of kidneys, treated males showed inflammation of renal pelvis more frequently than found in controls. Severe testicular atrophy with granulomata formation was a

consistent finding in all 2,4-TDA-treated males. The addition of 2,4-TDA to the diet resulted in a statistically significant increase in liver and mammary tumor development in both sexes, together with a significant increase of lung tumors (mostly adenomas) in males. An overall NOAEL was not demonstrated (Stula and Aftosmis 1976).

#### Long-term study (rat and mouse)

A bioassay of 2,4-TDA for possible carcinogenicity was conducted by administering the test chemical in food to F344 rats and B6C3F1 mice.

#### Rat

Groups of F344 rats (50 animals/sex/dose) were administered 2,4-TDA (purity 99.9%) at one of two doses, initially either 125 or 250 ppm (approx. 9 or 18 mg/kg bw/d) for 40 weeks. Because of excessive depression of amount of mean body weight gained in both low- and high-dose groups, doses were then reduced to 50 and 100 ppm (approx. 3.7 and 7.4 mg/kg bw/d). Calculated average intake was 5.9 and 13 mg/kg bw/day. Matched controls consisted of 20 untreated rats of each sex.

Survival was decreased in male and female rats. The number of rats which were still alive after 78 treatment weeks were as follows:

#### Number of rats which were still alive at week 78 on study

Dose	Males	Females
Low dose	42/50 (84%)	46/50 (92%)
High dose	32/50 (64%)	46/50 (92%)
Control	18/20 (90%)	20/20 (100%)

Mortality was dose related in both the male and female rats. Survival was decreased. All males given 250/100 ppm 2,4-TDA were dead by 79 weeks, and females given high doses were dead or were terminated due to morbidity by 84 weeks. Mean body weights of dosed male and female rats were lower than those of the corresponding controls and were dose related (mean weight as % of control: males: 125/50 ppm: 79%, 250/100 ppm: 58%; females: 125/50 ppm: 93%, 250/100 ppm: 66%). Nonneoplastic findings of this carcinogenesis bioassay in F344 rats revealed that 2,4-TDA was hepatotoxic. In the liver dosed animals exhibited various treatment induced nonneoplastic morphologic alterations which ranged from mild, scattered foci of lipidosis and focal necrosis of hepatocytes to severe, diffuse, toxic degenerative lesions. The incidence of primary hepatic lesions in rats was as follows:

#### Number of rats with primary nonneoplastic lesions in the liver

Dose	Males	Females

Low dose	25/49 (51%)	23/50 (46%)
High dose	36/50 (72%)	42/49 (86%)
Control	2/20 (10%)	1/20 (5%)

In addition kidney lesions were seen in both sexes (most marked in males). Microscopy of the kidneys revealed nonneoplastic lesions of different severity scored in the 1-5 grading system (Cardy 1979) (s. under the following table). The average numerical values are listed by dose and sex in the following table:

Scoring of chronic renal disease in rats

Dose	Males (mean average of severity grades/ number of animals tested)	Females
Low dose	3.7/49	2.0/50
High dose	3.9/50	2.8/49
Control	2.1/20	1.3/19

1-5 grading system mean: 1 = minimal changes detectable as slight basement membrane thickening seen most in Bowman's capsule. 2 = the stage of mild glomerular change and scattered, atrophic, dilated tubules with intratubular proteinaceous casts. 3 and 4 = subjective divisions of degree of the above changes along with glomerular atrophy and sclerosis, lymphoid aggregates, and a variable degree of interstitial fibrosis and architectural derangement. A score of 4 indicated severe involvement. 5 = reserved for end-stage kidneys.

Corresponding to the renal disease was a high incidence of associated secondary hyperparathyroidism in low- and high-dose males. The affected parathyroid glands were spherical and bulged from the surface of the cut thyroid gland. Associated lesions included metastatic calcification in numerous locations and absorption in bone with proliferation of osteoclasts and myelofibrosis. Further information on neoplastic effects to 2,4-TDA in rats is described in section 4.1.2.8. In F344 rats a average dose of 5.9 mg/kg bw/day 2,4-TDA showed hepatotoxic effects, induced the development of chronic renal disease in this strain, and an increased incidence of tumors. An overall NOAEL for rats was not demonstrated (NCI 1979; Cardy 1979).

Mouse

Mice were fed 100 or 200 ppm (approx. 15 or 30 mg/kg bw/d) 2,4-TDA for 101 weeks. Matched controls consisted of 20 untreated mice of each sex. Surviving mice were killed at the end of administration of the test chemical.

Mortality was not dose related in either the male and female B6C3F1 mice. Survival rates were comparable in dose groups and control groups. The number of mice which were still alive at termination of treatment after 101 weeks were as follows:

Number of mice which were still alive at termination of the study (week 101)

Dose	Males	Females
Low dose	45/50 (90%)	40/50 (80%)
High dose	43/50 (86%)	39/50 (78%)
Control	18/20 (90%)	15/20 (75%)

Mean body weights of dosed male and female mice were lower than those of the corresponding controls and were dose related except for the low-dose male mice, for which mean body weights were only slightly lower than those of controls. In female B6C3F1 mice, there was a dose-related delay in body weight gain of 23-50%. Female mice fed 100 or 200 ppm (approx. 15 or 30 mg/kg bw/d) 2,4-TDA developed a significant number of carcinomas of the liver. Treated mice also developed hyperplastic nodules in the liver. The incidence of hepatic lesions was not increased in male mice given 2,4-TDA. Further information on neoplastic effects to 2,4-TDA in mice is described in section 4.1.2.8. Histological examination of the kidneys did not reveal any 2,4-TDA-related change. An overall NOAEL was not demonstrated (NCI 1979; Reuber 1979).

- Inhalation

No data available.

- Dermal

No data available.

- Subcutaneous application

2-year study (rat)

Six aromatic amines or derivatives one of them was 2,4-TDA were tested for carcinogenicity in a subcutaneous injection test with Sprague-Dawley rats. 30 rats per dose and sex were administered by subcutaneous injections at doses of 0, 8.33, or 25 mg/kg bw 2,4-TDA (purity commercial grade) once a week until appearance of the first test substance-related tumor, with a maximum of a 2-year treatment. In addition to the control group receiving the vehicle (peanut oil), there was another, similar, untreated group, and a positive control group receiving benzidine. After cessation of the treatment rats were kept until their spontaneous death, or until they were in a moribund state and were killed.

The survival rates were comparable in dose groups and control groups excepted for the 25 mg/kg bw dose male rats, for which survival time was lower than those of controls. Mean body weights of dosed male and female rats were lower than those of the corresponding controls and were dose related. Microscopic examination of a number of organs and tissues revealed no treatment related lesions with the exception of the liver. 16 rats (males and females altogether) of the 25 mg/kg bw dose group and 7 rats of the 8.33 mg/kg bw dose group showed: focal necrosis of hepatocytes and/or single cirrhosis in the liver. Hepatocellular neoplasia was found in 1/120 rats. Furthermore, a dose-related statistically significant increased incidence of localized malignant tumors at the injection site in males was noted. An overall NOAEL was not demonstrated (Steinhoff and Dycka 1981).

### **Other information**

The toxicity of 2,4-TDA was examined from the beginning of the last century. These early studies with limited quality revealed liver damaging effects. In several experiments cited data such as on the specification and purity of the test substance used, detailed records of the investigation were incomplete. Most of them have been focused on selected parameters. Main findings of these studies showed good consistency and therefore, they were also considered for the effect assessment. In the following chapter, data from these early animal studies were summarised.

Rats were given 2.5% 2,4-TDA (approx. 1 mg test substance/g rice) in olive oil incorporated in their diet for up 80 days or longer. Increased mortality was described in rats after 5 days up to 3 weeks of treatment. No data on controls, haematology and clinical chemistry parameters were available. Liver histopathology was only reported. The continued feeding of 2,4-TDA to rats over 60 days resulted in a cirrhosis of the liver named by the author „cirrhosis hepatitis cholangioloplastica annularis“. When rats were given 2,4-TDA in the feed for 60 days, and thereafter untreated food, cirrhotic lesions quickly disappeared. Histomorphological findings in the liver were interpreted by the author as cancer-like atypical growth on the interlobular bile ducts (Nagata 1937, 1944).

### **Summary of animal toxicity data after repeated exposure to**

#### **2,4-TDA**

From numerous animal studies, the main toxic effects of 2,4-TDA were summarised in the Table 4.1.2.6A.

**Table 4.1.2.6 A**

#### **Summary table: Animal toxicity data after repeated exposure to 2,4-TDA**

<b>Species/strain (male/female) Group size Exposure Route</b>	<b>Dose Exposure duration</b>	<b>NOAEL for nonneoplastic effects</b>	<b>Adverse effects ↑ increase ↓ decrease</b>	<b>Reference</b>
Rat/Sprague-Dawley (9m) oral in feed	0, 30 mg/kg bw/day 1 week or 3 weeks	-	600 ppm (30 mg/kg bw/day) <u>after 1 week:</u> ↓ body weight gain	Varma et al. 1988

<p>(5m) oral in feed</p>	<p>daily</p> <p>0, 15 mg/kg bw/day 4, 6, 8, or 10 weeks, daily</p>		<p>↓ epididymal weight ↓ epididymal sperm content very small findings in Sertoli cell ultrastructure (few small cytoplasmic vacuoles) <u>after 3 weeks:</u> ↑ testes weight (more than doubled)</p> <p>↓ epididymal sperm count small findings in Sertoli cell ultrastructure</p> <p>300 ppm (15 mg/kg bw) <u>after 4, 6, or 8 weeks:</u> ↑ of testes/body weight ratios ↑ seminiferous tubule fluid volume <u>after 6, 8 or 10 weeks:</u> ↓ epididymal sperm content</p>	
<p>(9m) oral in feed</p>	<p>0, 15 mg/kg bw/day</p> <p>10 weeks, daily</p>		<p>300 ppm (15 mg/kg bw) ↑ rABP in testes and serum ↓ rABP in epididymidis variable degenerative effects in Sertoli cell ultrastructure</p>	
<p>Rat/F344 (5m/5f) oral in feed</p>	<p>0, 18, 36, 72, 144, 216 mg/kg bw/day 7 weeks, daily, one week recovery period</p>	<p>250 ppm (18 mg/kg bw/day)</p>	<p>2000 ppm (ca. 144 mg/kg bw/day) mortality (5/5m, 4/5f, no data on time point of death) 1000 ppm (ca. 72 mg/kg bw/day) ↑ liver cell vacuolation (m/f), bile duct hyperplasia (m) ≥ 500 ppm (ca. 36 mg/kg bw/day) ↓ body weight gain (m/f)</p>	<p>NCI 1979</p>

Table 4.1.2.6. A (contin.):

**Summary table: Animal toxicity data after repeated exposure to 2,4-TDA**

Species/strain (male/female) Group size Exposure Route	Dose Exposure duration	NOAEL for nonneoplastic effects	Adverse effects ↑ increase ↓ decrease	Reference
Rat/Sprague-Dawley (8-10m) oral in feed	0, 5, 15 mg/kg bw/day 10 weeks daily followed by recovery of 4 weeks	100 ppm (5 mg/kg bw/day) for light microscopic findings	300 ppm (15 mg/kg bw/day) ↓ body weight gain ↓ testes weights ↓ epididymal sperm count ↑ focal to diffuse hypospermia	Thysen 1985a
Rat/Sprague-Dawley (8-10m) oral in feed	0, 5, 15 mg/kg bw/day 10 weeks daily followed by recovery of 11 weeks	-	<u>After 10 weeks:</u> 300 ppm (15 mg/kg bw/day) ↓ body weight gain ↓ seminal vesicles and epididymal weights ↓ epididymal sperm count ↓ serum testosterone ↑ serum LH 100 ppm (5 mg/kg bw/day) ↓ epididymal sperm reserve <u>After 10 weeks + recovery:</u> 300 ppm (15 mg/kg bw/day) ↓ sperm count ↓ testes and epididymal weights ↓ serum testosterone ↑ serum LH	Thysen 1985b
Rat/Wistar (11m in low dose, 9m in high dose) oral in feed	0, 45, 75 mg/kg bw/day 36 weeks daily	-	600 ppm (45 mg/kg bw/day) ↓ survival time ↓ body weight gain ↑ liver weight, proliferation of oval cells, fatty degeneration, cirrhosis, cholangiofibrosis, nodular hyperplasia, hepatocarcinoma	Ito et al. 1969
Rat/ChP-CD (36m/36f per test group; 12m/12f in control group)	Calculate d average over total time: 28 mg/kg	-	367 ppm (28 mg/kg bw/day) ↑ mortality rate (m/f), ↓ body weight gain (m/f), slight anemia, leucocytosis (m/f),	Stula and Aftosmis 1976

oral in feed	bw/day 75 mg/kg bw/day for 2 weeks, 38 mg/kg bw/day for 5,5 months, 19 mg/kg bw/day for 9 months total exposure of 15 months daily		↑ serum ALP, GPT, bilirubin (m/f), focal necrosis of hepatocytes (m/f), cystic bile ducts (m/f), severe atrophy of the spleen (m/f), liver, and mammary tumors (m/f) severe testicular atrophy (m) inflammation on renal pelvis (m) ↑ lung tumors (m)	
Rat/F344 (50m/50f per test group; 20m/20f in control group) oral in feed	calculated average: 5.7, 13 mg/kg bw/day: 0, 9, 18 mg/kg bw/day for 40 weeks, then reduction to 3.7, 7.4 mg/kg bw/day due to depressed body weight gain until end of study, m: 79 weeks, f: 84 weeks, control m/f: 103 weeks; high dose exposure premature	-	≥ 5.9 mg/kg bw/day ↓ survival rate (m/f), ↓ body weight gain (m/f) liver cell degeneration, lipidosis (m/f) chronic renal disease (m/f) ↑ liver tumors (m/f) ↑ mammary gland tumors (f)	NCI 1979 Cardy 1979 Sontag 1981



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Table 4.1.2.6 A (contin.):

**Summary table: Animal toxicity data after repeated exposure to 2,4-TDA**

Species/strain Group size (male/female) Exposure route	Dose Exposure duration	NOAEL for nonneoplast ic effects	Adverse effects ↑ increase ↓ decrease	Reference
Mouse/CD-1 (10f) oral by gavage	0, 150, 175, 200, 225, 250 mg/kg bw/day 8 days, 8 days recovery period daily	150 mg/kg bw/day	≥ 175 mg/kg bw/day mortality: from the fifth dosing day to the fifth post-dosing day ↓ body weight gain (10%) rough coat, and hunched posture during recovery period	Smith 1983
Mouse/B6C3F 1 (f: no number per group available, a total of 864 females were used) oral by gavage	0, 25, 50, 100 mg/kg bw/day 14 days daily	- 25 mg/kg bw/day for liver effects	≥ 25 mg/kg bw/day <u>immune parameters:</u> ↓: splenic macrophage phagocytosis (45%), IgM (46%) and IgG (56%) responses in sheep erythrocytes, serum C3 production, host resistance to bacteria, NK cell activity (39%) ↑: number of blood leucocytes, proportion of lymphocytes in the blood, proportion of T and B lymphocytes in the spleen, delayed hypersensitivity response (123%), hepatic reticuloendothelial activity ≥ 50 mg/kg bw/day  ↑ liver weights (42%) mild to moderate centrolobular necrosis ↓ spleen weights <u>serum chemistries:</u> ↑ ALAT ↓ urea nitrogen	White et al. 1989 Burns et al. 1994

Mouse/B6C3F 1 (-5m/5f) oral in feed	0, 15, 30, 45, 75, 105, 150 mg/kg bw/day 7 weeks, one week recovery - daily	100 ppm (15 mg/kg bw/day)	≥ 1000 ppm (150 mg/kg bw/day) mortality (2/5m, 0/5f, no data on time point of death) 200 ppm (30 mg/kg bw/day) ↓ body weight gain (f)	NCI 1979
Mouse/B6C3F 1 (-50m/50f per test group; 20m/20f control group) oral in feed	0, 15, 30 mg/kg bw/day 101 weeks daily	-	≥ 100 ppm (≥15 mg/kg bw/day) ↓ body weight gain (m/f) ↑ nodular hyperplasia in the liver (f) ↑ hepatocarcinoma, lymphomas (f)	NCI 1979 Reuber 1979 Sontag 1981
Rat/Sprague- Dawley (30m/30f) subcutaneous application	0, 8.33, 25 mg/kg bw 2 years once a week	-	25 mg/kg bw ↓ survival time (m) ↓ body weight gain (m/f), focal necrosis of hepatocytes (m/f), single cirrhosis, liver tumor in 1/120 ↑ incidence of malign tumors at the injection site (m)	Steinhoff and Dycka 1981

m: male; f: female

Isomeric mixture 2,4-/2,6-TDA (80/20)

Experimental studies have demonstrated that the toxicity of the mixture of the isomers 2,4/2,6-TDA (80/20) is caused by the major constituent 2,4-TDA.

- *Oral*

No data available.

- *Inhalation*

The studies on inhalation toxicity were only conducted on a preliminary basis. These subacute inhalation studies were not performed in accordance to the test design of the current guidelines, B.8, OECD TG 412. Most of them have been focused on selected parameters. The data presented were only in summary form. No details were available for: lists of parameters examined, methods, and measuring instruments. Therefore, results of these studies were assessed as further information.

28-day study (rat)

A 28-day inhalation study was done with the mixture of the isomers 2,4-/2,6-TDA (80/20) vapourized at 100°C. The analytically measured concentrations were 9.5, and 83 mg/m<sup>3</sup>, so that the values mainly were above the saturated vapor concentration of 10 mg/m<sup>3</sup> at 20°C. 20 male Wistar rats were exposed to the mixture of isomers 2,4-/2,6-TDA (80/20) vapors 4 hours daily, 5 times a week for 4 weeks (whole body exposure). At an average air concentration of 83 mg/m<sup>3</sup> (approx. 9 mg/kg bw/d), exposed rats showed no effects compared to the controls (no data of number and sex) in appearance and condition, but had reduced body weight gain, and an increase of the relative weight of the liver, kidneys, and thyroid glands, and showed a relative lymphopenia in blood tests. At necropsy, there were no exposure-related gross lesions. At 9.5 mg/m<sup>3</sup>, approx. 1 mg/kg bw/day (calculated with respiratory volume of 6 l/h and 100% absorption) during 4 weeks, animals showed similar results compared to controls with respect to behavior and growth. At the end of the exposure, haematology, liver and kidney function tests, and gross pathology showed no 2,4-TDA-related findings. No data of histopathology were available. The overall NOAEC<sub>sys</sub> was 9.5 mg/m<sup>3</sup> (approx. 1 mg/kg bw/day) (Kimmerle and Solmecke 1971).

#### 21- and 28-day studies (cat)

2 male cats were exposed to 2,4-/2,6-TDA (80/20) vapors average 41.6 mg/m<sup>3</sup> (approx. 4.5 mg/kg bw/d), 4 hours daily, 5 consecutive days/week for 3 weeks (whole body exposure) followed by a 10 days recovery period. Cats showed increasing general deterioration and a decrease in body weight. One cat died post exposure (no more data). At the end of exposure severe methemoglobinemia (30%) were noted. No effects on blood parameters were recorded 10 days after the last exposure. At microscopic examination, there were lesions (not specified) in the lung, liver, and kidneys (no more data).

In another study 3 male and 3 female cats were exposed to 9.5 mg/m<sup>3</sup>, 4 hours/day for 5 days/week for 4 weeks. There was no body weight gain during the exposure. Low levels of methemoglobin formation (2.07%) were found after 2 weeks. The number of reticulocytes and Heinz bodies of exposed cats was similar to those of controls (no more data).

In summary, at 9.5 mg/m<sup>3</sup> 2,4-/2,6-TDA (80/20), a low level of methemoglobin formation (2.07%) were measured. At dose level of 41.6 mg/m<sup>3</sup>, cats showed severe methemoglobinemia (30%), retarded body weight gain, and pathomorphological findings (not specified) in the lungs, liver, and kidneys (no more data). An overall NOAEC was not derivable (Kimmerle and Solmecke 1971).

#### *Dermal*

No data available.

- *Subcutaneous application*

2-year study (rat)

In a subcutaneous injection test, 30 Sprague-Dawley rats per dose and sex were administered by subcutaneous injections at doses of 0, 8.33, or 25 mg/kg bw 2,4-/2,6-TDA (80/20) once a week until appearance of the first test substance-related tumor, with a maximum of a 2-year treatment. Further information is described in section 4.1.2.8.

The survival rates were comparable in the 8.33 mg/kg bw dose groups and control groups. At 25 mg/kg bw, male and female rats showed a decrease in survival time compared to controls. Mean body weights of all dosed male and female rats were lower than those of the corresponding controls and were dose related. Microscopic examination of a number of organs and tissues revealed no treatment-related lesions with the exception of the liver. 11 rats (males and females altogether) of the 25 mg/kg bw dose group and 8 rats of the 8.33 mg/kg bw dose group showed: focal necrosis of hepatocytes and/or single cirrhosis in the liver. An overall NOAEL was not demonstrated (Steinhoff and Dyca1981).

### **Other information**

Some short-term studies were carried out on Wistar rats, NMRI mice, Syrian golden hamsters, and rabbits, to give information to repeated dose toxicity of the mixture of isomers 2,4/2,6-TDA (80/20) by inhalation. Data from these studies were evaluated as further information.

Groups of 10 male Wistar rats, 20 male NMRI mice, 5 male Syrian golden hamsters, and 2 cross-bred rabbits were exposed to 2,4/2,6-TDA (80/20) vapors 4 hours daily, for 5 days (whole body exposure) to an average air concentration of 8.367 mg/l (rat, and mouse) or 7.557 mg/l (hamster, and rabbit) followed by a two weeks recovery.

Animals showed general deterioration and difficult breathing during the exposure. They recovered and no mortality was recorded. No more data were available (Kimmerle and Solmecke 1971).

### **Summary of animal toxicity data after repeated exposure to the**

#### **Isomeric mixture 2,4-/2,6-TDA (80/20)**

From animal studies, the main toxic effects of the mixture of the isomers 2,4-/2,6-TDA (80/20) were summarized in the Table 4.1.2.6. B.

**Table 4.1.2.6. B****Summary table: Animal toxicity data after repeated exposure to the mixture of the isomers 2,4-/2,6-TDA (80/20)**

<b>Species/strain group size (male/female) Exposure Route</b>	<b>Dose/Concentration Exposure duration</b>	<b>NOAEL / NOAEC for nonneoplastic effects</b>	<b>Adverse effects ↑ increase ↓ decrease</b>	<b>Reference</b>
<b>Rat/Wistar</b> (20m) inhalation (whole body)	0, 0.0095, 0.083 mg/l 28 days, 4 hours/day 5 days/week	9.5 mg/m <sup>3</sup> (1 mg/kg bw/day)	83 mg/m <sup>3</sup> (9 mg/kg bw/day) ↓ body weight gain ↑ relative organ weight of liver, kidney, and thyroid gland, lymphopenia	Kimmerle Solmecke 1971
<b>Cat/no data</b> (2m) Inhalation (whole body)	0.0416 mg/l 3 weeks, 4 hours/day, 5 days/week	-	41.6 mg/m <sup>3</sup> (4.5 mg/kg bw/day) general deterioration, ↓ body weight gain ↑ methemoglobin formation (30%) pathomorphological findings (not specified) in the lung, liver, and kidneys	Kimmerle, Solmecke 1971
<b>Cat/no data</b> (3m/3f) inhalation (whole body)	0.0095 mg/l 4 weeks, 4 hours/day, 5 days/week	-	9.5 mg/m <sup>3</sup> (1 mg/kg bw/day) low level of methemoglobin formation (2.07%) after 2 treatment weeks	Kimmerle Solmecke 1971
<b>Rat/Sprague-Dawley</b> (30m/30f) subcutaneous application	0, 8.33, 25 mg/kg bw 2 years once a week	-	≥ 8.33 mg/kg bw focal necrosis of hepatocytes, single cirrhosis ↑ incidence of malign tumors at the injection site 25 mg/kg bw ↓ survival time ↓ body weight gain	Steinhoff Dycken 1981

**m: male; f: female**Human data:

No data available.

**Summary of toxic effects after repeated exposure:**2,4-TDA

The repeated dose toxicity of 2,4-TDA by oral route of exposure has been well investigated in rodents. The primary target organ after short- and long-term dietary exposure of 2,4-TDA is the liver. 2,4-TDA damages the liver, and is able to accelerate the development of chronic

renal disease and to damage the male reproductive system in rats. In short-term studies, the toxic effects of 2,4-TDA are characterized by a decrease in body weight gain and an increase in the liver: body weight ratio. There was a decrease in body weight gain with dosages of approx. 15 mg/kg bw/d after 8-12 weeks of application in rats (Ito 1969, Stula 1976). Dietary exposure of 2,4-TDA at 5.9 mg/kg bw/day (time-weighted average dose after reduced in food from 125 to 50 ppm) for 2 years caused a clearly dose related delay of body weight gain and dose related decreased survival rates. At  $\geq 5.9$  mg/kg bw/day, hepatotoxic effects, and the development of chronic renal disease in the F344 strain were seen, an effect that contributed to a marked decrease in survival of dosed animals. Hepatotoxic effects were shown as fatty degeneration, cholangiofibrosis and liver cirrhosis (at  $\geq 45$  mg/kg bw/d, 36-week study; Ito 1969) as well as focal necrosis of hepatocytes to severe, diffuse, toxic degenerative lesions in the liver ( $\geq 5.9$  mg/kg bw/d, long-term study; Cardy 1979, NCI 1979). Kidney lesions were observed in both sexes (most marked in males) at  $\geq 5.9$  mg/kg bw/d. Corresponding to the renal disease was a high incidence of associated secondary hyperparathyroidism in dosed males (long-term study; NCI 1979). In addition, hepatocellular carcinomas or neoplastic nodules occurred at incidences that were dose related in both male and the female rats (Cardy 1979, NCI 1979).

Experimental data from rat studies demonstrated that 2,4-TDA induced serious health effects consisting of testicular atrophy at 28 mg/kg bw/d for 15 months (Stula and Aftosmis 1976) and inhibited spermatogenesis (66%) associated with a significant reduction in the weights of seminal vesicles and epididymides, morphological damage of Sertoli cells as well as with a diminished level of serum testosterone and an elevation of serum LH at doses of 15 mg/kg bw/day (10-week oral study, Thyssen et al. 1985a, 1985b, Varma et al. 1988). First adverse effects on epididymal sperm reserves were shown at dietary doses of 5 mg/kg bw/d in a 10-week oral study (Thyssen et al. 1985b).

The mouse is less sensitive to 2,4-TDA than rats. Mice exposed to dietary levels of 100 ppm (15 mg/kg bw/day) of 2,4-TDA for 101 weeks showed no significant differences in survival compared to the control animals. There was, however, a delay of body weight gain of 25-50%. This effect was dose related except for the low-dose male mice, for which mean body weights were only slightly lower than those of controls. In treated B6C3F1 mice, the incidence of hepatic lesions was not increased, and there were no treatment-related findings in the kidneys. Female mice fed 100 or 200 ppm (approx. 15 or 30 mg/kg bw/d) 2,4-TDA for 101 weeks developed a significant number of carcinomas of the liver. Treated mice of both sexes also developed hyperplastic nodules in the liver, but degenerative effects were not observed in mice ingesting 2,4-TDA up to 101 weeks (NCI 1979, Reuber 1979).

There were no repeated dose toxicity studies on rodents investigating 2,4-TDA effects after inhalation. No firm conclusions could be drawn from a mouse skin-painting test with respect to the dermal route of exposure described in section 4.1.2.8.

2,4-TDA produced local sarcomas after repeated subcutaneous injection once a week for 2 years in rats. At 25 mg/kg bw/day, males and females showed a decrease in survival time. A decrease in body weight gain, and in the liver focal necrosis of the hepatocytes and single cirrhosis were observed at  $\geq 8.33$  mg/kg bw/day.

Isomeric mixture 2,4/2,6-TDA (80/20)

The studies on inhalation toxicity to the mixture of the isomers 2,4-/2,6-TDA (80/20), conducted on preliminary basis only, gave 9.5 mg/m<sup>3</sup> (approx. 1 mg/kg bw/day, subacute study) as the NOAEC for systemic effects for the rat, whereas this dose already caused slight methemoglobin formation (2.07%) in the cat, which is more sensitive for this effect (NOAEC not derivable). The latter was confirmed in a further experiment. At dose level of 41.6 mg/m<sup>3</sup> (approx. 4.5 mg/kg bw/d, subacute study), cats showed severe methemoglobinemia (30%), retarded body weight gain, and pathomorphological findings (not specified) in the lungs, liver, and kidneys (no more data). At a concentration of 83 mg/m<sup>3</sup> (approx. 9 mg/kg bw/day, subacute study), rats showed reduced body weight gain, an increase of the relative weight of the liver, kidneys, and thyroid glands, and a relative lymphopenia in blood tests. Data for methemoglobin formation in tests with rats were not available (Kimmerle and Solmecke 1971).

In rats the mixture of isomers 2,4-/2,6-TDA (80/20) produced local sarcomas after repeated subcutaneous injection for 2 years. At 25 mg/kg bw/day, males and females showed a decrease in survival time. A decrease in body weight gain, and in the liver focal necrosis of the hepatocytes and single cirrhosis were observed at  $\geq$  8.33 mg/kg bw/day.

There were no animal studies using the oral or dermal route of exposure to commercial mixture of TDA 2,4-/2,6-TDA (80/20).

### **No/Lowest-observed-effect level/concentrations**

#### **2,4-TDA - Oral administration**

A LOAEL of 5.9 mg/kg bw/day (time-weighted average dose) was derived from a 2-year rat study (NCI 1979). A NOAEL was not estimated. Although this long-term feeding study was not in full agreement with the requirements needed for the base set studies of existing chemicals (only two doses tested, no data on haematology and clinical chemistry parameters), it represents the lowest effect level at which relevant toxic effects were observed. At this dosage there were a decreased survival rate, a clear delay in body weight gain, lesions of the liver and kidneys as well as tumors in the liver in high incidences.

The treatment of male rats with approximately 15 mg/kg bw/d for 10 weeks resulted in changes in Sertoli cell cytostructure, and after treatment for 4, 6, and 8 weeks in reduced body weight gain and increases in testes weights (nearly doubled testes/body weight ratios). At approximately 30 mg/kg bw/d 2,4-TDA for 3 weeks, male rats showed reduced epididymal sperm reserves, and increased alterations in Sertoli cell ultrastructure. The sperm counts and ultrastructural changes seen after three weeks of dietary TDA-treatment differed from those seen at 10 weeks of 2,4-TDA feeding with 15 mg/kg bw/d only in their severity (Varma et al. 1988). Toxic effects on spermatogenesis were reported in male rats given approximately 15 mg/kg bw/d for 10 weeks. At light microscopy focal or diffuse hypospermatocytogenesis in the seminiferous tubules and cauda epididymides were noted. No such findings were reported at approximately 5 mg/kg bw/d (Thyssen et al. 1985a). In a further experiment for 10 weeks significant decrease in body weight gain, seminal vesicles and mean epididymal weights, sperm count, and serum testosterone levels were determined in male rats receiving approximately 15 mg/kg bw/d 2,4-TDA, but not at 5 mg/kg bw/d. However, diminished sperm reserves were noted in male rats receiving approximately 5 mg/kg bw/d 2,4-TDA

(Thyssen et al. 1985b). Therefore, 5 mg/kg bw/d is considered to be as a marginal LOAEL for effects on spermatogenesis.

Other repeated dose toxicity studies of 2,4-TDA using medium-term treatment periods were able to identify a NOAEL. Some of them were not in (full) compliance with the current test guidelines, and have been focused on selected parameters. After subchronic administration the NOAEL in a limited study was 250 ppm (approx. 18 mg/kg bw/day) in rats and 100 ppm (approx. 15 mg/kg bw/day) in mice (7-week study NCI 1979). However, these values are not recommended because they fell into the range of the LOAEL and/or the database was considered less reliable than the 2-year study.

**LOAEL 5.9 mg/kg bw/day (103-week oral/F344 rat, NCI 1979)**

#### **Commercial mixture of the isomers 2,4-/2,6-TDA (80/20) - Inhalation**

There were no animal studies using the inhalation route of exposure to 2,4-TDA.

The existent studies with repeated inhalation exposures to the commercial mixture of the isomers 2,4-/2,6-TDA (80/20) were not in accordance to the test design of the current guidelines B.8; OECD TG 412. Therefore no valid data of effects via inhalation of commercial mixture of the isomers 2,4-/2,6-TDA (80/20) are available. The studies on inhalation toxicity, conducted on preliminary basis only, gave 9.5 mg/m<sup>3</sup> (approx. 1 mg/kg bw/day, subacute study) as the NOAEC for systemic effects for the rat, whereas this dose already caused methemoglobinemia of 2.07% in cats. In cats as a very sensitive species for methemoglobinemia a dose level of 41.6 mg/m<sup>3</sup> (approx. 4.5 mg/kg bw/d, subacute study) caused severe methemoglobinemia of 30%. So there was no NOAEC derivable because of methemoglobin formation in the cat (Kimmerle and Solmecke 1971). None of the existent studies investigated local effects on the respiratory tract.

**NOAEC<sub>sys</sub> 9.5 mg/m<sup>3</sup> (approx. 1 mg/kg bw/day) 28-day inhalation/Wistar rat**

#### **Classification**

There are several repeated dose toxicity studies with oral route of exposure and with different duration for 2,4-TDA and only few non-guideline compliant studies with inhalative administration of the isomeric 2,4-/2,6-TDA mixture. Due to the lack of data for the inhalative route for 2,4-TDA and for the oral route for 2,4-/2,6-TDA it is proposed to apply an identical classification proposal on chronic toxic effects of the pure substance and the mixture.

Oral



Human data on the repeated dose toxicity of 2,4-TDA or the 2,4-/2,6-TDA mixture are not available.

The proposal for classification is based on data from a well conducted carcinogenicity study in F344 rats (NCI, 1979, Cardy, 1979), and in addition from mid- and long-term diet studies using Sprague-Dawley, ChP-CD and Wistar rats comparable to guideline studies with acceptable restrictions (Ito et al. 1969, Stula and Aftosmis 1976).

The critical adverse effects in rats of both sexes after long-term administration of 2,4-TDA were hepatotoxicity and chronic renal failure that contributed to a marked decrease in survival at doses of  $\geq 5.9$  mg/kg bw/day. Hepatotoxic effects were shown as fatty degeneration, cholangiofibrosis and liver cirrhosis (at  $\geq 45$  mg/kg bw/day, 36-week study; Ito 1969) as well as focal necrosis of hepatocytes to severe, diffuse, toxic degenerative lesions in the liver ( $\geq 5.9$  mg/kg bw/day, long-term study; Cardy 1979, NCI 1979). Nephrotoxicity observed at  $\geq 5.9$  mg/kg bw/day was characterised as chronic renal glomerulonephrosis with glomerular atrophy and sclerosis, interstitial inflammation and fibrosis, and tubular degeneration and atrophy. Associated to the chronic renal disease, signs of secondary hyperparathyroidism such as bone resorption and hyperplasia of parathyroid glands were markedly expressed.

Additionally, experimental data from feeding studies with rats have demonstrated that 2,4-TDA induced dose related toxic effects on the testes. Testicular atrophy was observed at 28 mg/kg bw/day (15 months study; Stula and Aftosmis 1976). Disturbed spermatogenesis and Sertoli cell damage, atrophy of accessory sex glands, reduced serum level of testosterone and reflective increase in LH levels were associated to testis toxicity occurred at doses of 15 mg/kg bw/day (10-week feeding study; Thyssen et al. 1985a, 1985b, Varma et al. 1988). Even at the dose of approximately 5 mg/kg bw/day 2,4-TDA diminished sperm reserves indicating a depression of spermatogenesis were noted (10-week feeding study; Thyssen et al. 1985b).

The observed toxic effects such as mortalities and degenerative/necrotic lesions in liver, kidney and in male gonads are serious health damage according to the criteria for R48 (Annex VI of 67/548/EEC). They occurred at dosages below the R 48 guidance values, which is 50 mg/kg bw/day (90-day oral toxicity study in rodents). These effects were observed in a dose-related pattern at  $\geq 5.9$  mg/kg bw/day (liver, kidney, mortality) and 5 mg/kg bw/day (male reproductive system), respectively. Furthermore, there was clear dose-response relationship in frequency, intensity and severity for the observed effects.

According to the criteria of the Directive 67/548/EEC, 2,4-TDA is proposed to be classified and to be labelled as Xn, Harmful, R48/22 (Harmful: Danger of serious damage to health by prolonged exposure if swallowed).

#### Inhalation

There is a concern that 2,4-TDA and its isomeric 2,4-/2,6-TDA mixture may cause adverse systemic toxicity after prolonged inhalation exposure. On the present limited database, however, no firm conclusion on the need of classification for the repeated dose effects after prolonged inhalation can be drawn.

## Dermal

No data available for 2,4-TDA and its 2,4-/2,6-TDA mixture.

#### 4.1.2.7 Mutagenicity

##### In vitro tests

##### Bacterial gene mutations

Bacterial genotoxicity tests were clearly positive with S-9 mix in several tests.

2,4-Toluenediamine (2,4-TDA) was positive with S-9 mix in Salmonella typhimurium strains TA 98, TA 100, TA 1537 and TA 1538 with respect to induction of bacterial gene mutations for doses from 20 µg/plate upward (JETOC, 1996; George and Westmoreland, 1991; Shahin et al., 1985; Haworth et al., 1983; Shahin et al., 1980; Green et al., 1979). All investigations showed strong and dose-dependent effects; no toxic effects were observed. These Salmonella typhimurium strains were negative without S-9 mix up to a dose of 10000 µg/plate. 2,4-TDA was also positive in TA98 with S-9 mix in a microsuspension bioassay (George et al., 2001).

Cunningham and Matthews (1990) described the role of bacterial acetyltransferase on mutagenic effect of 2,4-TDA in bacterial mutation test with metabolic activation as shown by experiments with strain Salmonella typhimurium strain TA98/1,8-DNP6 (deficient in acetyltransferase), TA 98 (normal acetyltransferase) and the overproducer of acetyltransferase TA 98(pYG219). Compared with TA 98 the effect of strain TA98/1,8-DNP6 resulted in approximately 90% decrease in the mutagenic potency whereas the strain TA 98(pYG219) greatly enhanced the mutagenic effect. The authors concluded that after N-hydroxylation of 2,4-TDA by S-9 mix cytochrome P450 the resulting hydroxylamino intermediate is further activated by bacterial acetyltransferase to form the ultimate reactive intermediate, which is postulated to be 4-acetoxyamino-2-aminotoluene.

Negative results with and without S-9 mix were obtained in Salmonella typhimurium strain TA 1535 up to 10000 µg/plate (JETOC, 1996; Haworth et al., 1983) and in E. coli WP2uvrA up to 5000 µg/plate (JETOC, 1996).

**Table 1. In vitro tests: bacterial genotoxicity**

Test system	Concentration range		Result	Toxicity	Remarks	Reference
	with S-9 mix	without S-9 mix				
Gene mutation, Salm. typh. TA 98, TA 100, TA 1535, TA 1537, E.coli	0.07 63-5000 µg/plate	0.07 63-5000 µg/plate	positive	no toxic effects	positive only with S-9 mix	JETOC, 1996

WP2uvrA						
Gene mutation, Salm. typh. TA 98	100 - 3333 µg/plate	not done	positive	no toxic effects		George and Westmoreland, 1991
Gene mutation, Salm. typh. TA 97, TA 1537, TA 1538	50 - 500 µg/plate	not done	positive	no toxic effects		Shahin et al., 1985
Gene mutation, Salm. typh. TA 98, TA 100, TA 1535, TA 1537	10 - 10'000 µg/plate	10 - 10'000 µg/plate	positive	no toxic effects	positive only with S-9 mix	Haworth et al., 1983
Gene mutation, Salm. typh. TA 98, TA 100, TA 1538	5.0 -1000 µg/plate	5.0 - 1000 µg/plate	positive	no toxic effects	positive only with S-9 mix	Shahin et al., 1980
Gene mutations Salm. typh. TA 98; TA 100, TA 1535, TA 1537, TA 1538	500 - 5000 µg/plate	500 - 5000 µg/plate	positive	no toxic effects	positive only with S-9 mix	Green et al., 1979
Gene mutation, Salm. typh. TA 98	100 - 1666 µg/plate	not done	positive	no data		Cunningham and Matthews, 1990
Gene mutation, Salm. typh. TA 98, TA 100 (microsuspension bioassay)	10-500 µg/plate	10-500 µg/plate	positive	no data	positive only with S-9 mix	George et al., 2001

In vitro tests

Mammalian cell systems

#### Gene mutation tests

In general, 2,4-TDA was negative in mammalian cell mutation tests at the tk or hprt locus. A weak positive effect was obtained at the tk locus in L5178Y mouse lymphoma cells in the absence of S-9 mix; however, this effect was paralleled by strong cytotoxicity (Coppinger et al. 1984). In this study, 2,4-TDA was tested for a mutagenic potential at both the tk locus and the hprt locus in L5178Y mouse lymphoma cells and CHO-AT3-3 cells (Coppinger et al., 1984). Increases of the mutation frequency were found for positive controls in both test systems. At the tk locus of L5178Y cells a marginal positive response was observed without

S-9 mix. The effect was dose-dependent in the dose-range 87.8 - 1000 µg/ml: increase in the mutant frequencies from 1.3-fold at 87.8 µg/ml up to 4.1-fold at 1000 µg/ml. The genotoxic effect was paralleled by strong cytotoxicity: relative total growth (as compared to the solvent control) was 47% at 87.8 µg/ml and decreased down to 9% at 1000 µg/ml. With S-9 mix doses from 58.5 up to 1000 µg/ml were negative.

At the tk locus of CHO-AT3-2 cells 2,4-TDA was negative with and without S-9 mix for doses up to 6000 µg/ml. At the hprt locus 2,4-TDA was negative with and without S-9 mix both in L5178Y cells (up to 1000 µg/ml) and in CHO-AT3-2 cells (up to 10000 µg/ml).

A negative result at the hprt locus in V79 cells with and without S-9 mix was described by Fassina (1990) for a dose-range of 0.3 - 3.0 mmol/l (36.6 - 366.5 µg/ml). Increases in mutation frequency were found for positive controls.

**Table 2. In vitro tests: mammalian cell gene mutations**

Test system	Concentration range		Result	Toxicity	Remarks	Reference
	with S-9 mix	without S-9 mix				
tk locus, L5178Y cells (mouse lymphoma assay)	58.5 - 1000 µg/ml	58.5 - 1000 µg/ml	positive	with and without S-9 mix strong effects from 87.8 µg/ml upward	4-h treatment; positive only without S-9 mix: marginal response	Coppinger et al., 1984
tk locus, CHO-AT3-2 cells	2000 - 6000 µg/ml	2000 - 6000 µg/ml	negative	with and without S-9 mix at high concentrations	4-h treatment	
hprt locus, L5178Y cells	250 - 1000 µg/ml	250 - 1000 µg/ml	negative	no toxic effects	4-h treatment	

hpert locus, CHO-AT3-2 cells	1000 - 10'000 µg/ml	1000 - 10'000 µg/ml	negative	with and without S-9 mix at high concentrations	4-h treatment	
hpert locus, V79 cells	0.3 - 3.0 mmol/l  (36.6 - 366.5 µg/ml)	0.3 - 3.0 mmol/l  (36.6 - 366.5 µg/ml)	negative	without S-9 mix at doses of 1.0 mmol/l and higher	1-h treatment	Fassina, 1990

### Chromosomal aberration tests

Chromosomal aberration tests were positive with and without S-9 mix in mammalian cells.

Loveday et al. (1990) described that 2,4-TDA induced chromosomal aberrations in CHO cells with and without S-9 mix; unfortunately, no toxicity data were given and no differentiation was made of chromosomal aberrations with and without gaps. In the only one experiment with S-9 mix, after 2-h exposure increased aberration frequencies of 7% and 12% were found for doses of 1370 and 4550 µg/ml (12 h sampling; negative control, 1.0 % aberration frequency). Without S-9, in 3 experiments the results were positive after treatments for 2 h (sampling time, 20.5 h) and 8 h (sampling time, 10 h) for doses from 98.5 µg/ml upwards; the maximum aberration frequency was 41.7% (negative control, 4.0%).

A positive effect of 2,4-TDA on chromosome aberrations in CHO cells was described by Armstrong et al. (1992); the test was only done without S-9 mix. Doses from 245.4 µg/ml upwards induced chromosomal aberrations after a treatment of 3 h (sampling times, 10 h and 24 h) with a maximum chromosomal aberration frequency of ca. 15% (negative controls, 2.0 - 3.0 %). Toxic effects were observed at doses of 490.8 µg/ml and higher.

Bean et al. (1992) investigated the effect of sampling time on 2,4-TDA induced aberration frequencies in CHO cells without S-9 mix. The tested doses of 2.0 mmol/l and 6.0 mmol/l (244.3 µg/ml and 733.0 µg/ml) induced increases of chromosomal aberrations. After 3-h exposure maximum aberration frequencies were observed at 17 h sampling time (70% aberrant cells with 6.0 mmol/l, 12% aberrant cells with 2.0 mmol/l; mean frequency of aberrant cells in the negative controls was 3.4%).

In a study of JETOC (1996) chromosomal aberrations were analyzed in CHL cells only without S-9 mix for a dose-range of 0.78 - 50 µg/ml. At the highest tested doses of 13 and 50 µg/ml strong positive effects with dose-dependency were induced after exposure for 24 h and 48 h. The maximum aberration frequency was 65% (negative control, 0.5%); data on toxic effects were not given.

**Table 3. In vitro tests: chromosomal aberrations**

Test system	Concentration range	Result	Toxicity	Remarks	Reference
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	with S-9 mix	without S-9 mix				
Chromosomal aberration, CHO cells	455 - 4550 µg/ml	13.7 - 4920 µg/ml	positive	no data	positive with and without S-9 mix	Loveday et al., 1990
Chromosomal aberration, CHO cells	not done	1.0 - 10 mmol/l (123 - 1227 µg/ml)	positive	at doses of 4 mmol/l and higher		Armstrong et al., 1992
Chromosomal aberration, CHO cells	not done	2.0 - 6.0 mmol/l (244 - 733.0 µg/ml)	positive	no data		Bean et al., 1992
Chromosomal aberration, CHL cells	not done	0.78 - 50 µg/ml	positive	no data		JETOC, 1996

#### Sister chromatid exchange tests (SCE)

2,4-TDA was positive for induction of SCE with and without S-9 mix in mammalian cells.

Loveday et al. (1990) reported on induction of SCE in CHO cells with and without S-9 mix. With S-9 mix at all tested doses ranging from 468 up to 4680 µg/ml a weak positive effect was observed after a treatment for 2 h (sampling time, 26 h); the maximum SCE frequency was 1.6-fold that of the negative control. Without S-9 mix the SCE frequency increased dose-dependently in the dose-range 14.1 up to 130 µg/ml after continuous treatment for 26 h. The maximum SCE frequency determined at the highest tested dose of 130 µg/ml was 2.7-fold that of the negative control. No data about toxic effects were given.

**Table 4. In vitro tests: tests for induction of sister chromatid exchanges (SCE)**

Test system	Concentration range		Result	Toxicity	Remarks	Reference
	with S-9 mix	without S-9 mix				
CHO cells	468 - 4680 µg/ml	4.68 - 130 µg/ml	positive	no data	positive with and without S-9	Loveday et al., 1990

### Tests on unscheduled DNA synthesis (UDS tests)

2,4-TDA was positive for induction of UDS in primary hepatocyte cultures.

Selden et al. (1994) tested 2,4-TDA for induction of UDS in rat hepatocytes in a dose- range of 0.0005 - 10 mmol/l (0.06 - 1222 µg/ml) with help of the autoradiography procedure. No detailed data on the result were given; it was said that 0.01 mmol/l (1.2 µg/ml) was the lowest dose with observed positive effect; 22.1 net nuclear grains were found at this dose. Data about toxic effects were not given.

A 18-h treatment of primary rat hepatocytes with 2,4-TDA at doses from 0.01 up to 1.0 mmol/l (1.22 - 122.2 µg/ml) resulted in a positive UDS-response at 0.01 and 0.1 mmol/l (Bermudez et al.; 1979). Significant increased average net nuclear grains of 8.1 (0.01 mmol/l) and 7.6. (0.1 mmol/l) were determined by autoradiography procedure; the highest tested dose of 1.0 mmol/l induced a negative result (average net nuclear grains of 0.7). The average net nuclear grain of the solvent control was minus 3.2. Dimethylnitrosamine and 2-acetylaminofluorene were used as positive controls. Dimethylnitrosamine induced a significant increase in net grains to 6.7, and 2-acetylaminofluorene at a dose of 1µM induced a response to great to be quantified by the automatic counting system. There are no data on toxicity.

A positive response was also obtained by using human hepatocytes prepared from discarded surgical material (Butterworth et al., 1989). UDS was detected in hepatocytes from two donors at 0.1 and 1.0 mmol/l (12.2 and 122.2 µg/ml) after treatment of 18 h by autoradiography procedure. A maximum number of net grains per nucleus of 11.3 was determined in comparison to -4.7 in the solvent control. Data on toxicity were not given.

The measurement of UDS in a suspension of freshly isolated post-S-phase rat spermatides and primary spermatocytes exposed to 0.01 - 1.0 mmol/l (1.22 - 122 µg/ml) 2,4-TDA for 18 h gave negative results for both spermatogenic cell stages (Working and Butterworth, 1984). The tests with 2,4-TDA were only done without S-9 mix. The authors state that in this test system spermatocytes and spermatids underwent UDS only when exposed to direct-acting alkylating agents. No compound which required metabolic activation to be genotoxic caused UDS in either spermatogenic celltype. However, in the presence of a S9 activation system, UDS was induced. I.e. Aflatoxin B<sub>1</sub> and 2-aminofluorene were negative without S9-mix but gave a positive result in the presence of S9-mix.

**Table 5. In vitro tests: tests for induction of unscheduled DNA synthesis (UDS)**

Test System	Concentration range		Result	Toxicity	Remarks	reference
	with S-9 mix	without S-9 mix				
Primary rat hepatocytes	not applicable	0.0005 - 10 mmol/l (0.06 - 1222 µg/ml)	positive	no data	autoradiography procedure	Selden et al., 1994
Primary rat hepatocytes	not applicable	0.01 - 1.0 mmol/l (1.22 - 122.2 µg/ml)	positive	no data	autoradiography procedure	Bermudez et al., 1979
Human hepatocytes	not applicable	0.01 - 1.0 mmol/l (1.22 - 122.2 µg/ml)	positive	no data	autoradiography procedure	Butterworth et al., 1989
Rat spermatides & primary spermatocytes	not done	0.01 - 1.0 mmol/l (1.22 - 122.2 µg/ml)	negative	strong toxic effect at 1.0 mmol/l	autoradiography procedure	Working and Butterworth, 1984

DNA strand breaks

2,4-TDA was positive for induction of DNA strand breaks in mammalian cells with and without S-9 mix.

Nordenskjöld et al. (1984) showed that 2,4-TDA induced DNA strand breaks in human skin fibroblasts using the alkaline elution technique. The fibroblasts were exposed to 100 µmol/l (12.2 µg/ml) 2,4-TDA for 30 minutes in the absence of S-9 mix and in the presence of different activation systems (liver microsomes from either phenobarbital- or from 3-methylcholanthrene-induced rats; microsomes from ram seminal vesicles complemented with arachidonic acid). The strongest effect was obtained with microsomes from ram seminal vesicles, as indicated by a 21-fold increase of DNA strand breaks as compared to the negative control. A weak effect (3.7-fold increase of DNA strand breaks) was obtained in the presence of phenobarbital-induced rat liver microsomes while in the presence of 3-methylcholanthrene-induced rat liver microsomes a negative response was obtained. Without S-9 mix a weak effect of 2.3-fold increase of DNA strand breaks was induced. There are no data on toxicity.



Induction of DNA strand breaks in V79 cells with S-9 mix was analyzed by Swenberg (1981) with the help of alkaline elution technique. No detailed data were given; a positive response was obtained with rat liver S-9 mix in the tested dose range of 0.3 - 3.0 mmol/l (36.6 - 366.5 µg/ml) after treatment times of 2 h and 4 h. Data about toxic effects were not given.

Using also the alkaline elution technique, Sina et al. (1983) described that 2,4-TDA did not induce an increase of DNA strand breaks in primary rat hepatocytes in a dose range of 0.03 - 3.0 mmol/l (3.7 - 367 µg/ml) after a 3-h treatment. No toxic effects were observed.

**Table 6. In vitro tests: DNA strand breaks in mammalian cells**

Test system	Concentration range		Result	Toxicity	Remarks	Reference
	with S-9 mix	without S-9 mix				
Alkaline elution technique; human skin fibroblasts	100 µmol/l (12.3 µg/ml)	100 µmol/l (12.3 µg/ml)	positive	no data	positive with and without S-9 mix	Nordenskjöld et al., 1984
Alkaline elution technique; V79 cells	0.3 - 3.0 mmol/l (36.6 - 367 µg/ml)	not done	positive	no data		Swenberg, 1981
Alkaline elution technique; primary rat hepatocytes	not applicable	0.03 - 3.0 mmol/l (3.7 - 367 µg/ml)	negative	no data		Sina et al., 1983

### DNA adducts

2,4-TDA was able to form adducts with mammalian cell DNA in the absence and presence of S-9 mix.

Primary rat hepatocytes were used to assess the binding of <sup>14</sup>C 2,4-TDA to DNA in a dose range of 30 up to 300 µmol/l (3.6 - 36.6 µg/ml) with help of the liquid scintillation technique (Furlong et al., 1987). The DNA adduct formation was time-dependent and increased linearly for treatment times up to 24 h. Up to 100 µmol/l binding of 2,4-TDA to hepatic DNA correlated with the substrate concentration, then the effect plateaued.

The incubation of purified single-stranded calf thymus DNA with 2,4-TDA resulted in formation of DNA adducts as shown by chromatography (Citro et al., 1993); this investigation was done with S-9 mix only. Different concentrations of 2,4-TDA (12 - 200 g)

and different exposure times (12 h - 60 h) were used to find out that the maximum effect was obtained with 150 g 2,4-TDA at 20 h exposure time.

**Table 7. In vitro tests: DNA adducts formation**

Test system	Concentration range		Result	Toxicity	Remarks	Reference
	with S-9 mix	without S-9 mix				
Primary rat hepatocytes	not applicable	30 - 300 $\mu\text{mol/l}$  (3.6 - 36.6 $\mu\text{g/ml}$ )	positive	no data		Furlong et al., 1987
Single stranded calf thymus DNA	12 - 200 $\mu\text{g}$	not done	positive	no data		Citro et al., 1993

**Table 8. Overview on in vitro findings**

<u>Negative effects</u>	<u>Inconclusive effects</u>	<u>Positive effects</u>
<b><u>Mutation tests in vitro</u></b>		
		Bacterial gene mutations with S-9 mix
Mammalian cell mutagenicity (hprt locus) with and without S-9 mix		
Mammalian cell mutagenicity (tk locus) with S-9 mix	Mammalian cell mutagenicity (tk locus) without S-9	
		chromosomal aberrations with and without S-9 mix
<b><u>Indicator tests in vitro</u></b>		
		SCE in mammalian cells with and without S-9 mix
		UDS with primary hepatocytes
		DNA strand breaks in mammalian cells with and without S-9 mix
		DNA adducts in mammalian cells with and without S-9 mix

#### In vivo tests

##### Micronucleus assay

In general, results were negative in micronucleus assays. A weak positive effect in PVG rats was limited to a dose with high acute toxicity.

In a micronucleus assay on polychromatic erythrocytes in peripheral blood of male mice (strain: BDF1) 2,4-TDA led to a negative result after single intraperitoneal injection of doses ranging from 30 to 240 mg/kg bw (Morita et al., 1997). Sampling times were 24 h, 48 h and 72 h after treatment. The highest tested dose corresponded to 80% of the LD<sub>50</sub>. Informations on local cytotoxicity or toxic signs were not given.

George and Westmoreland (1991) reported on negative findings in an in vivo micronucleus test in bone marrow cells of male and female rats (strain: Fischer-344) after single oral administration of doses ranging from 50 up to 150 mg/kg bw. Sampling was 24 h and 48 h after treatment. The highest tested dose of 150 mg/kg bw induced an effect on local cytotoxicity (PCE/NCE) and in a pre-test this dose was lethal for one out of four animals. Adequate increases in micronucleus frequencies were reported for 5 and 10 mg/kg bw cyclophosphamide used as positive control.

For another in vivo micronucleus test on male rats (strain: PVG; inbred Piebald Virol Glaxo pigmented strain) George and Westmoreland (1991) reported on a small significant increase in the micronucleus frequency of bone marrow cells. A doubling of micronucleus frequency was observed at the highest tested dose of 300 mg/kg bw after a 24-h treatment: 0.34% micronucleated polychromatic erythrocytes as compared to 0.17% in the control. Doses of 150 and 225 mg/kg bw were negative. No cytotoxic effects were observed at either dose. A second sampling time, 48 h, was negative for all doses. No detailed data on general toxicity were given; it was noted, however, that treatment with 300 mg/kg bw killed half of the animals treated for the 48 h sampling time. Therefore, the weak micronucleus induction effect at 300 mg/kg bw is of low toxicological significance. Adequate increases in micronucleus frequencies were reported for 10 mg/kg bw cyclophosphamide used as positive control.

**Table 9. In vivo tests: Micronucleus (MN) test**

Test system	Doses	Expos. regimen	Sampl. times	Result	Local cytotox.	General toxicity	Remarks	Reference
BDF 1 mice, peripheral blood erythrocytes	30 - 240 mg/kg bw	1 x i.p.	24 h, 48 h, 72 h	negative	no data	240 mg/kg = 80% of LD <sub>50</sub>		Morita et al., 1997
Fischer 344 rats; bone marrow erythrocytes	50 - 150 mg/kg bw	1 x p.o.	24 h, 48 h	negative	at 150 mg/kg	pilot study: 1/4 animals dosed with 150 mg/kg died		George and Westmoreland, 1991
PVG rats; bone marrow erythrocytes	150 - 300 mg/kg bw	1 x p.o.	24 h, 48 h	positive	no effects	300 mg/kg = LD <sub>50</sub>	doubling of the MN frequency at 300 mg/kg bw only after 24-h treatment	George and Westmoreland, 1991

#### Sister-chromatid exchanges (SCE)

2,4-TDA was weakly positive for induction of SCE in mouse bone marrow cells.

2,4-TDA induced SCE in bone marrow cells of male Swiss mice after single intraperitoneal injection of the tested doses of 9.0 and 18 mg/kg bw (Parodi et al., 1983). The effects were weak: increases of SCE frequencies were 1.5-fold at 9.0 mg/kg bw and 1.3-fold at 18 mg/kg bw. The sampling time was 24 h. Data on toxicity were not given.

**Table 10. In vivo tests: Tests for induction of sister chromatid exchanges (SCE) in mice**

Test system	Doses	Expos. regimen	Sampl. times	Result	Local cytotox.	General toxicity	Remarks	Reference
Swiss mice, bone marrow cells	9.0 - 18 mg/kg bw	1 x i.p.	24 h	positive	strong toxicity at doses > 18 mg/kg		weak effect	Parodi et al., 1983

#### Induction of unscheduled DNA synthesis (UDS)

2,4-TDA was positive for induction of UDS in rat liver.

Mirsalis et al. (1982) reported on a strong positive result in hepatocytes of male rats (strain: Fischer-344) at the single tested dose of 150 mg/kg bw after single oral administration: 15.9 net grains/nucleus after a 2-h treatment and 11.0 net grains/nucleus after 12-h exposure (negative controls, -5.1 and -4.4 net grains/nucleus). No data on toxicity were given.

George and Westmoreland (1991) investigated the effects of single oral administrations of 150 mg/kg bw (2-h and 16-h treatment) and 300 mg/kg bw (16-h treatment) on hepatocytes of male rats (Fischer-344). A weak positive result was obtained at 16-h sampling with 150 mg/kg bw, no effect was found with 300 mg/kg bw (for 150 mg/kg the mean net grain value was 1.86 as compared to -3.19 in the negative control). Dosing of 150 mg/kg bw was negative at 2-h sampling time. No data about toxicity were given.

**Table 11. In vivo tests: Tests for induction of unscheduled DNA synthesis (UDS) in rats**

Test system	Doses	Expos. regimen	Sampl. times	Result	Local cytotox.	General toxicity	Remarks	Reference
Fischer-344 rat liver	150 mg/kg bw	1 x p.o.	2 h, 12 h	positive	no data	no data	autoradiography	Mirsalis et al., 1982

Fischer-344 rat liver	150 - 300 mg/kg bw	1 x p.o.	2 h, 16 h	positive	no data	pilot study: 1/4 animals dosed with 150 mg/kg died	autoradiography	George and Westmoreland, 1991
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### Rodent germ cell tests

2,4-TDA was negative in dominant lethal and sperm abnormality tests with mice.

According to Soares and Lock (1980) 2,4-TDA was negative in dominant lethal tests with male mice (strain: DBA/2J) both after oral or intraperitoneal administration of 40 mg/kg bw on two consecutive days. At 48 h after treatment, each treated male was paired with three CD-1 female mice. After seven days the females were replaced by new females. This mating scheme was repeated for a total of seven weeks post-treatment. There was no induction of abnormalities in sperm morphology after both types of administration. Sperms were investigated eight weeks post-treatment.

The i.p. application of 2,4-TDA doses from 111 mg/kg to 375 mg/kg bw to C57Bl/6xC3H mice (10 animals per dose group) resulted in a dose-dependent reduction of murine testicular DNA synthesis (Greene et al. 1981). The test substance was applied 3h prior to i.p. injection of 10 µCi [<sup>125</sup>I]iododeoxyuridine and 3.5 h prior to removal of testes; physiological saline was used as negative control and an oral dose of 100 mg/kg bw dimethylnitrosamine served as positive control. There are no data on toxicity in the main experiment. In a parallel experiment 2,4-TDA induced a dose-dependent reduction of body temperature. The authors conclude that the inhibition of murine testicular DNA synthesis induced by 2,4-TDA could not be entirely explained by drop in body temperature (which in itself may lead to reduced testicular DNA synthesis).

This study is regarded as additional information as inhibition of DNA synthesis is not a genotoxicity endpoint but a relatively unspecific parameter of cytotoxicity.

**Table12. In vivo tests: Rodent germ cell tests with mice**

Test system	Doses	Exposure regimen	Result	General toxicity	Reference
Testicular DNA synthesis	111, 167, 250, 375 mg/kg bw	1 x i.p.	positive	hypo-thermia	Greene et al., 1981
Dominant lethal test, DBA/2J mice	40 mg/kg bw	2 x p.o., treated on two consecutive days	negative	no data	Soares and Lock, 1980
		2 x i.p., treated on two consecutive days	negative	no data	

Sperm morphology test, DBA/2J mice	40 mg/kg bw	2 x p.o., treated on two consecutive days	negative	no data	Soares and Lock, 1980
		2 x i.p., treated on two consecutive days	negative	no data	

### Drosophila melanogaster

2,4-TDA induced positive effects in sex-linked recessive lethal tests with *Drosophila* (SLRL-test).

In sex-linked recessive lethal tests with *Drosophila* (strain: Berlin K) positive results were reported after feeding of males with doses of 5.9 mmol/l (721 µg/ml) 2,4-TDA and 15.2 mmol/l (1857 µg/ml) 2,4-TDA for 3 days (Blijleven, 1977).

Fahmy and Fahmy (1977) described that 2,4-TDA was positive in a test for sex linked recessive lethals in *Drosophila* (strain: Oregon-K) after single microinjection of doses ranging from 5.0 to 20 mmol/l (611 - 2443 µg/ml) into the haemocoel of males.

**Table 13. In vivo tests: tests with *Drosophila melanogaster***

Test system	Doses	Exposure regimen	Result	General toxicity	Reference
Sex-linked recessive lethal test	5.9 - 15.2 mmol/l (721 - 1857 µg/ml)	3 days; feeding of males	positive	no data	Blijleven, 1977
Sex-linked recessive lethal test	5.0 - 20 mmol/l; (611 - 2443 µg/ml)	1 x injection into the haemocoel of males	positive	no data	Fahmy and Fahmy, 1977

### DNA strand breaks

2,4-TDA was positive for induction of DNA strand breaks in several rodent tissues.

Induction of DNA strand breaks in cells of different mouse organs was analyzed by Sasaki et al. (1999; 1997) using the alkaline single-cell gel electrophoresis assay (Comet-assay).

Male mice (strain: ddY) were treated with the maximum tolerated doses of 60 mg/kg bw. After single oral administration a statistically significant increase in DNA damage was reported for cells of stomach, liver and kidney. The strongest effect was shown in liver. Negative results were observed in cells of colon, bladder, lung, brain and bone marrow. The

sampling times were 3 h, 8 h and 24 h; data on toxic effects were not given (Sasaki et al., 1999).

After single intraperitoneal administration of 240 mg/kg bw to male mice (strain CD-1) and exposure times of 3 h and 24 h positive effects were found in cells of liver, kidney and lung. In liver cells the strongest positive response was determined. No effects were observed in cells of spleen and bone marrow. No data on toxic effects were given (Sasaki et al., 1997).

Multiple organs of male Wistar rats were analysed for DNA strand breaks in the comet assay (Sekihashi et al., 2002). After a single oral gavage of 130 mg/kg bodyweight a statistically significant increase in migration of nuclear DNA was reported for stomach, colon, kidney and brain. The strongest effect was shown in stomach. No effects were observed in liver, bladder, lung and bone marrow. The dose was reported to be equivalent to the 0.5 x LD50 value.

Induction of DNA strand breaks in male rat liver (strain: Sprague-Dawley) was analyzed by Brambilla et al. (1985) using the viscometric technique, a measure of viscosity profiles of DNA. Animals were treated with single intraperitoneal injection of 37 and 147 mg/kg bw, sampling was after 2 h, 4 h, 12 h and 24 h. Both doses induced a positive effect; the highest tested dose was equivalent to the LD-50 value. There were no data on toxicity.

**Table 14. In vivo tests: DNA strand breaks**

Test system	Doses	Expos. regimen	Sampl. times	Result	General toxicity	Positive organs	Negative organs	Reference
Comet-assay on ddY mice	60 mg/kg bw	1 x p.o.	3 h, 8 h, 24 h	positive	no data	liver kidney stomach	lung bone marrow colon bladder brain	Sasaki et al., 1999
Comet-assay on CD-1 mice	240 mg/kg bw	1 x i.p.	3 h, 24 h	positive	no data	liver kidney lung	bone marrow spleen	Sasaki et al., 1997
Comet assay on Wistar rats	130 mg/kg bw	1 x p.o.	3h, 8 h, 24 h	positive	0.5 x LD50	stomach , colon, kidney, brain	liver, bladder, lung, bone marrow	Sekihashi et al., 2002
Viscometric technique, Sprague-Dawley rats	37 - 147 mg/kg bw	1 x i.p.	2 h, 4 h,	positive	147 mg/kg = LD <sub>50</sub>	hepato-cytes		Brambilla et al., 1985



			12 h, 24 h					
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### DNA adducts

2,4-TDA formed adducts with DNA of various organs of rats.

<sup>32</sup>P-post-labeling technique (with nuclease P1 enrichment):

Delclos et al. (1996) observed a time- and dose-dependent formation of adducts in DNA from livers and mammary glands of female rats (strain: Fischer-344) fed 10 up to 180 ppm (1.2 - 22.1 mg/kg bw) 2,4-TDA. Sampling times were 0.5, 1, 3 and 6 weeks after beginning of the test. The maximum yield of DNA adducts, about  $200 \times 10^7$  relative adduct level (RAL), was found at the highest tested dose after exposure for 6 weeks. A single major DNA adduct and two minor adducts were identified. No data on toxicity were given.

Wilson et al. (1996) reported on DNA-binding in hepatic cells of male rats (strain: Fischer-344) after single intraperitoneal injection of doses ranging from 0.5 up to 250 mg/kg bw; sampling time was 24 h. The effect was dose-dependent: the highest concentration of adducts (about 120 nmol/g DNA) was determined at the highest tested dose of 250 mg/kg bw. After a single intraperitoneal injection of 150 mg/kg bw a time course was investigated: adduct concentration was measured at different time points (0.5 - 30 days); maximum adduct level (about 60 nmol/g DNA) occurred 24 h post-exposure. No toxicity data were given.

Taningher et al. (1995) investigated 2,4-TDA for the ability to induce DNA-adducts in liver cells of male rats (strain: Fischer-344). After single intraperitoneal injection of 125 and 250 mg/kg bw a dose-dependent positive effect was observed after an exposure for 18 h. The quantitative evaluation of autoradiograms displayed one major and two faint spots. Data on toxicity were not given.

La and Froines (1994) examined DNA adduct formation in male and female rats (strain: Fischer-344) and compared the adduct formation between target organs for carcinogenicity (liver and mammary gland) and non-target organs (kidney and lung). The results demonstrated organ-specific and dose-dependent effects; there were no differences in adduct persistence. Relative adduct levels were given as ratios of adducted nucleotids to total nucleotids. With single intraperitoneal doses of 5.0 and 50 mg/kg bw the authors could show that the quantitative formation of the DNA adducts in rat liver cells is dose-dependent; maximum yields were obtained after 18 - 24 h at both doses and lasted over a 2-week period. Animals treated with a single intraperitoneal injection of 50 mg/kg bw showed a formation of three distinct DNA adducts in the liver and mammary gland at 18-h sampling; in kidney and lung only one adduct was observed. Among the organs examined, DNA binding was highest in liver (adduct 1:  $17.5 \times 10^7$  RAL; adduct 2:  $2.12 \times 10^7$  RAL; adduct 3:  $1.05 \times 10^7$  RAL) followed by mammary gland (adduct 1:  $1.8 \times 10^7$  RAL; adduct 2:  $0.3 \times 10^7$  RAL; adduct 3:  $0.15 \times 10^7$  RAL) and the two non-target organs lung (adduct 1:  $0.57 \times 10^7$  RAL) and kidney (adduct 1:  $0.37 \times 10^7$  RAL). Data on toxicity were not given.

Also in an earlier publication La and Froins (1992) described DNA adduct formation by 2,4-TDA in liver, lung and kidney of male rats and in mammary gland of female rats (strain:

Fische-344) after single intraperitoneal injection; sampling time was 18 h. DNA binding in liver cells was detected in a dose-range beginning at the lowest tested dose of 4.1 up to 2046  $\mu\text{mol/kg}$  bw (0.5 - 250  $\text{mg/kg}$  bw); the effect was dose-dependent. At a dose of 2046  $\mu\text{mol/kg}$  bw (250  $\text{mg/kg}$ ) DNA binding of was described in all four organs, with each producing one major and two minor adduct spots. The adducts induced were qualitatively identical among the organs, but quantitative differences were observed. The liver (RAL,  $29.2 \times 10^7$ ) and mammary gland (RAL,  $4.2 \times 10^7$ ) showed major adduct yields, which were up to 30 times higher than those for kidney (RAL,  $1.1 \times 10^7$ ) and lung (RAL,  $1.4 \times 10^7$ ). The yields of the minor adducts were approximately 1/10 that for the major adduct.

No adducts were detected by Delclos et al. (1996) in DNA of T-lymphocytes isolated from spleen of male rats (strain: Fischer-344) fed 40 or 180 ppm (4.9 or 22.1  $\text{mg/kg}$  bw) 2,4-TDA daily for 32 weeks.

#### Detection for tritiated DNA-adducts

A single intraperitoneal injection of 100  $\text{mg/kg}$  bw of tritiated 2,4-TDA (2,4- $^3\text{H}$ -TDA) to male rats (strain: Wistar) did not lead to interaction with DNA of liver cells but to binding to liver protein and ribosomal RNA in liver cells (Aune et al.; 1979). The detection limit for tritiated DNA-adducts is far less than that of the postlabelling method.

#### Hemoglobin adducts

Using the gas chromatography/mass spectrometry Wilson et al. (1996) showed a 2,4-TDA induced hemoglobin adduct formation in male rats (strain: Fischer-344) after single intraperitoneal injection. The hemoglobin adduct formation increased with dose and time: After administration of 150  $\text{mg/kg}$  bw a maximum adduct level was detected after exposure of 24 h (ca. 0.32  $\text{nmol/g}$  hemoglobin). To examine a relationship between administered doses and hemoglobin adduct levels, animals were exposed to doses in a range from 0.5 up to 250  $\text{mg/kg}$  bw for 24 h. The maximum adduct concentration of about 0.36  $\text{nmol/g}$  hemoglobin was determined at the highest tested dose of 250  $\text{mg/kg}$  bw. Data on toxicity were not given.

**Table 15. In vivo tests: DNA adducts in rats**

Test system	Doses	Expos. regimen	Sampl. times	Result	Positive tested organs	General toxicity	Reference
$^{32}\text{P}$ -post-labeling, Fischer-344 rats	10 - 180 ppm (1.2 - 22.1 $\text{mg/kg}$ day)	feeding daily for 6 weeks	week: 0.5, 1, 3, 6	positive	liver mammary gland	no data	Delclos et al., 1996
$^{32}\text{P}$ -post-labeling, Fischer-344 rats	0.5 - 250 $\text{mg/kg}$ bw	1 x i.p.	0.5 - 30 days	positive	liver	no data	Wilson et al., 1996

<sup>32</sup> P-post-labeling, Fischer-344 rats	125 - 250 mg/kg bw	1 x i.p.	18 h	positive	liver	no data	Taningher et al., 1995
<sup>32</sup> P-post-labeling, Fischer-344 rats	5.0 - 50 mg/kg bw	1 x i.p.	0.5 - 14 days	positive	liver	no data	La and Froines, 1994
	50 mg/kg bw	1 x i.p.	18 h	positive	liver mammary gland kidney lung	no data	
<sup>32</sup> P-post-labeling, Fischer-344 rats	4.1 - 2046 µmol/kg bw (0.5 - 250 mg/kg)	1 x i.p.	18 h	positive	liver mammary gland kidney lung	no data	La and Froines, 1992
<sup>32</sup> P-post-labeling, Fischer-344 rats	40 - 180 ppm (4.9 - 22.1 mg/kg/day)	feeding daily for 32 weeks	week: 1, 4, 8, 20, 32	negative (spleen T lymph.)		no data	Delclos et al., 1996
Tritiated DNA-adducts; Wistar rats	100 mg/kg bw	1 x i.p.	4 h	negative	liver	no data	Aune et al., 1979
Hb adducts, Fischer-344 rats	0.5 - 250 mg/kg bw	1 x i.p.	0.5 - 30 days	positive		no data	Wilson et al., 1996

### Transgenic mouse

2,4-TDA was positive in transgenic mouse assays.

Suter et al. (1996) described a weak genotoxic potential of 2,4-TDA on liver DNA of transgenic C57BL/6 Big Blue™ mice. Male and female mice were used to examine the induction of mutations in the lacI gene in liver. In consequence of determination of the maximal tolerated dose in non-transgenic C57BL/6 mice the test animals were given ten daily

oral doses of 80 mg/kg bw per day with a treatment free interval of 2 days between treatment five and six. Sampling times were 10 and 28 days after the last treatment. After an expression time of 28 days, 2,4-TDA treatments induced doublings of spontaneous mutation frequencies in both sexes (males,  $8.46 \times 10^{-5}$  as compared to  $4.32 \times 10^{-5}$  in the negative control; females,  $9.67 \times 10^{-5}$  as compared to  $4.32 \times 10^{-5}$ ). After 10 days expression a marginal response was obtained in females only ( $7.48 \times 10^{-5}$ ; negative control,  $5.15 \times 10^{-5}$ ). Ten days after treatment hepatic cell proliferation was induced.

In a study with Big Blue<sup>®</sup> transgenic B6C3F1 mice Hayward et al. (1995) reported on an increase of mutant frequency at lacI gene in liver. Male mice were exposed to 2,4-TDA at 1000 ppm (123 mg/kg bw) in the diet for 30 and 90 days. No effect was observed after a feeding of 30 days, while after 90 days the mutant frequency was higher (mean mutant frequency,  $12.1 \times 10^{-5}$  as compared to  $5.7 \times 10^{-5}$  in the control group). The tested dose corresponded to the highest non-toxic dose in a 90-day subchronic study.

**Table 16. In vivo tests: Transgenic mouse**

Test system	Doses	Expos. regimen	Sampl. times	Result	Tested organ	General toxicity	Reference
Big Blue <sup>™</sup> mouse	80 mg/kg bw/d	oral gavage (ten daily doses)	10 and 28 days after the last treatment	positive	liver	80 mg/kg = MTD	Suter et al., 1996
Big Blue <sup>®</sup> mouse	1000 ppm (123 mg/kg/d)	feeding for 30 and 90 days	on the day after feeding period	positive	liver	1000 ppm = highest non-toxic dose in a 90 day study	Hayward et al., 1995

**Table 17. OVERVIEW ON IN VIVO FINDINGS**

<u>Negative effects</u>	<u>Questionable effects</u>	<u>Positive effects</u>
<b><i>Mutation tests in vivo</i></b>		
Micronuclei in mice and rats (bone marrow; peripheral blood)	Micronuclei in rats at a highly toxic dose (bone marrow)	Gene mutations in transgenic mice (liver)
Dominant lethals		Drosophila

in mice		(SLRL test)
<b><u>Indicator tests in vivo</u></b>		
Sperm morphology in mice		SCE in mice (bone marrow)
		UDS in rats (liver)
		DNA strand-breaks in mice and rats (liver, kidney, lung, stomach)
		DNA adducts in rats (liver, mammary gland, kidney, lung)
		Reduction of testicular DNA- synthesis in mice

## Conclusion

2,4-Toluenediamine induces genotoxic effects in bacteria (gene mutations) and cultivated mammalian cells (chromosomal aberrations, SCE, UDS, DNA strand breaks, DNA adducts).

In general, rodent in vivo micronucleus tests were negative in bone marrow or peripheral blood; a weak positive effect in a rat strain was limited to a dose with high acute toxicity. In other tissues generally weak genotoxic effects were obtained, e.g. SCE in bone marrow cells, gene mutations in transgenic mice livers, UDS in rat liver, DNA strand breaks in liver, stomach, colon, lung, brain and kidney and DNA adducts in liver, mammary gland, kidney and lung were observed in rodent livers.

From a non-standard assay measuring a reduction of murine testicular DNA synthesis after 2,4-TDA application there is some indication for effects on the testes (Greene et al. 1981). Since hypothermia was found in a parallel experiment, reduced DNA synthesis is not a specific effect of DNA reactivity and the positive result was not supported by other in vivo tests on mutagenicity in germ cells (dominant lethal and sperm morphology tests) we do not regard these data as sufficient to classify 2,4-TDA as a category-2 mutagen. However, due to low sensitivity of dominant lethal and sperm morphology tests these test systems are not adequate for exclusion of germ cell mutagenesis. According to the revised TGD further germ cell mutagenicity testing is not required (conclusion i (on hold)). On the basis of the positive findings on somatic cells in vitro and in vivo we propose to classify the substance as a category 3 mutagen, R 68 (Possible risk of irreversible effects).

#### 4.1.2.8 Carcinogenicity

##### 2,4-TDA

##### Animal data:

The carcinogenic properties of 2,4-TDA have been investigated in a number of long-term animal studies using the oral route, and involving a variety strains of rats and mice. These studies provide clearly evidence that 2,4-TDA is carcinogenic in rats and mice. However, the majority of the long-term studies were not in (full) accordance to the test design of the current guidelines (B.30, 32, 33, OECD TG 451, 452, 453 testing carcinogenic or chronic toxic effects). Most of the studies have been focused on selected parameters, and no clinical chemistry and haematological parameters were measured. Largely testing procedures and documented results were comparable to testing efforts according to the current regulatory test protocols.

The carcinogenicity of 2,4-TDA following dermal application has been investigated in one study using Swiss-Webster mice. No firm conclusion could be drawn from this mouse skin-painting test due to limitations in study design including the small group size and use of a single dose level.

The carcinogenicity of 2,4-TDA by the inhalation route has not been studied in animals.

- *Oral*

##### Gavage study

##### Stop-test/30-day treatment (rat)

22 female Sprague-Dawley rats, 45 days of age, were given 10 intragastric doses of 100 mg/kg bw 2,4-TDA (purity commercial grade) over a 30-day treatment period. The test was terminated after an additional 9-month observation period. No data on haematology and clinical chemistry parameters were available. Only mammary tissue was examined histologically.

At histological examination of the mammary gland, fibroadenoma, or fibrosarcoma were seen. None of 20 control rats dosed with acetone/corn oil developed mammary tumors, whereas 23% of the treated females had mammary tumors (Stula Aftosmis 1976).

- *Diet studies*

##### 36-week study (rat)

In order to study development of carcinoma in the liver of rats treated with 2,4-TDA, two groups, each of 12 male Wistar rats, were fed diets containing 600 or 1000 ppm (approx. 45

or 75 mg/kg bw/day) of 2,4-TDA (purity commercial grade) for 30-36 weeks. A third group (6 male rats) was kept as control on the basis diet. This long-term feeding study was not in full accordance to the test design of the current guidelines testing carcinogenic or chronic toxic effects (e.g. dose regime, number of animals/dose). This study is reliable with restrictions. The study has been focused on selected parameters. As a carcinogenicity study, no clinical chemistry and haematological parameters were measured. However, testing procedures and documented results were comparable to testing efforts according to the current regulatory test protocols. Main findings of the study showed good consistency on data reported from a well conducted carcinogenicity study in F344 rats (NCI, 1979, Cardy, 1979). Further information on nonneoplastic effects to 2,4-TDA is described in section 4.1.2.6.

Oral treatment with 2,4-TDA over a period of 36 weeks was associated with tumor development in the liver in male Wistar rats. Liver carcinomas exhibited invasion and showed metastases in male Wistar rats, given dose levels of 600 ppm (approx. 45 mg/kg bw/d) or more, but not in control rats. Hepatocellular carcinomas varied from well-differentiated to poorly differentiated to undifferentiated carcinomas. Microscopic examination of the liver revealed further at both doses oval cell infiltration in the periportal areas, fatty changes of the liver parenchymal cells, bile duct proliferation, cirrhosis, nodular hyperplasia, and cholangiofibrosis. At 1000 ppm (approx. 75 mg/kg bw/d), carcinomas with numerous areas of nodular hyperplasia and cirrhosis of the liver were present in 100% (9/9) of rats, and with multiple metastases in 67% (6/9) to the lymph nodes, omentum, lungs, and epididymidis. In rats given 600 ppm (approx. 45 mg/kg bw/d), carcinomas of the liver, with metastases to the lymph nodes and omentum in 64% (7/11) of rats were seen. Metastatic areas in lymph nodes, omentum, epididymidis, and lung showed the same histological pattern of hepatocellular carcinoma with large irregular nuclei and many mitotic figures. The incidence of cancer in rat liver was higher on treatment with 1000 ppm (approx. 75 mg/kg bw/d) than on treatment with 600 ppm (approx. 45 mg/bw/d). The number of areas on nodular hyperplasia were also more with 1000 ppm than 600 ppm 2,4-TDA. Animals treated with 2,4-TDA had no primary neoplasms in organs other than the liver.

In summary, 2,4-TDA produced hepatocellular carcinomas in male Wistar rats at dose levels of 600 ppm approx. 45 mg/kg bw/d) or more (Ito et al.1969).

#### 15-month study (rat)

In a study to evaluate the long-term toxicity of a chlorotoluene diamine mixture, 2,4-TDA was used as a positive control. 36 male and 36 female ChP-CD rats were fed a diet containing 1000 ppm (approx. 75 mg/kg bw) 2,4-TDA (purity commercial grade) for two weeks, 500 ppm (approx. 38 mg/kg bw) for 5.5 months, followed by 250 ppm (approx. 19 mg/kg bw) for a period of 9 months. Calculated average daily intake was 367 ppm (approx. 28 mg/kg bw). Control group consisted of 12 untreated male and 10 female rats. This long-term oral toxicity study was not in accordance to the test design of the current guidelines (e.g. dose regime, number of animals/dose; only selected parameter). Testing procedures and documented results were comparable to testing efforts according to the current regulatory test protocols.

The addition of 1000→500→250 ppm 2,4-TDA to the diet of rats for up to 15 months resulted in a statistically significant increase in liver and mammary tumor development in both sexes, together with a significant increase of lung tumors (mostly adenomas) in males. In

females, the liver tumors were mostly neoplastic nodules whereas in males, there were moderately to well differentiated hepatocellular carcinomas. The mammary tumors were fibroadenomas. Chronic oral administration of 2,4-TDA to rats revealed in both sexes an increase in mortality rate, a marked reduction in body weight gain, slight anemia and leucocytosis, an increase in serum of ALP, GPT, and bilirubin. These findings are consistent with those found histopathologically in the liver: focal necrosis of hepatocytes, cystic bile ducts, cholangitis, cholangiofibrosis, hematopoiesis and hemosiderin. Severe atrophy of the spleen was seen in both sexes. Further information on nonneoplastic effects to 2,4-TDA is described in section 4.1.2.6 (Stula and Aftosmis 1976).

#### Long-term study (rat and mouse)

A bioassay for 2,4-TDA for possible carcinogenicity was conducted by administering the test substance in feed to F344 rats and B6C3F1 mice. Groups of 50 F344 rats of each sex were administered 2,4-TDA (purity 99.9%) initially either 125 or 250 ppm (approx. 9 or 18 mg/kg bw/day) for 40 weeks. Because of excessive depression of amount of mean body weight gain, thereafter doses were reduced to 50 and 100 ppm (approx. 3.7 and 7.4 mg/kg bw/day). Calculated average intake was 5.9 and 13 mg/kg bw/day. Administration of 50 ppm was continued for 63 weeks and surviving animals were killed. Surviving rats in the 100 ppm group were killed at the end of 39 (male) and 44 (female) weeks, due to morbidity. Matched controls consisted of 20 untreated rats of each sex. This combined chronic toxicity/carcinogenicity feeding study was not in (full) accordance to the test design of the current guidelines B.33, OECD TG 453 (only two dose levels were used; no haematological examination: especially no differential blood count was performed on the affected animals although observations suggest a deterioration in health of the treated animals during the study, no urinalysis, and no clinical biochemistry). Overall, testing procedures and documented results were comparable to testing efforts according to the current regulatory test protocols.

#### Rat

Mortality was dose related in the male and female rats. At both dose levels, male and female rats showed a decrease in survival time compared to controls. Mean body weights of dosed male and female rats were lower than those of the corresponding controls and were dose related. Nonneoplastic findings of this carcinogenesis bioassay in F344 rats revealed that 2,4-TDA was hepatotoxic and accelerated the development of chronic renal disease, an effect that contributed to a marked decrease in the survival of dosed animals. Corresponding to the renal disease was a high incidence of associated secondary hyperparathyroidism in low- and high-dose males. Further information on nonneoplastic effects of 2,4-TDA is described in section 4.1.2.6.

Dietary intake of 2,4-TDA for 79 weeks to F344 rats induced benign and malign hepatic neoplasms and high incidence of liver lesions in both sexes. These lesions consisted mainly of foci and larger areas of cellular alteration. In many of these lesions there was nuclear atypia, and in some there were areas that resembled transition from cellular alteration to neoplasia. In nearly all instances in which hepatic neoplasia was encountered, there were also foci of cellular alteration. The severity of these lesions and degree of cellular and nuclear atypia were greatest in females (42/49, 86%) given the high doses. Hepatocellular carcinomas or neoplastic nodules occurred at incidences that were dose related in both the males (P=0.014)



and the females ( $P=0.008$ ). In direct comparisons of incidences of the tumors in control and dosed groups, the incidence in the high-dose male group had a  $P$  value of 0.026, males: controls 0/20 (0%), low-dose 5/49 (10%), high-dose 10/50 (20%); females: controls 0/20 (0%), low-dose 0/50 (0%), high-dose 6/49 (12%). The incidence of liver neoplasia (benign plus malign) was significantly increased in male dose groups. In females, only the test for trend indicated significance. There was an increased incidence of proliferative lesions generally believed to be associated with the hepatocarcinogenesis that occurred to the test substance. The significant enhanced incidence of hepatocellular neoplasms in both sexes was supported by the high incidences of associated nonneoplastic lesions of the liver in the dosed groups.

In addition, 2,4-TDA caused statistically significant increase in the incidence of mammary tumors in females. In the female rats, carcinomas or fibroadenomas of the mammary gland occurred at incidences that were dose related ( $P=0.002$ ) and in direct comparisons were higher in the dosed groups ( $P=0.001$ ) than in the control group: controls 1/20 (5%), low-dose 38/50 (75%), high-dose 42/50 (84%). None of the mammary tumors metastasized. In the male rats, fibromas of the subcutaneous tissue occurred at incidences that were dose related ( $P=0.004$ ) and in indirect comparisons were higher in the dosed groups ( $P < 0.020$ ) than in the control group: controls 0/20 (0%), low-dose 15/30, high-dose 19/50. An increased incidence of lung tumors, carcinomas and adenomas (although statistically not significant) were seen in both sexes at the same time. The incidences of total lung tumors shown in treated animals were much higher than those seen in untreated controls (males: controls 0/20, low dose 5/50, high dose 5/50; females: controls 1/20, low dose 4/50, high dose 3/50).

Overall, 2,4-TDA was clearly carcinogenic for F344 rats, inducing proliferative hepatic findings and hepatocellular carcinomas in both sexes. It induced a high incidence of benign and malignant tumors of the mammary gland in females. In summary of the statistical findings, the incidences of fibromas of the subcutaneous tissue in male rats are associated with 2,4-TDA administration (NCI 1979; Cardy 1979).

### Mouse

Groups of 50 male and 50 female B6C3F1 mice were fed 100 or 200 ppm (approx. 15 or 30 mg/kg bw/day) 2,4-TDA (purity 99.9%) for 101 weeks. Matched controls consisted of 20 untreated mice of each sex. Mean body weights of dosed male and female mice were lower than those of the corresponding controls and were dose related except for the low-dose male mice, for which mean body weights were only slightly lower than those of controls. In female B6C3F1 mice, there was a dose-related delay in body weight gain of 23-50%. Mortality was not dose related in either the male and female mice. Survival rates were comparable in dose groups and control groups. Further information on nonneoplastic effects of 2,4-TDA is described in section 4.1.2.6.

Hepatocellular carcinomas occurred in the female B6C3F1 mouse at incidences that were dose related ( $P=0.002$ ) and in direct comparisons were higher in the dosed groups ( $P < 0.007$ ) than in the control group: controls 0/19 (0%); low-dose 13/47 (28%), high-dose 18/46 (39%). In males, hepatocellular carcinomas were found in the controls 5/20 (25%), low-dose 17/50 (34%), and high-dose group 13/49 (27%). Carcinomas varied from well-differentiated to poorly differentiated. Hyperplasia and nodular hyperplasia occurred in dosed males and females but not in corresponding controls. The number of mice with neoplasms of the liver are given in the table 4.1.2.8 A.

Table 4.1.2.8 A: Number of mice with neoplastic lesions of the liver

Sex	Dose	Hyperplasia	Hyperplastic nodules	Carcinomas	Hepatic neoplasms
Males	0	0/20	0/20	5/20	5/20 (25%)
	low dose	4/50	5/50	17/50	22/50 (42%)
	high dose	26/48	3/48	14/48	17/48 (33%)
Females	0	0/19	0/19	0/19	0/19 (0%)
	low dose	18/46	8/46	13/46	21/46 (48%)
	high dose	5/44	14/44	18/44	32/44 (64%)

Lymphomas occurred at a significant incidence ( $P < 0.001$ ) in the low-dose female mice, controls 2/19 (10%), low-dose 29/47 (62%), high-dose 11/46 (24%). No significant incidences occurred in male mice. Neoplasms of the hematopoietic system in the mice (not including hemangiomas and hemangiosarcomas of the lymph node) were found in the males: controls 2/20 (4%), low-dose 15/50 (30%), high-dose 8/49 (16%), and females: controls 2/19 (10%), low-dose 29/47 (62%), high-dose 11/46 (24%). Neoplasms of the lung and vascular system were slightly increased in dosed male mice. Carcinomas of the lung were well-differentiated papillary adenocarcinomas or poorly differentiated carcinomas. In the male mice, 0/20 (0%) controls, 9/50 (18%) low-dose, 6/49 (12%) high-dose animals had carcinomas of the lung.

Overall, 2,4-TDA was clearly carcinogenic for female mice, inducing in the liver hyperplasia, hyperplastic nodules, and hepatocellular carcinomas. Neoplasms of the lung and the vascular system were slightly increased in male mice and neoplasms of the hematopoietic system were slightly increased in male and female mice (NCI 1979; Reuber 1979).

Sontag (1981) summarized the results of thirteen substituted-benzenediamines (SBD) carcinogenicity studies, one of them was performed with 2,4-TDA. Results of the NCI studies (NCI 1979) were used in a comparison with the results of various assays for mutagenicity, chromosome damage and of cell transformation. All the SBD studied for genotoxic effects were positive in at least one assay system. For 2,4-TDA positive results of gene mutation tests (bacterial genotoxicity tests), sex-linked recessive lethal tests with *Drosophila* (SLRL-test) and in transformation of hamster embryo cells were presented. In addition for comparison tumor incidence data on SBD tested for carcinogenicity in rats and mice and control incidence data for the F344 rat and B6C3F<sub>1</sub> mouse were described. The control data were accumulated from results of NCI bioassay studies conducted over several years. The rat data are based on 1795 males and 1754 females and the mouse data on 2543 males and 2522 females. 2,4-TDA induced statistically significant incidences of tumors in both rats and mice. An overview on detailed tumor incidence data for 2,4-TDA (NCI study) and historical control incidence data in rats and mice is given in the following tables 4.1.2.8 B and 4.1.2.8 C:

**Table 4.1.2.8 B: Number of rats with neoplasms**

<b>Sex</b>	<b>Control</b>	<b>High</b>	<b>Low</b>	<b>Historical control</b>
<b>Urinary bladder</b>				
Male	-	-	-	2 (0.1%)
Female	0/20 (0.0%)	1/43 (2.3%)	0/43 (0.0%)	4 (0.2%)
<b>Kidney</b>				
Male	0/20 (0.0%)	0/50 (0.0%)	1/50 (2.0%)	4 (0.2%)
Female	-	-	-	3 (0.2%)
<b>Liver</b>				
Male	0/20 (0.0%)	10/50 (20%)	5/49 (10.2%)	31 (1.7%)
Female	0/20 (0.0%)	6/49 (12.2%)	0/50 (0.0%)	55 (3.1%)
<b>Mammary gland</b>				
Male	-	-	-	24 (1.3%)
Female	1/20 (5.0%)	42/50 (84.0%)	38/50 (76.0%)	300 (17.1%)
<b>Thyroid gland</b>				
Male	1/20 (5.0%)	1/47 (2.1%)	8/44 (2.1%)	129 (7.2%)
Female	0/20 (0.0%)	1/48 (2.1%)	2/49 (4.1%)	117 (6.7%)
<b>Skin and associated glands</b>				
Male	0/20 (0.0%)	0/50 (0.0%)	2/50 (4.0%)	29 (1.6 %)
Female	-	-	-	16 (0.9%)

**Table 4.1.2.8 C: Number of mice with neoplasms**

Sex	Control	High	Low	Historical control
<b>Liver</b>				
Male	5/20 (25.0%)	13/49 (26.5%)	17/50 (34.0%)	55 (21.7%)
Female	0/19 (0.0%)	18/46 (39.1%)	13/47 (27.7%)	98 (3.9%)
<b>Thyroid gland</b>				
Male	-	-	-	23 (0.9%)
Female	0/17 (0.0%)	0/44 (0.0%)	3/44 (6.8%)	36 (1.4%)

For 2,4-TDA there was a significant incidence of liver tumors in both dose groups for male rats (22% and 10.2% compared to 1.7% in historical controls) and only in the highest dose group for female rats (12.2% compared to 3.1% in controls). Mammary tumors were found in more than 80% of the female rats, compared to 17.1% in historical controls. A significant increased incidence of liver tumors was diagnosed in female mice as well (39.1% and 27.7% compared to 3.9% in historical controls).

- *Inhalation*

No data available.

- *Dermal*

2-year study (mouse)

To determine the toxicity and induction of epithelial tumors after repeated topical application of 2,4-TDA, a 2-year mouse-skin-painting study was conducted. 28 Swiss-Webster mice of both sexes were given once a week 0.05 ml of a 6% solution of 2,4-TDA (purity commercial grade, 3 mg/animal/week, approx. 75 mg/kg bw/week) or mixed with hair dye formulations containing the test substance in various concentrations in various groups to shaved back skin of the intrascapular region over 2 years. The treatment was discontinued after various times, depending on toxicity. In addition, there were an untreated control group and a positive control group receiving 9,10-dimethylbenzanthracene in acetone. No data on hematology and clinical chemistry parameters were available. Histopathology of selected organs and tissues was performed. This skin-painting model was not in accordance to the test design of the current guidelines with respect to the small group sizes and use of a single dose level.

The predominant neoplasms seen in 2,4-TDA treated mice were primary pulmonary adenomas and adenocarcinomas (males 17/21, control 6/21; females 6/9, control 3/9). Skin neoplasms were seen in most groups of mice, including untreated control mice at comparable

incidence rates. Dermal application of 2,4-TDA was nontoxic and noncarcinogenic to the skin of mice but was associated to higher tumor rates in the lungs (Giles and Chung 1976).

- *Subcutaneous application*

2-year study (rat)

In a subcutaneous injection test, 30 Sprague-Dawley rats per dose and sex were administered by subcutaneous injections at doses of 0, 8.33, or 25 mg/kg bw 2,4-TDA (purity commercial grade) once a week until appearance of the first test substance-related tumor, with a maximum of a 2-year treatment. In addition to the control group receiving the vehicle (peanut oil), there was another, similar, untreated group, and a positive control group receiving benzidine. After cessation of the treatment rats were kept until their spontaneous death, or until they were in a moribund state and were killed. Further information on nonneoplastic effects to 2,4-TDA is described in section 4.1.2.6.

The survival rates were comparable in dose groups and control groups excepted for the 25 mg/kg bw dose male rats, for which survival time was lower than those of controls. Mean body weights of dosed male and female rats were lower than those of the corresponding controls and were dose related. Microscopic examination of a number of organs and tissues revealed no treatment-related lesions with the exception of the liver. In the liver of all treated rats, focal necrosis of hepatocytes and in some animals cirrhosis were found. Hepatocellular neoplasia was found in 1/120 rats. Malignant tumors at the injection site were found in 16% of all treated rats. The test for trend indicated significance in males only (Steinhoff and Dycka1981).

**Other studies on carcinogenic effects in experimental animals**

Further studies were performed in two transgenic model namely the hemizygous Tg:AC mice and the p<sup>53def</sup> mice. In the first study 2,4-TDA (300 mg/kg bw) was applied for 24 weeks (vehicle ethanol/water 1:1) on shawed skin to hemizygous Tg:AC mice. In the second part of the study, 2,4-TDA was administered within feed at a concentration of 200 ppm to the p<sup>53def</sup> mice (Eastin et al, 1998). Fifteen animals/sex were treated 5x/week and another group of 15 animals/sex served as controls. The animals were observed twice daily, and body weights were recorded at weekly intervals. A complete necropsy was performed on all treated and control animals, but no organ weights were taken. Tissues from about 15 organ sites were examined by light-microscopy.

Survival was not different in 2,4-TDA and control groups of both studies. Terminal body weight was not different in animals receiving 2,4-TDA to the control Tg.AC mice. In p<sup>53def</sup> mice the body weights were lower (-25% males, - 12% females) as compared to controls. There were no statistically significant increases in the number of tumors in the Tg.AC or p<sup>53def</sup> mice. While no skin tumor was observed in controls of the Tg.AC mouse study, there were squamous cell papillomas in one male and in two female mice, and squamous cell carcinomas were found in two males of the 2,4-TDA group of Tg.AC mice. One male and two females of p<sup>53def</sup> mice receiving feed containing 2,4-TDA had malignant lymphomas, no tumor response was seen in the control p<sup>53def</sup> mice.

In both studies, the tumor response was lower as compared to the positive control substance TPA (12-O-tetradecanoylphorbol-13-acetate) in the same vehicle which in the NIEHS validation study induced 100% incidence of skin papillomas by the end of week 26 of treatment (Tennant et al., 2001). The slightly increased incidence of skin tumors in 2,4-TDA treated Tg.AC mice did not reach significance. The tumor response in the p<sup>53def</sup> mice could not be demonstrated in this study.

In conclusion, the study does not contribute to better understanding of the mechanism of action of 2,4-TDA. It is not clear whether the study itself was a so-called negative study because no internal validation by concurrent positive control has been performed or whether the models are not suited to demonstrate the known carcinogenic potential of 2,4-TDA.

2,4-TDA was examined in a study on the utility of a short term carcinogenesis test system in the Eker rat containing an insertion mutation in one allele of the tuberous sclerosis-2 (Tsc-2) tumor suppresser gene, which predisposes to the development of spontaneous renal cell carcinoma (Morton et al., 2002). The expected rate of spontaneous renal tumors is 100 % in Eker rats at one year of age. This animal model has some phenotypic characteristics of the human tuberous sclerosis where mutations on the TSC2 or TSC1 gene on chromosomes 16 and 9, respectively, are related to neurological disorders and various proliferative lesions in several organs (brain, heart, skin and kidneys) (review: Lendvay and Marshall, 2003). A group of 15 male Eker rats received orally 6.5 mg/kg bw/d 2,4-TDA on 7 days/week by gavage for a treatment period of 4 months beginning at an age of 10 weeks. In a control group 10 male Eker rats received the vehicle (0.01 M HCL) only. Complete necropsies were performed on all animals and a full set of tissues was collected and examined by light-microscopy. No signs of nephrotoxic effects were seen in 2,4-TDA treated rats, the mean number of renal hyperplastic tubules was 1.5 times higher in rats receiving 2,4-TDA. The mean numbers of renal adenomas were slightly higher in 2,4-TDA treated rats, two animals beard 3 renal cell carcinomas whereas no carcinoma was found in the control Eker rats at this time point. No other (nonrenal) tumors were observed. No effect was seen in the liver, a target organ of 2,4-TDA carcinogenic action, in this model.

Chemical-induced mutations in both alleles of the TSC2 genes were found in some renal cell carcinomas of Eker rats after nitrosamine treatment (Satake et al., 1999) that may give an explanation for increases in tumor rates and shortening of the latency period. Since no data on the mode of 2,4-TDA induced acceleration of tumor response were available, the interpretation of the study of Morton and co-workers remains uncertain. The kidney was not identified as a target for 2,4-TDA carcinogenicity in rat strains without predisposing gene abnormalities. However, it can not be excluded that humans with loss of function of the TSC tumor suppresser gene might have an increased susceptibility to develop renal carcinomas when exposed to 2,4-TDA.

To elucidate the source of the discordant results of 2,4-TDA and 2,6-TDA in the NTP bioassays studies were designed to determine the effects of these two chemicals on the cell proliferation in the liver (Cunningham et al., 1990, 1991). Male F344 rats received 9 daily gavage exposures of 12.5 or 25 mg/kg bw/d 2,4-TDA (purity >99%) and 25 or 50 mg/kg bw/d 2,6-TDA (purity >99%) in 0.01M HCl. Doses are equivalent to the starting bioassay doses of 125 and 250 ppm (2,4-TDA) or 250 and 500 ppm (2,6-TDA). Control groups received either 0.01M HCl as vehicle control or 0.4 ml carbon tetrachloride (CCl<sub>4</sub>) in corn oil (ip) as positive control. Osmotic minipumps containing BrdU (30 mg/ml) were implanted subcutaneously into the backs of all rats on the day following initiation of dosing. After 9 days rats were

killed and midlobe radial sections of the right anterior lobe of the liver were fixed, embedded in paraffin, and immunostained for BrdU incorporation into newly replicated cells. Random areas of the slides were chosen for counting labeled and unlabeled hepatocyte nuclei (>1000 hepatocytes/slide total), and the labelling index (LI) was calculated as the number of labelled hepatocytes/total number of hepatocytes (labelled plus unlabelled). Following 9 consecutive days of dosing liver weight/body weight ratio was increased at 25 mg/kg bw/d 2,4-TDA. Rats treated with 50 mg/kg bw/d 2,6-TDA showed a slight but significant decrease in the liver weight/body weight ratio. Vehicle and CCl<sub>4</sub> treated animals had average Labelling index in the liver of 1.1% and 50%, respectively. 2,4-TDA treatment induced a dose-dependent increase in cell proliferation in the liver. Animals receiving 2,4-TDA had an average LI of 12.5% at 12.5 mg/kg bw/d, and 20.4% at 25 mg/kg bw/d, whereas 2,6-TDA treatment induced no increase in cell turnover compared to vehicle control. Animals receiving 2,6-TDA had an average LI of 0.7% at both doses. These results indicate a positive correlation between increased cell proliferation and hepatocarcinogenesis induced by 2,4-TDA .

In further studies with Big Blue<sup>®</sup> transgenic B6C3F1 mice an increase of mutant frequency at *lacI* gene in the liver was reported after exposing to 2,4-TDA in feed for a period of 90 days, whereas the administration of 2,6-TDA did not increase the mutant frequency (Cunningham and Matthews 1995).

Taningher et al. (1995) investigated 2,4- and 2,6-TDA for the ability to induce preneoplastic foci in male F344 rat livers detected by GGT-staining in diethylnitrosamine (DENa)-initiated hepatocytes. For the preneoplastic foci test, groups of six rats were initiated at the same time by i.p. injection of 200 mg/kg bw DENa. After two weeks on basal diet, treated groups were given oral doses by gavage, 5 days/week of 25 mg/kg bw 2,4-TDA (purity 98%) or 50 mg/kg bw 2,6-TDA (purity 97%) in 0.01M HCl for a period of 6 weeks. Rats treated as positive controls were exposed to 500 ppm Phenobarbital sodium salt (PB) in their drinking water. Partial hepatectomy (PH) was performed at week 3 in rats of all groups, and the experiments were terminated at week 8. Liver sections obtained after sacrifice were fixed in 2-methyl butane which was cooled in liquid nitrogen and stored at -80 °C. In liver slices, foci positive for  $\gamma$ -glutamyltranspeptidase (GGT) of  $\geq 0.2$  mm mean diameter were scored, after staining. Four sections, one each from the right posterior and caudate lobes and two from the right anterior lobe, were used for scoring. Only 2,4-TDA was able to significantly enhance the growth of DENa-initiated hepatocytes, and induced significant increases in preneoplastic foci.

The potential of diaminitoluenes to induce the enzymes, primarily cytochrome P450 proteins, that catalyze their activation, and their ability to bind to the cytosolic aromatic hydrocarbon (Ah) receptor were reported by Cheung et al. (1996). The results indicate, that 2,4-TDA is a relatively weak inducer of CYP1A, displaces [<sup>3</sup>H] TCDD from the hepatic cytosolic Ah receptor, and could autoinduce its activation. In this study male Wistar rats were given a single intraperitoneal injection of different diaminitoluenes at dose levels of 10, 20, or 40 mg/kg bw, whereas controls received the corresponding volume of corn oil, the vehicle. All animals were killed 24 hr after administration. For the selective induction of distinct cytochrome P450 proteins, the animals received single daily intraperitoneal injections of benzo[a]pyrene (25 mg/kg bw), phenobarbitone (80 mg/kg bw), or clofibrate (80 mg/kg bw)

or by gavage isoniazid (100 mg/kg bw) or dexamethasone (100 mg/kg bw). Treatment was carried out for three days, all animals were killed 24 hr after the last administration.

There were further studies of limited validity on the carcinogenic effects of 2,4-TDA. In the following some early reports on experiments by various application routes in rodents to 2,4-TDA are summarized which were at least partly consistent to the results of other cancer studies. The occurrence of liver tumors in dosed male or female rats or female mice and of subcutaneous fibromas in dosed male rats, and further of carcinomas or adenomas of the mammary gland in the female rat of the carcinogenicity tests presented above is in agreement with the results of the following earlier studies. To complete the database these studies were reported here:

In order to estimate effects of repeated oral administration or subcutaneous injection of 2,4-TDA, long-term studies in rats and mice were performed by Pylev et al. (1979). No data on hematology and clinical chemistry parameters were available. Histopathology from 13 tissues and organs including mammary gland, adrenal gland, pituitary gland, intestine, bladder and the lung was reported. 35 male and 35 female albino interbred rats were administered orally by gavage with a starting level of 4 mg 2,4-TDA in 0.5 ml physiological saline per animal for 5 days/week which was gradually reduced to doses of 2.1, and 0.5 mg per rat for 3 days per week for up to 16 months. There were 39 male and 30 female control rats. Leukemia occurred in 5.5% of the animals and mammary tumors in 29.4% of the females.

58 female CC57 mice were gavaged with 2 mg 2,4-TDA in 0.2 ml physiological saline per animal for 5 days/week, and thereafter with 1 mg for 3 days/week for up to 16 months. There were 104 control mice (no data of sex). Treated mice showed fewer neoplasms than the untreated controls. It was assumed that early deaths of the treated animals may have reduced incidences of tumor development.

25 male and 25 female albino interbred rats were injected subcutaneously with 8 mg 2,4-TDA in 0.5 ml physiological saline/rat once a week for 16 months. During the experiment dose level was reduced. Rats received doses of 4, and thereafter 1 mg per rat. There were 39 male and 30 female untreated control rats. Subcutaneous injection of 2,4-TDA at dose regime below for 16 months produced leukemia in 12% of the treated animals, and mammary tumors were seen in 45.5% of the female rats.

26 female CC57 mice were injected subcutaneously with 2 mg 2,4-TDA in 0.2 ml physiological saline per mouse once a week for 16 months. There were 104 untreated control mice (no data of sex). The subcutaneous injection in mice for 16 months induced tumor formation at the injection site in 56% of the animals and slightly increased occurrence of leukemia.

Sarcoma was produced at the application site by subcutaneous injection of a 0.4% solution of 2,4-TDA in 1,2-propylene glycol (CAS No. 57-55-6) as vehicle into mixed strain of Saitama rats (20 rats/sex). The injections were made in doses of 0.5 ml once a week for 246 days on the back of rats. No contemporary controls were used. 11 rats died within 8 months, showing no specific treatment-related effects. 9 rats survived the treatment period of 246 days, receiving 28 injections during the study. No data on hematology and clinical chemistry parameters were available. Histopathology of selected organs and tissues was performed. At the injection site, developing sarcomas were detected in all survived rats. Subcutaneous



connective tissue became thickened and small hard consolidations of various size, and became palpable during the 8<sup>th</sup> to 12<sup>th</sup> months. These consolidations gradually turned into irregular nodules, which rapidly grew into fatal large tumors thereafter. Some of these tumors were rhabdomyosarcomas at least in part. In single tumors, additional fibrosarcomatous areas were seen. At necropsy, no metastasis was found, and no significant lesions were noted in internal organs. Histological examination of the liver showed hyperemia, and an increase of Kupffer cells, and in the later stages, hepatocellular vacuolation. However, hepatocellular neoplasia was not described in this study (Umeda 1955).

Limited information to liver toxicity following 2,4-TDA treatment was received from an early invalid study in rats (no data of number, sex and strain, no data of controls, and furthermore, no data on hematology and clinical chemistry parameters). Histopathology only from the liver was reported. Rats given 2.5% 2,4-TDA in olive oil incorporated in their diet for up 80 days or longer did not induce carcinoma or nodular hyperplasia of the liver, but they showed diffuse biliary cirrhosis of the liver. These histomorphological finding in the liver following daily oral intake of 2.5% 2,4-TDA with the food for more than 80 days was interpreted by the author as cancer-like atypical growth on the interlobular bile ducts (Nagata 1937, 1944).

Yoshida et al. (1941) inserted 2,4-TDA in collodium sack into the urinary bladder of rats with the intention to induce bladder tumors in parallel with the development of liver cancer. The substance was wrapped as crystals with collodium, and inserted into the bladder by operation, and then the bladder was closed. No cancer-like development was encountered after at least 125 days.

Little information was obtained from a study in original language Italian (Russo et al. 1981). Three aromatic amines, one of them was 2,4-TDA, were examined for their capacity of damaging rat liver DNA. Compounds were tested by the „in vivo“/DNA alkaline elution assay. 11 male Wistar rats were fed a diet containing 0, or 3.54 mg/kg bw 2,4-TDA for 9 months. It was reported that 2,4-TDA was „very positive“. Furthermore, it was directed to toxic dose level in the study.

#### Isomeric mixture 2,4/2,6-TDA (80/20)

##### *Oral*

No data available.

##### *Inhalation*

No data available.

##### *Dermal*

No data available.

### *Subcutaneous application*

#### 2-year study (rat)

In a subcutaneous injection test, doses of 0, 8.33, or 25 mg/kg bw of the mixture of the isomers 2,4/2,6-TDA (80/20) (purity commercial grade) were injected subcutaneously to 30 Sprague-Dawley rats per dose and sex once a week until appearance of the first test substance-related tumor, with a maximum of a 2-year treatment. In addition to the control group receiving peanut oil as vehicle, there was another, similar, untreated group, and a positive control group receiving benzidine. After cessation of the treatment the rats were kept until their spontaneous death, or until they were in a moribund state and were killed. No data on hematology and clinical chemistry parameters were available.

The survival rates of the 8.33 mg/kg bw dose groups were comparable to the control groups. At 25 mg/kg bw, male and female rats showed a decrease in survival time compared to controls. Mean body weights of all dosed male and female rats were lower than those of the corresponding controls and were dose related. Further information on nonneoplastic effects to the mixture of the isomers 2,4-TDA/2,6-TDA (80/20) is described in section 4.1.2.6. Malignant tumors (not specified) were seen predominantly at the injection site in 53% of all treated rats, being statistically significant in males only (Steinhoff and Dycka 1981).

Summary of animal toxicity data after long-term, long-life exposure to 2,4-TDA or to the commercial mixture of TDA, 2,4-/2,6-TDA (80/20)/considerations on the mode of action

There is sufficient evidence for the carcinogenicity of 2,4-TDA in experimental animals.

Oral intake to rats at doses of 5.9 mg/kg bw/day 2,4-TDA and more lead to a dose dependent increase of hepatocellular carcinomas or neoplastic nodules in both sexes. In addition, 2,4-TDA induced adenomas and carcinomas of the mammary gland, in females, further, fibromas of the subcutaneous tissue and an increased incidence of lung tumors (although statistically not significant) in males (Cardy 1979, NCI 1979, Sontag 1981, Ito et al. 1969, Stula and Aftosmis 1976, Pylev et al. 1979). In the mice hepatocellular carcinomas occurred significant in females after oral application of doses of 15 and 30 mg/kg bw/day for 101 weeks. In addition, oral long-life administration of 2,4-TDA may have increased the incidence of lymphomas in female mice. Neoplasms of the lung seen as adenomas and/or adenocarcinomas, and of the hematopoietic system were slightly increased in male mice. But no tumors occurred at significantly increased incidences in male mice (NCI 1979, Reuber 1979).

The carcinogenicity of 2,4-TDA by the inhalation route has not been studied in experimental animals.

The carcinogenicity of 2,4-TDA following dermal application has been investigated in one study using Swiss-Webster mice. Skin neoplasms were seen in most groups of mice, including untreated control mice. 2,4-TDA did not produce any abnormal proliferation or changes of the maturation of the squamous epithelium of the skin, but limitations in study

design do not allow firm conclusions to be drawn. Limitations of this study include the small group size and use of a single dose level.

No carcinogenicity studies to commercial mixture of TDA, 2,4-/2,6-TDA (80/20) were available using the inhalation, dermal, or oral route of exposure.

Repeated subcutaneous injection in rats with 2,4-TDA or the mixture of the isomers 2,4/2,6-TDA (80/20) for up to 2 years induced sarcomas at the application site in both males and females. In contrast to the oral dose studies there was no increased tumor incidence in the liver and mammary gland (Steinhoff and Dycka 1981).

### Liver tumors

2,4-TDA is a liver toxin and was carcinogenic for F344 rats in a 2-year NCI rodent carcinogenicity study by using the oral route of exposure. It induced benign and malignant hepatic neoplasms and also high incidence of liver lesions associated with hepatic neoplasia in both sexes. In the rat, hepatocellular carcinomas or neoplastic nodules occurred at incidences that were dose related in both the males and the females. The significant enhanced incidence of hepatocellular neoplasms in both sexes is proving the carcinogenic potential of 2,4-TDA because only a low liver tumor rate was supported in historical-control animals of the same strain. There was an increased incidence of proliferative lesions generally believed to be associated with the hepatocarcinogenesis. These lesions consisted mainly of foci of cellular alteration. In many of these lesions there was nuclear atypia, and in some there were areas that resembled transition from cellular alteration to neoplasia. Studies on hepatocellular proliferation showed that oral administration of 2,4-TDA in doses comparable to the bioassay starting doses for 9 days results in labelling indices up to >20% in the liver of F344 rats (Cunningham et al., 1990, 1991), and in studies with Big Blue<sup>®</sup> transgenic B6C3F1 mice an increase of mutant frequency at *lacI* gene in the livers was reported after exposing to 2,4-TDA in feed for a period of 90 days (Cunningham and Matthews 1995). The appearance of significant increases of altered foci in the livers of male F344 rats was observed after treatment with oral doses of 25 mg/kg bw 2,4-TDA, 5 days/week, for a period of 6 weeks (Taningher et al. 1995). In B6C3F1 mice, hepatocellular carcinoma occurred in the females at incidences that were dose related and their frequency was higher in dosed groups than compared to the control group. Mice with carcinomas often developed simultaneously diffuse hyperplasia in the liver, and hyperplastic nodules (NCI 1979). In mid-term toxicity studies, liver carcinomas exhibited invasion and showed metastases in male Wistar rats, given dose levels of 600 ppm (approx. 45 mg/kg bw/d) or more. No primary neoplasms were observed in organs other than the liver in the treated animals, and the livers of the controls were essentially normal (Ito 1969). The higher incidences and earlier onset of tumor formation are signs of the carcinogenic potential due to the doses employed. The addition of 1000→500→250 ppm (approx. 28 mg/bw/d) 2,4-TDA to the diet of rats for up to 15 months resulted in a statistically significant increase in liver tumors in both sexes. In females, the liver tumors were mostly neoplastic nodules whereas in males, there were moderately to well differentiated hepatocellular carcinomas (Stula and Aftosmis 1976). There are numerous early carcinogenicity studies on 2,4-TDA using the oral route which showed that 2,4-TDA produces liver cirrhosis and sometimes liver adenomas in rats and mice, but indisputable malignant tumors were not encountered.

### Mammary tumors

2,4-TDA induced a high, statistically significant incidence of benign and malignant mammary gland tumors in females F344 rats. None of the mammary tumors metastasized (NCI 1979). 23% of the treated female Sprague-Dawley rats (10 intragastric doses of 100 mg/kg bw 2,4-TDA over a 30-day treatment period, followed by an additional 9-month observation period) developed fibroadenoma, or fibrosarcoma of the mammary gland. In a further study the addition of 1000→500→250 ppm (approx. 28 mg/kg bw/d) 2,4-TDA to the diet of rats for up to 15 months resulted in a statistically significant increase in mammary tumor development in both sexes. Histologically the tumors were also fibroadenomas (Stula and Aftosmis 1976). A study of limited validity reports an incidence of 29.4% mammary gland tumor rate in female rats (strain not specified) (Pylev et al. 1979).

### Lung tumors

A statistically not significant incidence of lung tumors was seen in F344 rats (both sexes) administered approximately ±5.9 mg/kg bw/d for 103 weeks by oral route. Neoplasms of the lung were also slightly increased in male B6C3F1 mice. Carcinomas of the lung were well-differentiated papillary adenocarcinomas or poorly differentiated carcinomas (NCI 1979). The addition of 1000→500→250 ppm (approx. 28 mg/kg bw/d) 2,4-TDA to the diet of rats for up to 15 months resulted in a significant increase of lung tumors (mostly adenomas) in males. These findings are in accordance with the results of a previous mouse study. In this 2-year mouse-skin-painting study, primary pulmonary adenomas and adenocarcinomas were found in treated Swiss-Webster mice (Giles and Chung 1976).

### Mechanism of tumor development

The mechanism of liver tumor development remains unclear. The available genotoxicity data give a substantial concern that 2,4-TDA is a genotoxic carcinogen. On the other hand, the experiments on 2,4-TDA suggest a link between hepatocellular necrosis and the development of hepatocellular tumors, especially in male rats. In the cancer study in rats (NCI 1979, Cardy 1979) at the lowest dose of 5.9 mg/kg bw/d (time-weighted average dose after reduced in food from 125 to 50 ppm) with both sexes there were decreased survival rates and a clearly delay of body weight gain (approx. 25%). At the same dose necrosis and neoplasia in the liver in both sexes were observed. Hepatotoxic effects were shown as fatty degeneration, cholangiofibrosis, and liver cirrhosis (mid-term study, Ito 1969) as well as degeneration of the liver (long-term study, NCI 1979, Cardy 1979). In this study nearly all instances in which hepatic neoplasia was encountered, there were also foci of cellular alteration. The severity of these lesions and degree of cellular and nuclear atypia were greatest in females (42/49, 86%) given the high dose (13 mg/kg bw/d). In mice, treated animals also developed hyperplastic hepatic nodules, but precursor hepatic lesions were not observed. In conclusion, tumor induction after cell injury in the liver may be interpreted as indication of a nongenotoxic mechanism of action. On the other hand there are positive genotoxic data in vitro and in vivo. Therefore, it is unlikely to assume that there is a link between hepatocellular necrosis and the development of hepatocellular tumors for 2,4-TDA because a correlation of toxicity and development of tumors in the liver was seen in rats only for males but not in mice of both sexes.

Besides, 2,4-TDA induced the development of tumors in other organs independent of cytotoxicity. There were a high, statistically significant incidence of benign and malignant mammary gland tumors in female rats, and a frequently appearance of lung tumors (statistically not significant) in rats and mice .

In summary, based on the results of experimental studies in rats and mice and the results of genotoxicity studies to 2,4-TDA it is assumed that a genotoxic mechanism is involved for the tumor development of each target organ. The experiments on 2,4-TDA give also suggestion to other possible mechanisms than genotoxicity. The effect of cytotoxicity and the development of tumors does not appear to have been investigated in depth. At present there are no data which give reasons to assume that these mechanisms are species-specific so that they may also apply for humans.

#### Human data:

data not available

#### Cell transformation test

Results for cell transformations tests only done without S-9 mix are contradictory.

Le Boeuf et al. (1996) observed an increase in the transformation frequency using Syrian hamster embryo cells (SHE cells) by culturing at pH 6.7 (instead pH 7.1 to 7.3 which is conventionally used). With a 7-day exposure, 2,4-TDA was tested at doses ranging from 0.5 up to 65 µg/ml. All concentrations from 20 µg/ml upward caused a statistically significantly increase in morphological transformation frequency (MTF): highest MTF 3.21% as compared to 0.39% in the negative control. Toxic effects were observed at the highest tested concentrations.

In conventionally conducted SHE cell transformation tests (pH value 7.1 - 7.3), negative results were shown by Holen et al. (1990) in the dose range 5.0 - 30 µg/ml; doses higher than 10 µg/ml induced toxic effects) and by Green and Friedman (1979) at doses ranging from 62.5 up to 1000 µg/ml; no data on toxic effects were given.

2,4-TDA was positive in a cell transformation test with a non-standard stable tranfected mouse embryo fibroblast cell line (Kowalski et al., 2000). 2,4-TDA was tested in the dose range 0.1 – 100 µg/ml. According to the authors a statistically significant number of foci was induced from 10 µg/ml upwards. A dose of 100 µg/ml was indicated as toxic dose.

Table 4.1.2.8 D: In vitro tests: cell transformation

Test system	Concentration range		Result	Toxicity	Remarks	Reference
	with S-9 mix	without S-9 mix				
SHE cells	not done	0.5 - 65 µg/ml	positive	at the highest concentrations	pH 6.7	Le Boeuf et al., 1996
SHE cells	not done	5.0 - 30 µg/ml	negative	> 10 µg/ml	pH 7.1-7.3	Holen at al., 1990
SHE cells	not done	62.5 - 1000	negative	no data	pH 7.1-7.3	Green and

		µg/ml				Friedman, 1979
Stable transfected mouse embryo fibroblast cell line	not done	0.1-100 µg/ml	positive	at 100 µg/ml (decrease in number of foci)		Kowalski et al., 2000

**Conclusion:**

**Classification:**

2,4-TDA is already classified and labelled as: Carcinogen Category 2, T, Toxic, R 45, May cause cancer. This classification will be confirmed by:

- 2,4-TDA is carcinogenic in rats and mice of both sexes.
- Carcinogenic potential of 2,4-TDA was demonstrated for administration by oral route. There are no data available for inhalation and no relevant information for dermal route of exposure.
- Valid data from guideline-compliant life-time studies (NCI, 1979) and supportive data from other long-term studies (Ito et al. 1969, Stula and Aftosmis 1976) indicated that oral treatment with 2,4-TDA was associated with tumor development in the liver and lung (in rat and mouse), and in the mammary gland (rat), the subcutis (rat), hematopoietic and vascular system (mouse). 2,4-TDA induced hepatocellular carcinomas or neoplastic nodules (synonym to hepatomas) in rats of both sexes and in female mice. In addition, three rat studies in different strains revealed increases in tumor incidences in the mammary gland. Dose-dependent increases in subcutaneous fibromas were seen in male rats. Also, lung tumor incidences of carcinomas and adenomas were higher in treated male and female rats and in male mice compared to the control values. Increases in lung tumor rates were also observed in non-conventional studies in male ChP-CD rats (Stula and Aftosmis, 1976). In mice, higher incidences of hemangiomas and haemangiosarcomas (in males only) and lymphomas (in females only) than the control incidences were seen in both treatment dose groups. Except for the murine liver tumors, other tumor rates in the mouse did not increase dose-dependently.
- 2,4-TDA has been shown to be mutagenic in tests with bacteria (gene mutations) and cultivated mammalian cells (chromosomal aberrations, SCE, UDS, DNA strand breaks, DNA adducts). So, 2,4-TDA is considered as a genotoxic carcinogen.
- Furthermore, other mechanisms of tumor induction may be involved. Increased cell proliferation following liver cell necrosis might also be active in hepatocarcinogenesis by 2,4-TDA. However, the lack of toxic precursor lesions in mice livers questioned the significance of cytotoxicity induced mitogenic effects. No indications on other possible modes of action than genotoxicity were identified for the tumors at other tumor target sites than the liver.

- There are no human data on the carcinogenic effects of 2,4-TDA. No mechanistic arguments are known to indicate that these findings would be restricted to animals.

According to the EC criteria of Directive 93/21/EEC for classification and labelling guide 2,4-TDA is classified as a carcinogen, category 2 and labelled as T, Toxic, R 45 (May cause cancer).

Isomeric mixture 2,4-/2,6-TDA (80/20)

No carcinogenicity studies on the commercial mixture of TDA, 2,4-/2,6-TDA, was available using the inhalation, dermal, or oral route of exposure.

The commercial grade TDA, which was an isomeric mixture of the 2,4- and 2,6-TDA, is suspected to have carcinogenic properties due to the main compound 2,4-TDA. Therefore, the current classification of 2,4-TDA is also proposed to the commercial mixture 2,4-/2,6-TDA .

#### **4.1.2.9 Toxicity for reproduction**

##### 2,4-TDA

##### Animal data:

##### Fertility impairment

Guideline-according generation studies are presently not available. However, 2,4-toluene diamine had been investigated for effects on male fertility in a series of several feeding studies in Sprague- Dawley rats (Thysen et al. 1984; Thysen 1985 a, b; Varma et al. 1988). Further details on these studies are provided in chapter 4.1.2.6.

In a first preliminary study, an unspecified number of animals were fed a laboratory chow containing 0.1% 2,4-TDA for nine weeks, equivalent to a daily intake of approximately 50 mg/kg bw (Thysen et al. 1984, only abstract available). Several mating trials followed and resulted in reproductive failure. All of the treated males revealed to be sterile since the vaginal smears of all females were devoid of sperm and no pregnancies resulted. It was reported that at this level 2,4-TDA caused body and testicular weight loss. Pathologic studies in the treated males showed arrested spermatogenesis, the seminal vesicles contained little fluid, the ventral prostate was filled with a rubbery coagulum, and the epididymal fat pad showed extensive regression. Increased plasma LH levels were noted while plasma testosterone levels were normal.

In a second experiment diets admixed with 0, 0.03 and 0.01% 2,4-TDA (98% purity) were fed ad libitum over a period of 10 weeks to groups of 8 to 10 males according to a daily intake of approximately 15 and 5 mg 2,4-TDA/kg bw (Thysen et al. 1985a). After 10 weeks of treatment mating trials followed over a period of 2 weeks with a total of 4 untreated virgin females mated with each male. Body weights were measured weekly. The females were killed 2 weeks after confirmed insemination and examined for the presence and number of implantation sites, viable implants and resorptions. The males were sacrificed 2 weeks after

mating, i.e., 4 weeks after return to regular feed. The testes and epididymides were taken for histopathological investigation. The rats that were fed 0.03% 2,4-TDA showed a retardation in weight gain during weeks 4 -10 probably due to some reduced food consumption. Five out of the ten males treated with 0.03% 2,4-TDA were infertile (evidence of implantations on day 14 after mating / number of sperm positive females for males considered as infertile was 0/2, 0/4, 0/3, 0/3 and 0/4), while the remaining animals in this group exhibited generally unimpaired fertility. All animals in the control and low dose groups were fertile. The mating index (provided as ratio of sperm-positive females/mated females) was statistically significantly ( $p < 0.01$ ) reduced to 0.73 in the group fed 0.03% 2,4-TDA in comparison to 0.95 and 1.00 in the 0.01% dose group and in the controls. The fertility index (provided as ratio of pregnant females/sperm-positive females) was statistically significantly ( $p < 0.05$ ) reduced to 0.41 in the group fed 0.03% 2,4-TDA in comparison to 0.74 and 0.72 in the 0.01% dose group and in the controls. The pregnancies derived from treated males were not significantly different from those produced from control males in terms of viable implants. Also the mean number of resorptions were similar in all of the groups. Light-microscopic examinations of the testes of rats treated with 0.03% 2,4-TDA revealed focal or diffuse hypospermatocytogenesis in the seminiferous tubules and in cauda epididymides.

These latter results were further supported by an additional quantitative study in which the effects of dietary exposure to 0, 0.01 and 0.03% 2,4-TDA on epididymal sperm counts, reproductive organ weights, and on circulating levels of gonatropins and of testosterone were evaluated after a period of 10 weeks of exposure as well as after an additional period of 11 weeks on normal chow (Thysen et al. 1985b). Also in this trial after 10 weeks of exposure the rats that received 0.03% 2,4-TDA had reduced weight gain (36% less than the controls) presumably due to some reduced food consumption. Since food deprivation in male rats for longer periods has not been found to affect spermatogenesis it is rather unlikely that 2,4-TDA treatment associated sperm count reductions can be explained as an effect secondary to reduced increase in body weight gain. For the 0.03% 2,4-TDA treatment group mean organ weights were statistically significantly ( $p < 0.05$ ) lower for epididymidis ( $525 + 129$  mg versus  $650 + 57$  mg in controls) and seminal vesicles ( $197 + 19$  mg versus  $238 + 22$  mg in controls) whereas testes and prostate weights of the treated animals were similar to those of controls. Diminished epididymal sperm reserves (total sperm count/cauda epididymidis) were found in animals even at the 0.01% 2,4-TDA dose level ( $267 \pm 70 \times 10^6$  versus  $323 \pm 52 \times 10^6$  in controls), and there was a substantial and statistically significant ( $p < 0.05$ ) decrease in sperm count to  $110 \pm 87 \times 10^6$  total sperm/cauda epididymidis in the high dose group with oligospermia as the major effect during the treatment period. After the recovery period of 11 weeks the sperm count in the 0.03% 2,4-TDA treatment group remained statistically significantly ( $p < 0.001$ ) depressed ( $139 \pm 144 \times 10^6$ /cauda epididymidis versus  $342 \pm 58 \times 10^6$ /cauda epididymidis in the controls). The sperm concentrations correlated well with testis and epididymidis weights, which were both significantly reduced. No significant changes were found for mean seminal vesicle and ventral prostate weights. There was a statistically significant ( $p < 0.05$ ) decrease in serum testosterone ( $2.24 \pm 1.56$  ng/L versus  $4.48 \pm 0.95$  ng/L in the controls) in the 0.03% 2,4-TDA treatment group after 10 weeks on diet and it remained statistically non-significantly low ( $1.66 \pm 0.51$  ng/L versus  $2.33 \pm 0.81$  ng/L in the controls) even after a recovery period of 11 weeks. The reduced androgen levels were in accord with statistically non-significantly elevated LH concentrations. There was no significant treatment related effect on FSH levels.

The studies of Thysen et al. were further extended to the determination of effects of 2,4-TDA on androgen-binding protein (rABP) production, on ultrastructural changes in seminiferous tubules, and on early changes in testicular morphology and spermatogenesis in a series of



additional studies (Varma et al. 1988). In the first experiment groups of 9 rats were given 0 or 0.03% 2,4-TDA via diet for a period of 10 weeks and serum, medium of seminiferous tubule cultures, and testicular and epididymal cytosol were analyzed for rABP. Serum rABP and testicular cytosol concentrations from treated rats were 3.8- respectively 8.9-fold higher than from control rats. Media from cultures of TDA-exposed seminiferous tubules contained 7 times as much rABP as those of control tubules. Epididymal cytosol from treated animals however showed a 67% decrease in rABP content. Testes examination by transmission electron microscopy revealed variable degenerative changes in Sertoli cells as compared with controls. Germ cells, where present, demonstrated normal maturational changes. In the second experiment groups of 5 rats were given 0 or 0.03% 2,4-TDA via diet and sacrificed at 2 week intervals up to 10 weeks on diet. After 4, 6 or 8 weeks there was a doubling of testes/body weight ratios. The testes weight gain correlated closely with the highly significant 2- to 3-fold increase in seminiferous tubule fluid volume. After 10 weeks of exposure testicular weight was reduced to that of controls, but seminiferous tubule fluid volume was still elevated. An approximately 50% decrease in epididymal sperm reserves was found when TDA feeding was extended to 6, 8, or 10 weeks. The observed changes in testicular characteristics were interpreted as an indication for 2,4-TDA effects on Sertoli cell function, on rABP release from the testes and epididymides, and possibly on tubular fluid transport. In the third experiment groups of 9 rats were given 0 or 0.06% 2,4-TDA via diet for 1 or 3 weeks. After 1 week in the treated animals there was a 25% decrease in body growth and epididymal sperm content, reduced epididymal weight, and minor structural changes in Sertoli cells. After 3 weeks of exposure the sperm counts were further reduced, and were accompanied by a doubling of testes weight, and ultrastructural changes in Sertoli cells. There were no significant changes in serum testosterone levels. The results of this study demonstrated 2,4-TDA toxicity on testicular spermatogenesis to occur within 3 weeks of treatment suggesting that the early inhibition of spermatogenesis by 2,4-TDA is mediated through Sertoli cell damage.

Additional information can be taken from short-term investigations with an *in vivo* model proposed as a "rapid -test" to identify carcinogenic activity (Topham 1980), where 2,4-TDA failed to induce sperm-head abnormalities in a special inbred strain of mice.

Furthermore in a dominant lethal test with male DBA/2J mice treated orally or with *i.p.* injections with doses of 2 x 40 mg 2,4-TDA/kg bw on two consecutive days there was no change in percent of fertile matings, the number of implantations or mean percentage of postimplantation death up to 7 weeks post treatment. Also there was no indication of an increase in morphologically abnormal sperm at 8 weeks after treatment (Soares and Lock 1980). After single *i.p.* injections of high doses of 111 to 375 mg 2,4-TDA to C57Bl/6xC3H mice an inhibition of testicular DNA synthesis of 53 to 86% was observed within a period of 6.5 hr after administration (Greene et al. 1981). However, because of the acute exposure to high doses and the different parameters which were investigated a comparison with the data generated in rats is not possible.

#### Developmental toxicity:

Guideline-according developmental toxicity studies are presently not available. However, 2,4-toluene diamine had been investigated in a screening assay covering a total of 60 chemicals at one dose level only, where a group of 50 CD-1 albino mice was treated orally by gavage with

150 mg 2,4-TDA/kg bw/d in corn oil in a volume of 10 ml/kg bw on gestational days 6 to 13 (Hardin et al. 1987 a, b). This dose was equivalent to the LD<sub>10</sub> predicted from a preceding dose-finding study in non-pregnant animals and revealed signs of clear-cut maternal toxicity as 17 out of the 50 dams died and a significantly reduced mean body weight change was noted. In the surviving dams treatment with 2,4-TDA significantly reduced the number of live litters (5 out of 20) as well as the number of live born per litter. In the remaining offspring birth weight, weight gain, and viability through the first three postpartum days were not significantly different from that of the control group.

Other information for completeness of the database:

In a compilation of 517 chemicals, that had been screened for estrogenic activity in vitro using a yeast two-hybrid assay, also 2,4-TDA is listed (Nishihara et al., 2000). Whereas 64 out of the 517 tested compounds were evaluated as positive, for 2,4-TDA no estrogenic activity was revealed.

#### Human data:

There are several studies available from the United States upon male employees from several plants manufacturing diaminotoluenes concerning the rates of spontaneous abortion, stillbirths, and congenital malformations in their offspring and including the investigation of semen specimens (Ahrenholz 1980; Anonymous 1980; Ahrenholz and Meyer 1982; Hamill et al. 1982; Levine et al. 1985, reviewed by WHO 1987). Occupational exposure in these studies usually involved exposure to both diaminotoluenes (without indication of the isomers) and dinitrotoluene without discrimination. During the course of these studies spermatocyte-damage effects for the exposed groups and an increased rate of spontaneous abortions among the wives of exposed workers were indicated in some of the studies, while from other studies no significant differences in semen analysis and in miscarriage rates were reported. The overall evaluation of these occupational exposure studies is not possible because of the mixed exposure and the fact that testes damaging effects are also being discussed for dinitrotoluene. Furthermore, the results of those studies indicating any reproductive impairment are of limited value for several reasons, e.g., the cohorts were of limited size only and there were some risks of selection bias, because the populations were restricted to those who volunteered. Therefore, the presently available human data are not assessed to be suitable for reproductive hazard identification of 2,4-TDA. Any human data on females are not available.

#### **Conclusion:**

Animal studies revealed that the repeated oral intake of 2,4-TDA affects male fertility and spermatogenesis in rats dose-dependently. Dietary dose-levels of approximately 15 mg/kg bw/d over a 10 week period significantly reduced mating and fertility indices and produced obvious toxic effects on spermatogenesis (66% reduction) associated with a significant reduction in the weights of seminal vesicles and epididymides, morphological damage of Sertoli cells as well as with a diminished level of serum testosterone and an elevation of serum LH. Sperm count and testosterone levels remained low even after a recovery period of 11 weeks. Since first effects on epididymal sperm reserves were shown at a dietary level of

approximately 5 mg/kg bw/d this dose is assumed to represent the marginal LOAEL. A NOAEL for the effects on sperm parameters could not be shown.

Based on the evaluation of the available animal data 2,4-TDA should be classified as Reprotox. Cat. 3, R 62 (Possible risk of impaired fertility).

Experimental data related to female fertility are not available.

Hazard identification with respect to reproduction cannot be completed at present because the available data from a single screening investigation in mice are not valid for the assessment of any developmental impairment. Since the presently available results from epidemiological studies are inconclusive and some of the studies were of restricted validity, hazard identification with respect to reproduction is primarily based on the experience from animal studies.

#### Isomeric mixture 2,4/2,6-TDA (80/20)

##### Animal data

Fertility impairment:

There are no data available.

Developmental toxicity:

There are no data available.

##### Other information for completeness of the database:

Investigations on 2,6-TDA revealed that in the testes of mice there was a 40 to 56% inhibition of DNA synthesis after a single i.p. injection of 30 to 100 mg 2,6-TDA/kg bw (Greene et al. 1981). Examination of the body temperature of the animals led to the hypothesis that this inhibition might have been caused by chemically induced body temperature decrease. There were no further data on toxicity on testes reported.

##### **Conclusion:**

Hazard identification with respect to toxicity for reproduction can not be performed since there are no investigations available with 2,4-/2,6-TDA (80/20). Also the presently available results from epidemiological studies are inconclusive and some of the studies were of restricted validity.

Animal studies with rats on the predominant isomer however revealed that the repeated oral intake of 2,4-TDA affects male fertility and affects spermatogenesis already at a dose level of approximately 5 mg/kg bw/d. Since 2,4-TDA is the major constituent of 2,4-/2,6-TDA

(80/20), also this mixture of isomers should be classified as Reprotox. Cat. 3, R 62 (Possible risk of impaired fertility).

### 4.1.3 Risk characterisation

#### 4.1.3.1 General aspects

Toluene-2,4-diamine (2,4-TDA) is almost completely absorbed via the gastrointestinal tract in animals and well absorbed via the skin (in man 24% over an exposure time of 24 h). No data are available on inhalation. In rats the highest tissue concentrations were measured in liver and kidney after oral or i.p. administration. Concentrations in heart, lungs, spleen, and testes were significantly lower. The maximum concentration in blood was determined in rats after 1 to 8 h following i.p. injection. There are no species-related differences in tissue distribution between mice and rats.

In rats, rabbits, and guinea pigs unchanged 2,4-TDA was excreted via urine in concentrations from 0.1 to 3%. 2,4-TDA is mainly hydroxylated at the ring under formation of aminophenols (major pathway) and additionally N-acetylation occurs. Mono- as well diacetyl derivatives were observed in different quantities in the urine of rats, mice, rabbits, and guinea pigs. In dogs, however, only very small amounts of the monoacetyl derivative were detected. Elimination of sulfate conjugates was shown in the 24-hour urine in rats and mice, whereas glucuronic acid conjugates occurred at a higher level in mice than in rats. The excretion of the 2,4-TDA metabolites predominantly occurs via urine in rats and mice. A study in rats with oral administration or i.v. injection of 3 mg/kg bw resulted in an urinary elimination half-life of 4.6 h. An oral dose of 60 mg/kg bw showed an elimination half-life of 8 hours.

In animal tests, 2,4-TDA has proven to be toxic (tests with rats and mice), resulting in oral LD50 values between 73 and 350 mg/kg bw. In a study with dermal application, a dermal LD50 value of 1200 mg/kg bw was detected. No human nor animal data are available on acute inhalation toxicity of pure 2,4-TDA. However, taking into account the fact that a mixture of 80% 2,4-TDA and 20% 2,6-TDA has a similar acute toxicity profile as pure 2,4-TDA, results of tests with that 80/20 mixture are considered sufficient to assess the acute inhalation toxicity of the pure 2,4-TDA: The inhalation toxicity of that mixture is considered to be of no concern as judged on the basis of tests with rats, mice and rabbits. The 80/20 mixture of 2,4-/2,6-TDA was toxic to harmful in tests with rats, mice, rabbits and cats, based on oral LD50 values between 50 and 500 mg/kg bw. With dermal application a LD50 value of 463 mg/kg bw was determined for rats. No mortality occurred after a 4 hour inhalation to concentrations of appr. 5.57 mg/l, but all animals appeared in a bad health state. Thus, a concentration of 1.8 mg/l will be used for risk characterisation.

Enhanced methaemoglobin formation was detected after a single oral dose application of the 80/20 mixture of 2,4-/2,6-TDA to cats ranging from 70% increase after a 50 mg dose and a 5.4% increase after a 0.5 mg dose.

In Draize tests with rabbits, 2,4-TDA did not cause skin irritation and demonstrated only slight conjunctival redness after instillation to the eye. However, the 80/20 mixture of 2,4-/2,6-TDA has demonstrated severe corrosive properties when instilled into the eyes of rabbits.

In a Magnusson Kligman test with 2,4-TDA up to 100% of the guinea pigs demonstrated a positive reaction. Human data demonstrate a possible cross sensitivity to p-phenylenediamine.

Studies in experimental animals have shown that main toxic effect associated with dietary exposure of 2,4-TDA is hepatotoxicity. In short-term studies effects were characterized by a decrease in body weight and an increase in the liver: body weight ratios. In long-term studies toxic effects on the liver accelerated the development of chronic renal disease in rats, an effect that contributed to a marked decrease in survival. In a 2-year feeding study in rats, the lowest dose of 5.9 mg/kg bw/day showed toxic effects in the liver and kidneys and increased tumor incidences in the liver (male rats, female rats, female mice), and in the mammary gland (female rats) (LOAEL). Severe testicular atrophy was a finding at 28 mg/kg bw/day in rats in a 15 months study. Inhibited spermatogenesis (66%) associated with a significant reduction in the weights of seminal vesicles and epididymides, morphological damage of Sertoli cells as well as with a diminished level of serum testosterone and an elevation of serum LH was observed at dose level of 15 mg/kg bw/day in rats in a 10-week study. The dose of 5 mg/kg bw/day is considered as marginal LOAEL for effects on reproductive organs as it causes a decrease in epididymal sperm reserves.

In subacute studies of limited test design with inhalation exposure to the isomeric mixture 2,4-/2,6-TDA (80/20) gave 0.0095 mg/l (approx. 1 mg/kg bw/day) as a NOAEL for systemic effects in the rat. Whereas, in cats as a very sensitive species for methemoglobinemia this dose already caused a slight methemoglobin formation of 2.07%.

In vitro 2,4-TDA induces genotoxic effects in bacteria (gene mutations) and cultivated mammalian cells (chromosomal aberrations, SCE, UDS, DNA strand breaks, DNA adducts). In general, rodent in vivo micronucleus tests were negative in bone marrow or peripheral blood; a weak positive effect in one rat strain was limited to a dose with high acute toxicity. In other tissues generally weak genotoxic effects were obtained, e.g. SCE in bone marrow cells, gene mutations in transgenic mice livers, UDS in rat liver, DNA strand breaks in liver, stomach, colon, lung, brain and kidney and DNA adducts in liver, mammary gland, kidney and lung were observed in rodent livers.

From a non-standard assay measuring a reduction of murine testicular DNA synthesis after 2,4-TDA application there is some indication for effects on the testes (Greene et al. 1981). Since hypothermia was found in a parallel experiment, reduced DNA synthesis is not a specific effect of DNA reactivity and the positive result was not supported by other in vivo tests on mutagenicity in germ cells (dominant lethal and sperm morphology tests) we do not regard these data as sufficient to classify 2,4-TDA as a category-2 mutagen. However, due to low sensitivity of dominant lethal and sperm morphology tests these test systems are not adequate for exclusion of germ cell mutagenesis. On the basis of the positive findings on somatic cells in vitro and in vivo we rather propose to classify the substance as a category-3 mutagen, R 68 (Possible risk of irreversible effects).

2,4-TDA is carcinogenic in standard animal studies. In rats, 2,4-TDA produced liver tumors in both genders and mammary tumors in females after oral administration, and local sarcomas after subcutaneous injection. 2,4-TDA was also carcinogenic for female mice, inducing hepatocellular carcinomas. The incidence of lymphomas in the female mice suggested that these tumors also may have been related to administration of 2,4-TDA.

It was concluded that the mixture of the isomers 2,4-/2,6-TDA (80/20) has to be considered as potential carcinogen due to content of the constituent 2,4-TDA (80%).

Results from human epidemiological studies concerning reproductive health are inconclusive and some of the studies are of restricted validity only. With respect to fertility impairment the available data from studies in male rats revealed 2,4-TDA to affect male fertility (in terms of reduced fertility and impaired spermatogenesis) in a dose related manner (LOAEL for effects on spermatogenesis of 5 mg/kg bw/d). Hazard characterisation for reproductive toxicity cannot be completed due to the lack of valid data for the assessment of the endpoint developmental toxicity.

According to the hazard characterisation of 2,4-TDA it was concluded that also the mixture of isomers 2,4-/2,6-TDA (80/20) has to be considered to be a reproductive toxicant.

### 4.1.3.2 Workers

#### 4.1.3.2.1 Introductory remarks

2,4-TDA is a colourless solid with a vapour pressure of 0.017 Pa at room temperature, which is soluble in water and organic solvents. The substance is predominantly used as an intermediate in the chemical industry. The major part (98–99%) is processed as a 2,4/2,6-TDA (80/20) mixture to toluylene diisocyanate (TDI). A minor amount is used as a starting product for the production of acridine, sulfur and azo dyes.

The occupational exposure scenarios have been described and discussed in detail in section 4.1.1.2. Exposure to 2,4-TDA may occur during production and further processing of 2,4-TDA as a chemical intermediate and in the production and use of 2,4-TDA pastilles. The exposure routes to be considered in connection with the workplace are inhalation and dermal contact especially during cleaning, repair and decoupling of transfer lines or handling of the TDA pastilles. For workers the inhalation exposure levels reported in table 4.1.1.2.2.A are taken forward to risk characterisation. Dermal exposure is assessed with the EASE model. The used values for the dermal risk assessment represent the worst case situation (see table 4.1.1.2.2.B).

The toxicological data on 2,4-TDA are described and discussed in section 4.1.2. If studies are performed with the 2,4/2,6-TDA (80/20) mixture, the toxicological effects are generally ascribed to the component 2,4-TDA, otherwise it is mentioned separately. Quantitative human toxicity data are not available. Risk estimations are therefore based on animal data. The experimental threshold levels identified in the hazard assessment part of the report are taken forward to occupational risk assessment. Carcinogenicity is addressed as the most significant effect in the toxicological profile of 2,4-TDA.

#### **Systemic availability for different routes of exposure**

For the majority of toxicological endpoints 2,4-TDA data originate from oral studies. Since workers are exposed either by inhalation or by skin contact, route to route transformation is essential for worker risk assessment.

According to elimination studies in rats 2,4-TDA is assumed to be readily absorbed via the gastrointestinal tract. 60–65% of an orally administered dose is excreted within 48 hours via the urine. The excretion of the activity in the feces of rats after i.v. or i.p. injection was 20 – 30% after 2 to 5 days, thus supporting that the absorption of 2,4-TDA in rats after oral dosing is almost complete.. For skin penetration a value of 24% is reported from male human volunteers (see chap. 4.1.2.1) without further information. From comparison of oral and dermal LD50 values (a comparison that is only valid for a high dose range) dermal dosing seems to be clearly less effective than oral application, however the difference does not reach one order of magnitude (see chap. 4.1.2.2). No data are reported for absorption by inhalation.

For risk assessment purposes the following assumptions of systemic availability are taken forward for the calculation of MOS.

Systemic availability after oral intake: ca. 100% (experimental data)



Systemic availability after dermal contact: ca. 25 % (experimental data)  
Systemic availability after inhalation: ca. 100 % (default assumption)

### Occupational exposure and internal body burden

In table 4.1.3.2.A. the exposure levels of table 4.1.1.2.2 are summarised and the route specific and total internal body burden is identified.

Table 4.1.3.2.A: 2,4-TDA exposure levels which are relevant for occupational risk assessment and internal body burden

	Inhalation shift average (mg/m <sup>3</sup> )	Dermal contact shift average (mg/p/d) <sup>(2,3)</sup>	Internal body burden of workers after repeated exposure (mg/p/d)		
			Inhalation <sup>(1)</sup>	Dermal <sup>(2,3)</sup>	Combined
1) Production and further processing as a chemical intermediate	0.00075	0.15	0.0075	0.0375	0.045
2) Production 2,4-TDA pastilles (4 weeks/year)	0.025	0.42	0.25	0.105	0.355
3) Use of 2,4-TDA pastilles for the production of dyes (not daily)	0.025	0.42	0.25	0.105	0.355

<sup>(1)</sup> based on the assumption of 100% inhalative absorption; breathing volume of 10 m<sup>3</sup> per shift

<sup>(2)</sup> EASE use of suitable gloves

<sup>(3)</sup> 25% dermal absorption

Because of the assumption, that the systemic availability of 2,4-TDA after inhalation is 100%, the inhalative exposure and internal body burden by inhalation are equal. In the case of dermal risk assessment the systemic availability for dermal exposure is assumed to lie in the range of 25%. The internal body burden is therefore lower than exposure at the workplace by the factor 4.

### Default values for physiological parameters

Body weight, rat	250 g
Body weight worker	70 kg
Respiratory rate, rat at rest	0.8 l/min/kg
Respiratory rate, worker at rest	0.2 l/min/kg
Respiratory volume of worker during 8 hours at rest	6.7 m <sup>3</sup>
Respiratory volume of worker during 8 hours of light activity	10 m <sup>3</sup>

## **Calculation of MOS values**

MOS values are calculated as quotient of experimental NOAEL (or LOAEL) from animal or human studies and workplace exposure levels. If the route of application in animal or human studies is different from the actual occupational exposure, the dose units of the experimental and exposure data have to be adapted previously to MOS calculation. As result of this adaptation a “starting point” for the MOS calculation is identified.

The exposure routes considered in occupational risk assessment are inhalation and dermal contact. The MOS values for exposure by each route are considered separately. The combined MOS-value is calculated as quotient of the internal NAEL (i.e. the external NOAEL multiplied with the percentage of absorption) and the total 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. Against this background it is recognized that combined risks only rarely determine concern. However, for the sake of completeness, all combined MOS values are given in this report on 2,4-TDA.

## **Evaluation of MOS values**

Risk assessment based on MOS values implies the identification of a minimal MOS as decision mark between conclusion ii and iii. In order to get consistent conclusions for different chemicals, substance-specific adjustment factors, which may vary depending on data availability and the specific toxicological endpoint to be evaluated, are identified. Scientifically based adjustment factors are used for the extrapolation of animal data to the worker population (e.g. adaptation of scenarios, route-to-route extrapolation, interspecies extrapolation and duration adjustment). The uncertainties in the specific calculations are weighed by expert judgement and expressed as an additional “uncertainty factor”. The multiplicative combination of these different factors and the uncertainty factor yield the minimal MOS value.

If the MOS value for a certain exposure scenario is below the minimal MOS, the corresponding risk situation is considered to be of concern. A MOS value higher than the minimal MOS indicates no concern.

In a parallel procedure, which gives identical but more direct results, the toxicological starting point carried forward to risk characterisation may be divided by the endpoint-specific assessment factors. As a result, an exposure level is identified, which may serve as trigger for decisions when compared directly with the occupational exposure levels. In the context of this risk assessment report it will be called “critical exposure level”. Concern will be expressed for scenarios above this trigger value.

### ***Interspecies differences***

Species differences might exist concerning the susceptibility for 2,4-TDA toxicity. However no information on the relative sensitivity of humans is available. There is no mechanistic argument to suggest that findings are restricted to animals and should not be transferred to

humans. For the purpose of occupational risk assessment, scaling on the basis of metabolic rate is used as a default assumption for interspecies extrapolation.

For interspecies extrapolation of oral or dermal data metabolic rate scaling results in lower effective dose levels in mg per kg bodyweight for humans compared to experimental animals. The scaling factor depends on  $[\text{bodyweight}_{\text{human}}/\text{bodyweight}_{\text{animal}}]^{0.25}$ , e.g. for rats a factor of 4 will be used (for details of calculation see NO\_NL, 1999).

For inhalation the principle of metabolic rate scaling implies that a specific inhalation exposure level (in  $\text{mg}/\text{m}^3$ ) is toxicologically equivalent in experimental animals and humans. However, care has to be taken to rely the extrapolation between species on directly comparable conditions: under study conditions rats are thought to be at a state of reduced activity; the corresponding human breathing volume in 8 hours is  $6.7 \text{ m}^3$  ( $0.2 \text{ l}/\text{min}/\text{kg} \times 60 \text{ min}/\text{h} \times 70 \text{ kg}$ ). Workers are assumed to breathe  $10 \text{ m}^3$  during a normal working day under conditions of light to moderate activity. Thus for workers the amount of substance inhaled must be spread over a 1.5 times higher breathing volume. Maintaining toxicological equivalence means that, compared to the experimental levels, the corresponding occupational air concentrations will be 1.5 times lower.

### ***Duration adjustment***

Since studies with suitable experimental design are available for 2,4-TDA there is no need for a specific duration adjustment step in extrapolation. Where adaptation of daily or weekly doses is necessary, e.g. in the calculation of totally administered amounts of 2,4-TDA, a linear adjustment is used.

### ***Uncertainty considerations***

The default adjustment factors outlined above are based upon evaluation of literature data for different chemicals. From a statistical point of view the individual parameters have to be understood as point estimates belonging to probability density functions. Each factor is taken as geometric mean (point near maximum) from its density function. The multiplicative combination of all factors is therefore supposed to result in a central tendency point estimate, addressing a situation which is likely to occur. However, the actual risks may either be less or more pronounced than estimated.

To complete the assessment, the uncertainty included in the procedure outlined above should be addressed and, if necessary, be used to modify the minimal MOS in terms of precaution. On that purpose several aspects should be taken into account, which by their nature are not easy to quantify. Examples are the reliability of the data base, the variability in assessment factors, the different steps necessary to bridge data gaps, the biological relevance of the observed effects.

### ***Intraspecies variability***

There are no substance-specific data which might permit quantification of possible differences in sensitivity among workers. For the evaluation of MOS values a specific intraspecies extrapolation factor is not used. To a certain extent, the aspect of human variability will be covered by uncertainty considerations introduced to the risk evaluation.

## **MOE approach:**

The formal structure of the MOE approach (Margin Of Exposure) for non-threshold carcinogens is comparable to the MOS approach for threshold effects. In both risk assessment approaches MOS or MOE values are compared with minimal MOS or MOE values.

Calculation of the MOE values starts with the dose descriptor chosen (T25, BMDO5). The dose descriptor is divided by the exposure levels resulting in scenario-specific MOE values. These values have to be compared with a standard, which is called the minimal MOE. This minimal MOE contains the overall information that bridges the gap between the (animal) dose descriptor chosen and the „very low concern“ situation of specified exposure groups.

The higher the MOE in comparison to the minimal MOE, the lower (is) the anticipated carcinogenic risk. The minimal MOE is a decision criterion that aims at a risk level already considered to be of very low concern. If the actual scenario-specific MOE is significantly greater than the minimal MOE, it might be indicated to the risk managers that current risk reduction measures are already very effective and presumably have already resulted in very low risks.

On its own, the scenario-specific MOE value does not allow for reaching conclusions on concern. For that purpose the scenario-specific MOE values are compared with a standard called minimal MOE which reflects those health-related aspects which are expressed in quantitative terms (e.g. interspecies extrapolation, adjustment for exposure schedules and low-risk extrapolation). Formally, the minimal MOE is the product of the selected adjustment factors.

The adjustment factor for low risk extrapolation is specific for risk assessment of non-threshold carcinogens. Contrary to the risk assessment for threshold effects, for the non-threshold effects a dose without effect cannot be derived. Against that background, the low risk extrapolation factor and, consequently, the minimal MOE aims at a risk level considered „already to be of very low concern“. This factor expresses the target margin between the high risk related to the dose descriptor and a very low risk for the population exposed.

Of crucial importance is the decision on the magnitude of this low risk extrapolation factor. This factor additionally covers elements of risk evaluation, which clearly is of societal concern. Thus, for this low risk extrapolation factor, policy guidance is needed. There might be a common understanding that a reference risk for the low dose region might be in the pragmatic range of a cancer lifetime risk of  $10^{-4}$  down to  $10^{-6}$ . Different levels may be suggested as reference risk for different subpopulations. The following conversion of a risk level to the corresponding factor is given for illustrative purposes: Starting with the dose descriptor T25 (lifetime cancer risk of 25:100) and assuming a linear dose response down to a reference risk level of e.g.  $10^{-5}$  (1:100,000) results in a low risk extrapolation factor of 25,000. The low risk extrapolation factor, being a function of the policy-driven reference risk level, is anticipated to have the greatest impact on the minimal MOE.

The concept of the minimal MOE principally is open to modifications of the assumption of a linear dose-response relationship. If there is scientific evidence indicating deviations from linearity, e.g. supporting a sublinear dose response at low doses, then this information may be accounted for by correspondingly decreasing the low-risk extrapolation factor. In consequence, the minimal MOE will be decreased to the same extent. If a non-linear dose response is assumed, but available data are not adequate to quantitatively modify the low-risk

extrapolation factor, then the available information should be qualitatively accounted for following the comparison of MOEs and the minimal MOE.

Reaching conclusions on the degree of concern for non-threshold carcinogens is supported by comparison of MOEs with the minimal MOE. The higher the MOE and the lower the minimal MOE, the lower is the scenario-specific concern for cancer risk.

It is acknowledged that readers of the risk assessment reports have to get accustomed to the MOE terminology. Comparing the scenario-specific MOEs with the minimal MOE only indirectly points at a „critical“ exposure level. You easily get the latter health-based yardstick by rearrangement of the MOE terms: the critical exposure level is calculated by dividing the selected dose descriptor by the minimal MOE. A scenario-specific exposure level lower than the critical exposure level results in very low concern; while a scenario-specific exposure level greater than the critical exposure level is of substantial concern. It is acknowledged that there is no clear-cut line of decision between „substantial concern“ and „very low concern“. This background of uncertainty may argue for the MOE terminology. However, for specific risk communication purposes it might be advantageous to additionally use the terminology of the critical exposure level.

#### **4.1.3.2.2 Occupational risk assessment**

##### **Acute toxicity**

##### *Systemic effects (inhalation)*

No human data are available concerning the acute inhalation toxicity of 2,4-TDA. Animal data are rare, too. One of two rabbits died after exposure to a concentration of approximately 17.2 mg/l/4h of a vapour-dust-mixture of 2,4/2,6-isomers (80/20). For mice and rats a LC50 of > 5.57 mg/l/4h was calculated. No animals died at this concentration, but all appeared in a poor state of general health and exhibited laboured respiration. At a concentration of approximately 1.8 mg/l/4h no clinical signs were detected (see chap. 4.1.2.2). This experimental value is selected as NOAEC for the risk assessment of acute inhalation toxicity. The air concentration of 1,800 mg/m<sup>3</sup> is taken as starting point for MOS calculation. The following assessment factors are applied for the identification of the minimal MOS:

- the study duration was 4 hours compared to occupational exposure of 8 hours, resulting in an adaptation factor of 2
- physiological differences between humans at rest and workers account for a factor of 1.5
- following a precautionary approach, an uncertainty factor of 10 is proposed, because in acute studies compared to repeated dose studies less detailed information is obtained concerning the no effect level.

Altogether the minimal MOS results in 30 (2 x 1.5 x 10). The corresponding critical exposure level is 60 mg/m<sup>3</sup> (1,800 mg/m<sup>3</sup> / 30).

The highest inhalation shift average values result from scenario 2 and 3 with 0.025 mg/m<sup>3</sup>. The corresponding MOS value lies in the range of 72,000 (see table 4.1.3.2.B). Compared to

the minimal MOS this value indicates that risks due to acute inhalation toxicity are not expected under normal workplace conditions.

Conclusion: ii

### *Systemic effects (dermal)*

No human data are available concerning the acute dermal toxicity of 2,4-TDA. In a rat study with dermal application, a dermal LD50 value of 1,200 mg/kg bw is reported. No further information is available. From another study with a mixture of 2,4/2,6-isomers (80/20) a dermal LD50 of 463 mg/kg is reported for female rats, 50 mg/kg are tolerated without macroscopically visible organ changes. This value is used for dermal risk assessment, taking the fact into account that a mixture of 80% 2,4-TDA and 20% 2,6-TDA has a similar acute toxicity profile as pure 2,4-TDA. As starting point for MOS calculation the human dose corresponding to this dermal NOAEL is identified as 3,500 mg/person (50 mg/kg x 70 kg). The following assessment factors are applied for the identification of the minimal MOS:

- metabolic rate scaling from rats to humans reveals a factor of 4
- according to a precautionary approach an uncertainty factor of 10 is proposed because acute studies provide less detailed information about the no effect level than repeated dose studies.

By multiplication of these factors the minimal MOS results in 40 (4 x 10). The corresponding critical exposure level is calculated at 88 mg/person (3,500 mg/person / 40).

Compared to the minimal MOS the MOS values (see table 4.1.3.2.B) do not indicate any concern for dermal exposure with respect to acute toxicity of 2,4-TDA.

Conclusion ii

### *Combined exposure*

The above described dermal acute study with the mixture of 2,4/2,6-TDA-isomers (80/20) is chosen in order to determine the critical exposure level for combined exposure (inhalation and dermal contact of 2,4-TDA). The reported NOAEL of 50 mg/person corresponds to a human dose of 3500 mg/person (50 mg/kg x 70 kg). This value resembles the external dose. The corresponding internal dose corresponds to 875 mg/person, including the aspect of 25% dermal absorption. The following assessment factors are applied for the identification of the minimal MOS:

- metabolic rate scaling from rats to humans reveals a factor of 4
- according to a precautionary approach, an uncertainty factor of 10 is proposed because acute studies provide less detailed information about the no effect level than repeated dose studies.

By multiplication of these factors the minimal MOS results in 40 (4 x 10). The corresponding critical exposure level is 22 mg/person (875 mg/person / 40).

The highest combined internal body burden results from scenario 2 (production of 2,4-TDA pastilles) with a value of 0.355 mg/person (see table 4.1.3.2.A). The corresponding MOS value is calculated as 2,500 (see table 4.1.3.2.B). These values indicate no a reason for concern.

Conclusion ii

Table 4.1.3.2. B: MOS values for acute toxicity of TDA, systemic effects

	Inhalation			Dermal			Combined		
Starting point for MOS calculation	1800 mg/m <sup>3</sup>			3500 mg/p/d (external dose)			875 mg/p/d (internal dose)		
Minimal MOS	30			40			40		
Critical exposure level	60 mg/m <sup>3</sup>			88 mg/p/d (external dose)			22 mg/p/d (internal dose)		
	Exposure (mg/m <sup>3</sup> )	MOS	Conclusion	Exposure (mg/p)	MOS	Conclusion	Internal body burden (mg/p)	MOS	Conclusion
1) Production and further processing as a chemical intermediate	0.00075	2,400,000	ii	0.15 <sup>(1)</sup>	23,300	ii	0.045	19,500	ii
2) Production 2,4-TDA pastilles	0.025	72,000	ii	0.42 <sup>(1)</sup>	8,300	ii	0.355	2,500	ii

<sup>(1)</sup>EASE, use of suitable gloves

## Irritation/Corrosivity

### *Dermal, eye, and inhalative irritation of pure toluene-2,4-diamine*

Human data are not available. In Draize tests with rabbits the pure 2,4-TDA did not cause skin irritation and demonstrated only slight conjunctival redness after instillation to the eye. The duration (and observation) period for the slight conjunctival irritation caused by 2,4-TDA was 72 hours. No data are available concerning the inhalation of pure 2,4-TDA. However, skin and eye irritation studies have been performed. The results show that there is no reason to expect severe local effects by this application route. In summary it is concluded that the irritant properties of pure 2,4-TDA are of no concern for the workplace. There is no need for classification. The current classification as irritant to the eyes (R 36) is not confirmed.

Conclusion: ii

***Dermal and eye irritation of the mixture of 2,4/2,6-isomers (80/20)***

In a Draize eye test with 6 rabbits the substance demonstrated severe corrosive properties when instilled to the eye. However, this study was regarded to be invalid (see chapter 4.1.3.2). Taking into account the result of a former study with rabbits, which noted pronounced erythema and chemosis after application of 50 mg 2,4-/2,6-TDA (80/20), reversible within 4 days (Kimmerle and Sonneke, 1971), lead to the conclusion that the existing classification with R36 should be warranted. Eye contact critically depends on proper handling of the fluid and the proper use of safety goggles. Even though suitable personal protective equipment (PPE) should usually be available in the relevant workplaces, unintended contact by non-proper use may occur. Therefore a risk from eye irritation has to be considered for the 2,4/2,6-TDA mixture. Based on the labelling with R36, control measures exist for the 2,4/2,6-TDA mixture. These should be able to minimize the exposure of the eyes and therefore reduce concern. Therefore conclusion ii is proposed. However, these control measures must be implemented and complied with. For skin sensitisation no classification is needed.

Conclusion: ii

***Inhalation of the mixture of 2,4/2,6-isomers (80/20)***

In rat inhalation studies clinical symptoms indicating obvious severe local damage were not observed up to a concentration of 83 mg/m<sup>3</sup> of the 2,4/2,6-TDA mixture (80/20). However in cats there were microscopic visible lesions in the lung after exposure to 41.6 mg/m<sup>3</sup> for 4 hours daily, 5 days a week for 3 weeks. Such lung effects were not reported at 9.5 mg/m<sup>3</sup>. This air concentration is used for preliminary risk assessment and calculation of the MOS value.

A factor of 10 might be applied in the assessment to account for uncertainties. The minimal MOS would thus result as 10, the corresponding critical air concentration would lie in the range of 1 mg/m<sup>3</sup> (9.5 mg/m<sup>3</sup> / 10).

The highest inhalation exposure values result from scenario 2 and 3 with 0,025 mg/m<sup>3</sup>, the MOS is calculated as 360 (9.5 / 0,025). This value does not provide reasons for concern with respect of irritating properties acting on the airways in the case of exposure to a mixture of 2,4/2,6-isomers (80/20) under normal workplace conditions.

Conclusion: ii

**Sensitisation**

***Dermal***



Animal skin tests reveal sensitising properties for 2,4-TDA. In addition, a high rate (67.5%) of cross sensitisation to 2,4-TDA was reported from patch tests, performed on patients who were hypersensitive to p-phenylenediamine.

Considerations about skin sensitisation are connected with the assumption that a possible threshold lies at low, but unknown doses. Because of extensive technical and organisational risk reduction measures dermal exposure, and thus the risk of skin sensitisation, is considered to be small. However, because the corresponding risk cannot be quantified or excluded, there is a general concern for skin sensitisation.

Conclusion: iii

### ***Inhalation***

No information on respiratory sensitisation is available. Although 2,4-TDA has demonstrated a sensitising potential in skin tests it is not suspected of being a potent respiratory sensitiser in humans. In view of the fact that, during all the years of use, no knowledge of specific case reports have been reported, respiratory sensitisation after the inhalative exposure of workers to 2,4-TDA is not expected.

Conclusion: ii

### **Repeated dose toxicity**

#### ***Local effects (inhalation, dermal):***

A 6% solution of 2,4-TDA, with doses of approximately 75 mg/kg/week, was nontoxic to the skin of mice in a 2-year mouse-skin-painting study (see chap. 4.1.2.8). For additional information, see chapter Irritation. The current classification as irritant, R 36 is not confirmed.

In summary: under normal workplace conditions, the available data do not indicate a special risk for local effects triggered by long-term exposure.

Conclusion: ii

#### ***Systemic effects by inhalation***

Several studies with repeated application, mainly by the oral route, have been performed in mice and rats. The primary target organ after short- and long-term dietary exposure of 2,4-TDA is the liver: 2,4-TDA damages hepatocytes, leading to cellular necrosis and cirrhosis. In addition, 2,4-TDA is able to accelerate the development of chronic renal disease and to damage the male reproductive system.

No valid inhalation data are available for the assessment of the repeated inhalative toxicity of 2,4-TDA. Preliminary information on inhalation toxicity of the 2,4/2,6-TDA mixture (80/20) comes from a 28-day inhalation study, conducted by Kimmerle and Solmecke (1971). The data presented are in summary form. No details are available for lists of examined parameters, histopathologic examinations, and measuring instruments. Therefore, results of these studies are used as preliminary information. 20 male Wistar rats were exposed to the mixture of the

isomers 2,4/2,6-TDA (80/20) that was vaporized at 100°C, 4 hours a day, 5 times a week, for 4 weeks (whole body exposure). At an average air concentration of 83 mg/m<sup>3</sup> (approx. 9 mg/kg/day see chapter 4.1.2.6), exposed rats showed reduced body weight gain, and an increase in the relative weight of the liver, kidneys, and thyroid glands. At 9.5 mg/m<sup>3</sup> (approx. 1 mg/kg/day see chapter 4.1.2.6) no signs of changes compared to the controls were observed.

The study which is judged to serve as the key study for risk assessment concerning inhalative repeated dose toxicity is a 2-year oral gavage carcinogenicity study in rats and mice (NCI, 1979). In this oral study, F344 rats and B6C3F1 mice (50 animals/sex/dose) were administered average doses of 0, 5.9 and 13 mg/kg/day 2,4-TDA (rats) and 0, 15 and 30 mg/kg/day 2,4-TDA (mice). The dose of 5.9 mg/kg/day, identified as the LOAEL, revealed hepato- and nephrotoxic effects (nonneoplastic morphologic alterations of different severity) and a number of different tumours (liver, mammary gland, hematopoietic system, lung and subcutis) in rats. A NOAEL was not determined. The observed tumours were not taken into account in the assessment of repeated dose toxicity (this point is discussed in detail in the carcinogenicity chapter). The LOAEL of 5.9 mg/kg/day will be used, to assess the inhalative risks of repeated exposure of 2,4-TDA.

As starting point for MOS calculation the corresponding internal human dose is identified as 413 mg/person/day (5.9 mg/kg/day x 70 kg). Expressed as airborne concentration, the starting point is 41 mg/m<sup>3</sup> (413 mg/person/day / 10 m<sup>3</sup>/day). The following assessment factors are applied for the identification of the minimal MOS:

- adaptation of scenarios (experimental 7 days/week to 5 days/week for workers) reveals a factor of 5/7
- to extrapolate from the LOAEL to the NAEL a standard assessment factor of 3 (ECETOC, 1995) seems to be fairly small with respect to the high incidences and severe nature of effects at the dose level of 5.9 mg/kg/day (see chapter 4.1.2.6). Therefore an extrapolation factor of 6 is used in the risk assessment.
- metabolic rate scaling from rat to human uses a factor of 4
- main short-comings result from the fact that the most relevant studies used the oral application route and a NOAEL was not detected in the experiment. An uncertainty factor of 5 seems adequate to account for these aspects.

The multiplication of these factors produces a minimal MOS of 86 (5/7 x 6 x 4 x 5). The corresponding critical exposure level calculates to 0.5 mg/m<sup>3</sup> for inhalation (41 mg/m<sup>3</sup> / 86).

The MOS values (see table 4.1.3.2.C) do not indicate any concern for inhalation with respect to repeated dose toxicity of 2,4-TDA.

Conclusion ii

### ***Systemic effects by dermal contact, combined exposure***

Preliminary information on dermal effects results from a 2-year subcutaneous injection test on Sprague-Dawley rats with the isomer mixture 2,4-/2,6-TDA (80/20) which will be used for plausibility considerations. It has to be kept in mind that the study overcomes dermal

absorption by subcutaneous application. At 8.33 mg/kg/week, 8/30 animals showed focal necrosis of hepatocytes and/or single cirrhosis in the liver.

Likewise for repeated inhalation, the toxicity of 2,4-TDA after repeated dermal and combined exposure will be assessed by taking the 2-year oral gavage study from NCI (see above). The LOAEL of 5.9 mg/kg, revealing hepato- and nephrotoxic effects in rats, will be used to assess dermal and combined risks after repeated exposure.

The starting point for MOS calculation is identified as 413 mg/person/day (5.9 mg/kg/day x 70 kg). Expressed as dermal dose (external value) it calculates to 1,650 mg/person (413 mg/kg/day x 4), taking 25% dermal absorption into consideration. With the minimal MOS of 86 (derivation see systemic effects by inhalation) the corresponding critical exposure levels are calculated as 19 mg/person/day for the assessment of the external dose for skin contact (1,650 mg/person/day / 86), and 5 mg/person/day as the internal dose for evaluation of combined exposure (413 mg/person/day / 86).

The MOS values (see table 4.1.3.2.C) do not indicate any concern for dermal and combined exposure with respect to repeated dose toxicity of 2,4-TDA.

Conclusion: ii

Table 4.1.3.2.C: MOS values for repeated dose toxicity of TDA, systemic effects

	Inhalation			Dermal			Combined		
Starting point for MOS calculation	41 mg/m <sup>3</sup>			1650 mg/p/d (external dose)			413 mg/p/d (internal dose)		
Minimal MOS	86			86			86		
Critical exposure level	0.5 mg/m <sup>3</sup>			19 mg/p/d (external dose)			5 mg/p/d (internal dose)		
	Exposure (mg/m <sup>3</sup> )	MOS	Conclusion	Exposure (mg/p)	MOS	Conclusion	Internal body burden (mg/p)	MOS	Conclusion
1) Production and further processing as a chemical intermediate	0.00075	54,700	ii	0.15 <sup>(1)</sup>	11,000	ii	0.045	9,200	ii
2) Production 2,4-TDA pastilles	0.025	1600	ii	0.42 <sup>(1)</sup>	3900	ii	0.355	1,200	ii
3) Use of 2,4-TDA pastilles for the production of dyes	0.025	1,600	ii	0.42 <sup>(1)</sup>	3,900	ii	0.355	1,200	ii

<sup>(1)</sup>EASE, use of suitable gloves

## Mutagenicity

In vitro studies demonstrate a significant mutagenic potential of 2,4-TDA, which is only weakly expressed in standard tests in vivo. However, genotoxic effects in vivo are reported by several indicator tests.

Only limited experimental data are available for an assessment of heritable genetic damage in germ cells of humans. Negative results were found in dominant lethal and sperm morphology tests; however, due to low sensitivity these findings are not adequate for exclusion of germ cell mutagenesis. There is some indication for effects on the testes from a non-standard assay measuring the reduction of murine testicular DNA synthesis after 2,4-TDA. In summary: it cannot be excluded that 2,4-TDA has genetic effects on germ cells.

Since the nature of the effect in general is considered to be severe, there is reason for concern in connection with all exposure scenarios, even those that only occur occasionally. Available data do not allow for a quantitative risk assessment. However, when discussing the need and priority of further risk reduction activities, the evaluation of 2,4-TDA cancer risk is proposed to be taken into consideration.

Conclusion: iii for somatic cell mutagenicity  
Conclusion: i (on hold) for germ cell mutagenicity

## **Carcinogenicity**

Several studies in mice and rats with 2,4-TDA clearly indicate that 2,4-TDA is carcinogenic. The target organs include liver, mammary gland, hematopoietic system, lung and subcutis, indicating that 2,4-TDA is a multipotent animal carcinogen. Studies mainly used dietary application. However, studies of limited validity with skin painting and subcutaneous injection are also available which likewise demonstrate a carcinogenic potential of 2,4-TDA at different sites. Information from inhalation studies support the idea that 2,4-TDA is easily taken up by inhalation and, to a limited extent, by dermal application - thereby causing systemic cancer risks. Occupational risk assessment will rely on the results of the 2-year oral gavage study from NCI (see above).

With reference to chap. 4.1.2.7 2,4-TDA has a mutagenic potential. The assumption is that the genotoxicity is responsible for tumour initiation and development. Thus as a plausible mode of action a non-threshold mechanism is presumed.

To describe the tumour-risks of 2,4-TDA the minimal MOE-concept is used (for explanation of this method see chapter 4.1.3.2.1). T25-values for different tumour types are calculated and shown in table 4.1.3.2.D. In the case of this study 2 mg/kg/day (dose where 25% of the female rats develop tumours of the mammary gland) and 14 mg/kg/day (dose where 25% of the male rats develop liver tumours) are chosen for further risk considerations. The reason for not taking only the lowest value forward but also the T25 value of the male liver tumours is the uncertainty about the biological relevance of the mammary gland tumours. Therefore for both values (for mammary gland and liver tumours) a MOE calculation will be done.

Table 4.1.3.2.D: Tumour incidences and calculation of T25 in rats and mice (NCI 1979)

Study design	Dose mg/kg/d	Tumour incidence <sup>(1)</sup>	T25 <sup>(2)</sup> mg/kg/d	Nonneoplastic effects (selected)
Rat, diet 103 wk, 7d/wk	0	liver(m) 0/20 liver(f) 0/20 mammary gland(f) 1/20		Dose dependent effects: - reduced body weight gain - decreased survival time - chronic renal disease - liver lesions
	5.9 <sup>(3)</sup>	liver(m) 5/49	14	
		liver(f) 0/50 mammary gland(f) 38/50	2 <sup>(4)</sup>	
	13 <sup>(3)</sup>	liver(m) 10/50 liver(f) 6/49 mammary gland(f) 42/50	17	
Mouse, diet 103 wk, 7d/wk	0	liver carc., (m) 5/20 liver carc., (f) 0/19 lymphoma(f) 2/19 lung carc., (m) 0/20 hematopoietic system(m) 2/20 hematopoietic system(f) 2/19		Dose dependent effect: - reduced body weight gain
	15	liver carc., (m) 17/50	40	
		liver carc., (f) 13/47	13	
		lymphoma(f) 29/47	7	
		lung carc., (m) 9/50	21	
		hematopoietic system(m) 15/50	18	
	hematopoietic system(f) 29/47	7		
	30	liver carc., (m) 13/49		
		liver carc., (f) 18/46		
lymphoma(f) 11/46				
lung carc., (m) 6/49				
hematopoietic system(m) 8/49				
hematopoietic system(f) 1/46				

<sup>(1)</sup> Only tumours with high incidences reported here; incidences summarise malign and benign neoplasms with the exception of mice liver and lung tumours which are carcinomas.

<sup>(2)</sup> T25 = daily dose level (corrected for duration of experiment vs standard life span) x 25% x (net tumour incidence in %)<sup>-1</sup>, for details see Dybing et al., 1997

<sup>(3)</sup> Doses had to be reduced from initially 9 and 18 mg/kg/d in diet to 3.7 and 7.4 mg/kg/d respectively because of excessive depression of mean body weight gain after 40 weeks of study, for details see chap. 4.1.2.8, values given represent the calculated average daily intake.

<sup>(4)</sup> Calculation of the 2 mg/kg/day is: 5.9 mg/kg/d (no correction for duration of experiment vs. standard life span was necessary) x 25% / 75%. The 75% value is the result of the observed tumour incidence of 38 from 50 animals corrected by the factor for spontaneous tumour incidence (1 in 20 animals).

The corresponding starting points of the T25-value of 2 mg/kg/day, derived from the mammary gland tumours of the female rats and the T25-value of 14 mg/kg/day from the liver tumours of the male rats are 140 mg/person/day (2 mg/kg x 70 kg), and 980 mg/person/day (14 mg/kg x 70 kg). Expressed as airborne concentration the starting points are 14 mg/m<sup>3</sup> (140 mg/person/day / 10 m<sup>3</sup>/day) and 98 mg/m<sup>3</sup> (980 mg/person/day / 10 m<sup>3</sup>/day). The corresponding dermal doses (external value) calculate to 560 mg/person/day (140 mg/person/day x 4) and 3,920 mg/person/day (980 mg/person/day x 4) including the aspect of 25% dermal absorption.

The following assessment factors are applied for the identification of the minimal MOE:

- metabolic rate scaling from rat to human uses a factor of 4
- a factor of 25,000 is applied to the T25 for risk extrapolation from high to low doses (probability for cancer lifetime risk of 10<sup>-5</sup> as reference value, see also chapter 4.1.3.2.1 Introductory remarks, MOE approach).
- the correction factor for “standard life span humans” versus duration of exposure at work is 1/2.84 (40y x 48w x 5d) / (75y x 52w x 7d), constants taken from DECOS (1995).

Multiplication of these factors gives the minimal MOE of 35,200 (4 x 25,000 / 2.84).

The corresponding critical exposure level for mammary gland tumours would calculate to 0.0004 mg/m<sup>3</sup> for inhalation (14 mg/m<sup>3</sup> / 35,200), 0.02 mg/person/day as external dose for skin contact (560 mg/person/day / 35,200) and 0.004 mg/person/day as internal dose for the evaluation of combined exposure (140 mg/person/day / 35,200).

If the liver tumours would be the basis for calculation the corresponding critical exposure level would be higher by the factor of 7 compared with the mammary gland tumours: 0.003 mg/m<sup>3</sup> for inhalation (98 mg/m<sup>3</sup> / 35,200), 0.11 mg/person/day as external dose for skin contact (3,920 mg/person/day / 35,200) and 0.03 mg/person/day as internal dose for the evaluation of combined exposure (980 mg/person/day / 35,200).

The following three tables 4.1.3.2.E, 4.1.3.2.F, and 4.1.3.2.G present the specific data relevant for the carcinogenicity risk characterisation with the minimal MOE-concept. It should be noticed that these tables use a minimal MOE which is equivalent to a cancer lifetime risk of 1 : 100,000 and furthermore allow for a comparison between two types of tumours (mammary gland and liver tumours). For 2,4-TDA related carcinogenicity there is the general conclusion iii for all scenarios, because of the genotoxic properties of 2,4-TDA. This conclusion iii will be modified in “concern” (MOE significantly lower than the minimal MOE), “borderline situation” (MOE in the range of the minimal MOE, with a deviation of a factor of about 2), and “very low concern” (MOE significantly higher than the minimal MOE).

Table 4.1.3.2.E: MOE values after inhalative exposure of 2,4-TDA

	Mammary gland tumours			Liver tumours		
Starting point for MOE calculation	14 mg/m <sup>3</sup>			98 mg/m <sup>3</sup>		
Minimal MOE	35,200			35,200		
Critical exposure level	0.0004 mg/m <sup>3</sup>			0.003 mg/m <sup>3</sup>		
	Exposure (mg/m <sup>3</sup> )	MOE	Conclusion	Exposure (mg/m <sup>3</sup> )	MOE	Conclusion
1) Production and further processing as a chemical intermediate (daily)	0.00075	18,700	borderline <sup>(1)</sup>	0.00075	130,700	very low concern <sup>(1)</sup>
2) Production 2,4-TDA pastilles (4 weeks/year)	0.025 <sup>(2)</sup>	5,600 <sup>(2)</sup>	concern <sup>(2)</sup>	0.025 <sup>(2)</sup>	39,200 <sup>(2)</sup>	borderline <sup>(2)</sup>
3) Use of 2,4-TDA pastilles for the production of dyes (not daily)	0.025 <sup>(3)</sup>	>560 <sup>(3)</sup>	concern <sup>(3)</sup>	0.025 <sup>(3)</sup>	>3,920 <sup>(3)</sup>	concern <sup>(3)</sup>

<sup>(1)</sup> Borderline situation is reached, if the main focus is set on the results of the mammary gland tumours. By looking to the liver tumours, the result of the risk assessment is less critical by the factor of 7 and reaches the very low concern.

<sup>(2)</sup> The frequency of exposure at scenario 2 is limited to 4 weeks a year (see also chapter 4.1.1.2). To account for this aspect of a reduced annual dose, the MOE values in scenario 2 are multiplied with the factor 10. Concern is reached, if the main focus is set on the results of the mammary gland tumours. By looking to the liver tumours, the result of the risk assessment is less critical by the factor of 7 and reaches borderline situation.

<sup>(3)</sup> The frequency of exposure at scenario 3 is not daily (see also chapter 4.1.1.2). To account for this aspect of a reduced annual dose, which however cannot be further specified, the MOE values in scenario 3 are marked with a > symbol. Concern is reached, if the main focus is set on the results of the mammary gland tumours. By looking to the liver tumours, the result of the risk assessment is less critical by the factor of 7 but reaches nevertheless concern.

Table 4.1.3.2.F: MOE values after dermal contact to 2,4-TDA

	Mammary gland tumours			Liver tumours		
Starting point for MOE calculation	560 mg/p/d (external dose)			3,920 mg/p/d (external dose)		
Minimal MOE	35,200			35,200		
Critical exposure level	0.02 mg/p/d (external dose)			0.11 mg/p/d (external dose)		
	Exposure (mg/p)	MOE	Conclusion	Exposure (mg/p)	MOE	Conclusion
1) Production and further processing as a chemical intermediate (daily)	0.15	3,700	concern <sup>(1)</sup>	0.15	26,000	borderline <sup>(1)</sup>
2) Production 2,4-TDA pastilles (4 weeks/year)	0.42	13,300 <sup>(2)</sup>	concern <sup>(2)</sup>	0.42	93,000 <sup>(2)</sup>	very low concern <sup>(2)</sup>
3) Use of 2,4-TDA pastilles for the production of dyes (not daily)	0.42	>1,330 <sup>(3)</sup>	concern <sup>(3)</sup>	0.42	>9,300 <sup>(3)</sup>	borderline <sup>(3)</sup>

<sup>(1)</sup>Concern is reached, if the main focus is set on the results of the mammary gland tumours. By looking to the liver tumours, the result of the risk assessment is less critical by the factor of 7 and reaches borderline.

<sup>(2)</sup> The frequency of exposure at scenario 2 is limited to 4 weeks a year (see also chapter 4.1.1.2). To account for this aspect of a reduced annual dose, the MOE values in scenario 2 are multiplied with the factor of 10. Concern is reached, if the main focus is set on the results of the mammary gland tumours. By looking to the liver tumours, the result of the risk assessment is less critical by the factor of 7 and reaches very low concern.

<sup>(3)</sup> The frequency of exposure at scenario 3 is not daily (see also chapter 4.1.1.2). To account for this aspect of a reduced annual dose, which however cannot be further specified, the MOE values in scenario 3 are marked with a > symbol. Concern is reached, if the main focus is set on the results of the mammary gland tumours. By looking to the liver tumours, the result of the risk assessment is less critical by the factor of 7 and reaches borderline.



Table 4.1.3.2.G: MOE values after combined dermal and inhalative contact of 2,4-TDA

	Mammary gland tumours			Liver tumours		
Starting point for MOE calculation	140 mg/p/d (internal dose <sup>(1)</sup> )			980 mg/p/d (internal dose <sup>(1)</sup> )		
Minimal MOE	35,200			35,200		
Critical exposure level	0.004 mg/p/d (internal dose <sup>(1)</sup> )			0.03 mg/p/d (internal dose <sup>(1)</sup> )		
	Internal body burden (mg/p)	MOE	Conclusion	Internal body burden (mg/p)	MOE	Conclusion
1) Production and further processing as a chemical intermediate (daily)	0.045 <sup>(1)</sup>	3,100	concern <sup>(2)</sup>	0.045 <sup>(1)</sup>	2,200	concern <sup>(2)</sup>
2) Production 2,4-TDA pastilles (4 weeks/year)	0.355 <sup>(1)</sup>	3,900	concern <sup>(2)</sup>	0.355 <sup>(1)</sup>	27,600	borderline <sup>(2)</sup>
3) Use of 2,4-TDA pastilles for the production of dyes (not daily)	0.355 <sup>(1)</sup>	>390	concern <sup>(2)</sup>	0.3 <sup>(1)</sup>	>3300	concern <sup>(2)</sup>

<sup>(1)</sup>The values of the internal body burden include an inhalative and a dermal part. For the calculation of the combined risks of 2,4-TDA the values for the inhalative and dermal part are taken from tables 4.1.3.2.E and 4.1.3.2.F. It has to be kept in mind that the fraction of the dermal internal value is 1/4 from the dermal exposure value, because the dermal adsorption is 25%.

<sup>(2)</sup>Depending on which tumour type the assessment is based on, the conclusions range between concern and a borderline situation. Concern is reached, if the main focus is set on the results of the mammary gland tumours. By looking to the liver tumours, the result of the risk assessment is less critical by the factor of 7 and reaches the borderline situation in scenario 2 (for considerations revealing the calculations for scenario 2 and 3 see also the tables above).

As outlined in the chapter on occupational exposure, extensive technical and organisational risk reduction measures have already resulted in very low levels of exposure (by dermal contact and inhalation). In order to translate this technical information of very low exposure into terms of risk, for 2,4-TDA a quantitative risk assessment approach was performed (see tables before).

Although this minimal MOE of 35,200 was generally tolerated, there was no formal decision as to this minimal MOE, in particular because such a formal decision is beyond the competency of the TC NES. The specification of a minimal MOE, which means risk evaluation, needs policy guidance; subject to risk acceptance different minimal MOE values might be chosen.

Based on the most sensitive type of tumours (the mammary gland tumours), tables 4.1.3.2.H and 4.1.3.2.I additionally present the conclusions for higher and lower minimal MOE values. Compared to the chosen minimal MOE of 35,200, a 10 times higher minimal MOE of 352,000 is equivalent to a chosen risk level of 1 : 1,000,000; a 10 times lower minimal MOE

of 3,520 is equivalent to a chosen risk level of 1 : 10,000. These relationships are only valid for the assumption of low-dose linearity.

Table 4.1.3.2.H: Conclusions for inhalation exposure (mammary gland tumours and different MOE values)

	Exposure in mg/m <sup>3</sup>	Minimal MOE of 352,000	Minimal MOE* of 35,200	Minimal MOE of 3,520
1) Production and further processing as a chemical intermediate (daily)	0.00075	concern	borderline	very low concern
2) Production 2,4-TDA pastilles (4 weeks/year)	0.025	concern	concern	borderline
3) Use of 2,4-TDA pastilles for the production of dyes (not daily)	0.025	concern	concern	between very low concern and borderline

\*This minimal MOE is used in the tables above and is equivalent to a cancer risk level of 1 : 100,000

Table 4.1.3.2.I: Conclusions for dermal exposure (mammary gland tumours and different MOE values)

	Exposure in mg/p/d	Minimal MOE of 352,000	Minimal MOE* of 35,200	Minimal MOE of 3,520
1) Production and further processing as a chemical intermediate (daily)	0.15	concern	concern	borderline
2) Production 2,4-TDA pastilles (4 weeks/year)	0.42	concern	concern	very low concern
3) Use of 2,4-TDA pastilles for the production of dyes (not daily)	0.42	concern	concern	between borderline and concern

\*This minimal MOE is used in the tables above and is equivalent to a cancer risk level of 1 : 100,000

It is evident from these tables (table 4.1.3.2.H and 4.1.3.2.I) that the specific conclusions for the different occupational exposure scenarios critically depend on the chosen level of risk acceptance. This comparison may be helpful for risk managers in order to evaluate the necessity and priority of further risk reduction measures beyond those that has already been successfully implemented.

Conclusion: iii

## Reproductive toxicity

### *Fertility impairment*

Information from dermal or inhalation studies is not available, the limited human data are not judged to be suitable for matters of risk assessment (chap. 4.1.2.9). However, a sufficient picture of the fertility effects of 2,4-TDA on male rats can be derived from a series of feeding studies in rats though the design of the studies does not meet guideline requirements:

In a one-generation study, a daily intake of 50 mg/kg for several weeks resulted in total reproductive failure of the male rats, at 15 mg/kg/day mating and fertility indices were significantly reduced, down to a level of 50%. Pathologic investigation showed arrested spermatogenesis associated with a significant reduction in the weight of seminal vesicles and epididymis, morphological damage to the Sertoli cells and altered hormone levels. A dose of 5 mg/kg/day showed no histopathological changes (chap. 4.1.2.6). However, even at this dose level, diminished sperm reserves were still detected (chap. 4.1.2.9). For men this effect might be of higher significance for fertility impairment than for rats due to species differences in reserve sperm pools. Thus, in summary, a dietary dose in rats of 5 mg/kg/d for several weeks is assumed to be the LOAEL for male fertility. There are no data of dose- related impairment of female fertility.

The LOAEL of 5 mg/kg/day will be used for risk assessment. The corresponding starting point for MOS calculation is identified as 350 mg/person/day (5 mg/kg/day x 70 kg). Including the aspect of 25% dermal absorption the corresponding dermal dose (external value) is calculated as 1,400 mg/person/day (350 mg/person/day x 4). Expressed as air concentration the starting point is 35 mg/m<sup>3</sup> (350 mg/person/day / 10 m<sup>3</sup>/day).

The following assessment factors are applied for the identification of the minimal MOS:

- a default value of 3 (according to ECETOC, 1995) is applied at the LOAEL because a NOAEL cannot be derived from the dose-response-relationship
- metabolic rate scaling from rat to human uses a factor of 4
- fertility impairment in general is evaluated to be a severe adverse effect. In addition, the slope of the dose-response curve is steep: little dose deviations (from 5 to 15 mg/kg/day) had significant effects on fertility (from 100% to 50%). Therefore a precautionary approach appears indicated. A factor of 10 is selected for uncertainty considerations.

Multiplication of these factors produces the minimal MOS of 120 (3 x 4 x 10). The corresponding critical exposure levels are calculated as to 0.3 mg/m<sup>3</sup> for inhalation (35 mg/m<sup>3</sup> / 120), 12 mg/person/day as external dose for skin contact (1,400 mg/person/day / 120) and 3 mg/person/day as internal dose for evaluation of combined exposure (350 mg/person/day / 120).

With respect to fertility impairment there is no concern after inhalation dermal contact and combined exposure of 2,4-TDA at the above described workplaces (see table 4.1.3.2.J).

Table 4.1.3.2.J: MOS values for male workers concerning fertility

	Inhalation			Dermal			Combined		
Starting point for MOS calculation	35 mg/m <sup>3</sup>			1,400 mg/p/d (external dose)			350 mg/p/d (internal dose)		
Minimal MOS	120			120			120		
Critical exposure level	0.3 mg/m <sup>3</sup>			12 mg/p/d (external dose)			3 mg/p/d (internal dose)		
	Exposure (mg/m <sup>3</sup> )	MOS	Conclusion	Exposure (mg/p/d)	MOS	Conclusion	Exposure (mg/p/d)	MOS	Conclusion
1) Production and further processing as a chemical intermediate	0.00075	46,700	ii	0.15 <sup>(1)</sup>	9,300	ii	0.045	7,800	ii
2) Production 2,4-TDA pastilles	0.025	1,400	ii	0.42 <sup>(1)</sup>	3,300	ii	0.355	1,000	ii
3) Use of 2,4-TDA pastilles for the production of dyes	0.025	1,400	ii	0.42 <sup>(1)</sup>	3,300	ii	0.355	1,200	ii

<sup>(1)</sup>EASE, use of suitable gloves

Conclusion: ii

### Developmental toxicity

Reliable data concerning developmental toxicity are at present not available. According to the revised TGD the results of reproductive toxicity testing of germ cell mutagens (Category 1 or 2) and genotoxic carcinogens (Category 3 mutagens and Category 1 or 2 carcinogens) are unlikely to influence the outcome of the risk assessment. This is because the risk characterisation for such substances will be based on the assumption that a threshold exposure level for adverse health effects cannot be identified, which will normally lead to a recommendation for the most stringent risk management measures. Therefore, reproductive testing will not normally be required for germ cell mutagens and genotoxic carcinogens, unless there are case-specific reasons to indicate that the information gained from testing will be needed for the risk characterisation. Germ cell mutagens and genotoxic carcinogens not tested for reproductive toxicity should be regarded as potentially toxic to reproduction.

Conclusion: i (on hold)

#### 4.1.3.2.3 Summary of conclusions for the occupational risk assessment of 2,4-TDA

As result of the occupational risk assessment for 2,4-TDA, concern is risen for specified toxicological endpoints (table 4.1.3.2.I).

Table 4.1.3.2.K Endpoint-specific overall conclusions for the occupational risk assessment of 2,4-TDA

Toxicological endpoints		concern for at least one scenario
Acute toxicity	inhalation	
	dermal	
	combined	
Irritation/ Corrosivity	dermal	
	eye	
	acute respiratory tract	
Sensitisation	skin	iii
	respiratory	
Repeated dose toxicity	inhalation, local	
	inhalation, systemic	
	dermal, local	
	dermal, systemic	
	combined, systemic	
Mutagenicity	for somatic cells	iii
	for germ cells	i (on hold)
Carcinogenicity	inhalation	iii
	dermal	iii
	combined	iii <sup>(1)</sup>
Fertility impairment	inhalation	
	dermal	
	combined	
Developmental toxicity	inhalation	i (on hold)
	dermal	i (on hold)
	combined	i (on hold)

<sup>1)</sup> conclusion iii already results from inhalative and dermal exposure

<sup>2)</sup> blank fields: conclusion ii

Tables 4.1.3.2.L and 4.1.3.2.M show the critical exposure levels for inhalation and dermal contact as identified in the report and used for decisions. These tables try to visualize the risk profile of 2,4-TDA for inhalation and dermal contact. The risk situations (defined by exposure scenario and the critical exposure level for a specific toxicological endpoint) are arranged in such a way, that the “high risk” situations principally are located in the left upper corner of the table, whereas the “low” risk situations are located in the lower right area of the table (grey areas for conclusion iii “concern” and conclusion iii “borderline situation”). This type of table may help to reach consistent conclusions for different endpoints and scenarios. Because

a critical exposure level could not be identified for mutagenicity and skin sensitisation, these endpoints are not included in the tables.

Table 4.1.3.2.L: Ranking of the critical exposure levels for 2,4-TDA with respect to inhalative exposure at the workplace

Exposure scenario	Exposure level in mg/m <sup>3</sup>	Carcinog. mammary gland tumours minMOE: 352,000	Carcinog. mammary gland tumours minMOE: 35,200	Carcinog. mammary gland tumours minMOE: 3,520	Fertility	Repeated dose toxicity	Acute toxicity	
		Critical exposure level in mg/m <sup>3</sup>						
		0.00004	0.0004	0.004	0.3	0.5	60	
2) Production of 2,4-TDA pastilles (4 weeks/year)	0.025 <sup>(1)</sup>	concern	concern	borderline				
3) Use of 2,4-TDA pastilles for the production of dyes (not daily)	0.42	concern	concern	between very low concern and borderline				
1) Production and further processing as a chemical intermediate	0.0075	concern	borderline	very low concern				

<sup>(1)</sup>reduced values are taken (see table 4.1.3.2.E)

<sup>(2)</sup>blank fields: conclusion ii

Table 4.1.3.2.M: Ranking of the critical exposure levels for 2,4-TDA with respect to dermal exposure at the workplace

Exposure scenario	Exposure level in mg/p/d	Carcinog. mammary gland tumours minMOE: 352,000	Carcinog. mammary gland tumours minMOE: 35,200	Carcinog. mammary gland tumours minMOE: 3,520	Fertility	Repeated dose toxicity	Acute toxicity	
		Critical exposure level in mg/p/d						
		0.002	0.02	0.2	12	19	88	
3) Use of 2,4-TDA pastilles for the production of dyes (not daily)	0.2	concern	concern	between borderline and concern				
1) Production and further processing as a chemical intermediate	0.15	concern	concern	borderline				

2) Production of 2,4-TDA pastilles (4 weeks/year)	0.42 <sup>(1)</sup>	concern	concern	very low concern			
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<sup>(1)</sup>reduced values are taken (see table 4.1.3.2.F)

<sup>(2)</sup>blank fields: conclusion ii

Concern is risen as the result of the occupational risk assessment for 2,4-TDA. The toxicological endpoints which lead to concern are carcinogenicity in combination with mutagenicity (classification as category 2 carcinogen) and the dermal sensitisation. For the skin sensitisation the corresponding risks are considered to be small, because of extensive technical and organisational dermal risk reduction measures. However, because the corresponding risk cannot be quantified or excluded, a general concern for skin sensitisation is expressed.

For 2,4-TDA-related carcinogenicity there is a general conclusion iii for all three occupational exposure scenarios. As outlined in the chapter on occupational exposure, extensive technical and organisational risk reduction measures have already resulted in very low levels of exposure (dermal and inhalative). In order to translate this technical information of low exposure into terms of risk, for 2,4-TDA a quantitative risk assessment approach was performed (for detailed explanation see chapter carcinogenicity). Additionally a risk evaluation for this endpoint was done by calculating with different levels of risk acceptance. The specific conclusions for the different occupational exposure scenarios critically depend on the chosen level of risk acceptance. This comparison may be helpful for risk managers in order to evaluate the necessity and priority of further risk reduction measures beyond those that has already been successfully implemented.

#### 4.1.3.3 Consumers

Since a consumer exposure seems not to exist, a health risk of consumers regarding Acute toxicity, Irritation, Corrosivity, Sensitization, Repeated dose toxicity, Mutagenicity, Carcinogenicity, and Reproductive toxicity is not expected.

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.

#### 4.1.3.4 Man exposed indirectly via the environment

Indirect exposure via the environment is calculated using data for oral intake via food (fish) and drinking water. Following the local scenario data (at a point source, cf. 4.1.1.4) a total daily intake of total TDA for humans of 0.026 µg/kg bw/d is calculated. Calculations for the regional scenario resulted in a total daily dose of  $2.6 \times 10^{-7}$  mg/kg bw/d.

##### Repeated dose toxicity

A NOAEL has not been established; the LOAEL of 5.9 mg/kg bw/day (time-weighted average dose) was derived from a 2-year rat study on 2,4-TDA (NCI 1979). This study represents the lowest effect level at which relevant toxic effects were observed. At this dosage there was a decreased survival rate, a clear delay in body weight gain, lesions of the liver and kidneys as well as tumors in the liver in high incidences. This long term study was considered to be the most appropriate one for risk assessment although the study did not include haematology and clinical chemistry parameters.

Other repeated dose toxicity studies of 2,4-TDA using medium-term treatment periods were able to identify a NOAEL. Some of them were not in full compliance with the current test guidelines, and have been focused on selected parameters. After subchronic administration the NOAEL in a limited study was 250 ppm (approx. 19 mg/kg bw/day) in rats and 100 ppm (approx. 15 mg/kg bw/day) in mice (7-week study, NCI 1979). The treatment of rats with 300 ppm (approx. 15 mg/kg bw/day) for 10 weeks resulted in a toxic effect on spermatogenesis due to an adverse effect on Sertoli cell cytostructure. No alterations in Sertoli cell ultrastructure were reported at 100 ppm (approx. 5 mg/kg bw/day), however, first effects on epididymal sperm reserves were shown (Thysen et al. 1985a).

In the following text the data base on repeated dose toxicity of 2,4-TDA is considered to explain the conclusion about the appropriateness of the MOS for this endpoint.

- 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 data were published in peer reviewed journals or submitted to the Competent Authority in private reports being adequately detailed and in accordance with internationally recognized guidelines and to GLP.



The findings of all studies are not contradictory so that the judgment can be based on the database (cf. sections 4.1.2.6 and 4.1.2.8).

There are no reasons to assume limited confidence.

- uncertainty arising from the variability in the experimental data

The studies cited above allow to conclude on the LOAEL of severe toxicity from on rats and mice. The main findings of the other studies which are not in full compliance to current test guidelines showed good consistency.

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

- intra- and interspecies variation

Comparing the effect levels for nonneoplastic lesions no clear sex preference seems to exist.

Data on kinetics of the substance do not allow to calculate the intraspecies and interspecies variability by applying modern approaches. However, the available data give no hint on a particular high variability in kinetics. The variability of the data on the toxicodynamics has been described above and has been considered not to justify an increased MOS. For establishing the MOS, the LOAEL of the most sensitive animal study (rats) has been used.

- the nature and severity of the effect

The carcinogenic action of 2,4-TDA in rats and mice is proven. However, there are no data on carcinogenicity in humans.

The effects described in rats as „observed adverse effects“ are decreased survival rate, a clear delay in body weight gain, lesions of the liver and kidneys as well as tumors in the liver in high incidences. These effects have to be considered to be severe health effects.

There are no reasons to assume that the non-carcinogenic effects shown in the animal experiments are limited to the species tested, thus being not of relevance for humans. Therefore there is concern, which has to be expressed in the magnitude of the MOS.

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

The estimated total body burden with an assumed absorption of 100% is compared with an oral LOAEL from a 2-year study.

There are no reasons to assume that special concern can be derived from this procedure.

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

Following the exposure scenario there is no reason to assume a special risk for elderly or children.

- other factors

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

MOS for the exposure scenario:

Man exposed indirectly via the environment

Local scenario

The total daily intake was calculated to be 0.000026 mg/kg bw/d. The margin of safety between the

exposure level of	0.000026 mg/kg bw/d
and the	
oral LOAEL of	5.9 mg/kg bw/d

is judged to be sufficient, even if special considerations on intra- and interspecies variation, nature and severity of the effects and possible human populations at risk are taken into consideration. Thus, 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.

Regional scenario

The total daily intake was calculated to be  $2.6 \times 10^{-7}$  mg/kg bw/d. The margin of safety between the

exposure level of	$2.6 \times 10^{-7}$ mg/kg bw/d
and the	
oral LOAEL of	5 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.

Genotoxicity

Toluene-2,4-diamine has been shown to be a mutagen *in vitro* in tests with bacteria and mammalian cell cultures. *In vivo* 2,4-TDA induced only weak effects in cells from bone marrow even at nearly acute toxic doses. However, several results from tests analyzing genetic effects in other organs, like liver, mammary gland and kidney, prove that 2,4-TDA forms DNA-adducts, induces DNA-strand breaks and also mutants in transgenic mice. In studies with repeated application, time related effects were observed already at rather low doses. Taking into account that 2,4-TDA influenced murine testicular DNA synthesis, genetic effects on germ cells cannot be excluded.

Considering the positive results from the *in vivo* mutagenicity tests in somatic cells supported by the clear evidence for mutagenic properties from mammalian cells *in vitro* 2,4-TDA has to be classified as category 3 mutagen (R 68, possible risk of irreversible effects).

Conclusion:

For somatic all mutagenicity

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

For germ cell mutagenicity

- i) (on hold) There is need for further information and/or testing.

Carcinogenicity

2,4-TDA is carcinogenic to rats and mice. In F344 rats 2,4-TDA produced dose-dependently higher incidences of hepatocellular carcinomas or neoplastic nodules in males and females and mammary tumors in females after oral administration (LOAEL for hepatocellular carcinoma 5.9 mg/kg bw/d, 103-week rat study, cf. 4.1.2.8). Hepatocellular carcinomas have also been diagnosed in female B6C3F1 mice. Mice with carcinomas often had hyperplasia in the liver, sometimes diffuse, and hyperplastic nodules. In rats, 2,4-TDA or the mixture of isomers 2,4/2,6-TDA (80/20) produced localized sarcomas at the application site after subcutaneous injection. 2,4-TDA is considered as a genotoxic carcinogen. There are no mechanistic arguments to indicate that these findings would be restricted to animals and not to humans.

The commercial grade TDA, which was an isomeric mixture of the 2,4- and 2,6-TDA (80/20), is suspected to have carcinogenic properties due to the constituent 2,4-TDA, which actually is classified to be Carcinogen Category 2, and is labelled with T, Toxic, R 45, May cause cancer.

Based on the available effect data and the daily intake value a margin of exposure (MOE) of about  $2.3 \times 10^5$  can be derived for the local scenario with regard to carcinogenicity. A value of greater than 10,000 has been proposed by the EFSA Scientific Committee (EFSA, 2005) to characterise low risk if started from BMDL10. Given the fact that calculation of MOE starts with a LOAEL risk reduction measures have to be taken.

Conclusion:

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

Reproductive toxicity

Fertility

Hazard identification with respect to toxicity for reproduction can not be performed since there are no investigations available with 2,4-/2,6-TDA (80/20). Also the presently available results from epidemiological studies are inconclusive and some of the studies were of restricted validity.

Animal studies with rats on the predominant isomer 2,4-TDA however revealed that the repeated oral intake of the substance affects spermatogenesis already at a dose level of approximately 5 mg/kg bw/d (LOAEL).

MOS for the exposure scenario:

Man exposed indirectly via the environment

Local scenario

The total daily intake was calculated to be 0.000026 mg/kg bw/d. The margin of safety between the

exposure level of	0.000026 mg/kg bw/d
and the	
oral LOAEL of	5 mg/kg bw/d

is judged to be sufficient even taking into account that a LOAEL is used for derivation of the MOS.

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.

Regional scenario

The total daily intake was calculated to be  $2.6 \times 10^{-7}$  mg/kg bw/d. The margin of safety between the

exposure level of	$2.6 \times 10^{-7}$ mg/kg bw/d
and the	
oral LOAEL of	5 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.

Developmental toxicity

Reliable data for hazard assessment concerning developmental effects are at present not available. Thus, a risk characterisation for this endpoint cannot be performed. However, 2,4-TDA is classified as a Carcinogen Category 2.

Risk reduction measures are required in view of the carcinogenic properties of the substance, the need for a test to evaluate developmental toxicity should be revisited in the light of risk reduction strategy.

Conclusion:

i) (on hold) There is need for further information and/or testing.

**4.1.3.5 (Combined exposure)**

**4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)**

**4.2.1 Exposure assessment**

**4.2.1.1 Occupational exposure**

Refer to 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**

2,4-TDA is not explosive.

**4.2.2.2 Flammability**

2,4-TDA is not highly flammable.

**4.2.2.3 Oxidising potential**

Due to its chemical structure, 2,4-TDA is not expected to possess any oxidizing properties.

**4.2.3 Risk characterisation**

**4.2.3.1 Workers**

Risk reduction measures beyond those which are being applied already are not considered necessary.

Conclusion: ii

**4.2.3.2 (Combined exposure)**

**4.3 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)**

**4.3.1 Exposure assessment**

**4.3.1.1 Occupational exposure**

Refer to chapter 4.1.1.1

**4.3.1.2 Consumer exposure**

**4.3.1.3 Indirect exposure via the environment**

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

**4.3.2.1 Explosivity**

2,4-TDA is not explosive.

**4.3.2.2 Flammability**

2,4-TDA is not highly flammable.

**4.3.2.3 Oxidising potential**

Due to its chemical structure, 2,4-TDA is not expected to possess any oxidizing properties.

**4.3.3 Risk characterisation**

**4.3.3.1 Workers**

Risk reduction measures beyond those which are being applied already are not considered necessary.

Conclusion: ii

**4.3.3.2 Consumers**

**4.3.3.3 Man exposed indirectly via the environment**

## 5 CONCLUSIONS / RESULTS

- ( 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 or for risk reduction measures beyond those which are being applied already
- ( X ) 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 the site dye1 that process 2,4-TDA to dyes.

PEC/PNEC ratios for wastewater treatment plants, surface water and sediment are above 1 for the scenario “processing of 2,4-TDA to dyes” at site 1. As this scenario is fully based on default values, improvement of the exposure data basis is possible. Information on TDA emission from this site should be provided.

**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 to the aquatic compartment and for waste water treatment plants for all other sites and the environmental compartments atmosphere and soil and secondary poisoning.

### **Human Health**

#### Workers

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

There is a need for better information to adequately characterise the risks regarding the mutagenicity (germ cell mutagenicity) and developmental toxicity because the current database does not adequately cover these endpoints. The collection of additional information



should, however, not delay the implementation of appropriate control measures needed to address the concern related to other endpoints (conclusion (i) on hold).

**Conclusion (iii)** There is a need for specific measures to limit the risks

There is concern for mutagenicity (somatic cell mutagenicity) and carcinogenicity as a consequence of dermal and inhalation exposure arising from all investigated occupational exposure scenarios. Extensive technical and organisational reduction measures have already led to very low levels of exposure.

Carcinogenicity risk assessment was conducted with a quantitative approach. Additionally a risk evaluation for this endpoint was done by calculating with different levels of risk acceptance. The specific conclusions for the different occupational exposure scenarios critically depend on the chosen level of risk acceptance. This comparison may be helpful for risk managers in order to evaluate the necessity and priority of further risk reduction measures beyond those that has already been successfully implemented.

There is concern for skin sensitisation as a consequence of dermal exposure arising from all investigated occupational exposure scenarios. Risks of skin sensitisation are considered to be small. However, because the corresponding risk cannot be quantified or excluded, a general concern for skin sensitisation is expressed.

#### Consumer

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

Since a consumer exposure seems not to exist, a health risk of consumers is not expected.

#### Man exposed indirectly via the environment

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

There is a need for better information to adequately characterise the risks regarding the mutagenicity (germ cell mutagenicity) and developmental toxicity because the current database does not adequately cover these endpoints. The collection of additional information should, however, not delay the implementation of appropriate control measures needed to address the concern related to other endpoints (conclusion (i) on hold).

**Conclusion (iii)** There is a need for specific measures to limit the risks

The risk assessment shows that the margin of exposure could be assumed to be sufficient for mutagenicity (somatic cell mutagenicity) and carcinogenicity, but that risks cannot be excluded at any exposure, as the substance is considered as genotoxic carcinogen.

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# **Appendix A I**

of the Risk Assessment Report

TDA

**Distribution and fate**

**January 2003**



## Distribution and Fate

d := Tag

### Substance: 2,4-TDA

melting point:	MP := 363·K
vapour pressure:	VP := 0.017·Pa
water solubility:	SOL := 38000·mg·l <sup>-1</sup>
part. coefficient octanol/water:	LOGP <sub>OW</sub> := 0.074
moleculare weight:	MOLW := 0.122·kg·mol <sup>-1</sup>
gas constant:	R := 8.3143J·(mol·(K)) <sup>-1</sup>
temperature:	T := 293.15K
conc. of suspended matter in the river:	SUSP <sub>water</sub> := 15·mg·l <sup>-1</sup>
density of the solid phase:	RHO <sub>solid</sub> := 2500·kg·m <sup>-3</sup>
volume fraction water in susp. matter:	F <sub>water_susp</sub> := 0.9
volume fraction solids in susp.matter:	F <sub>solid_susp</sub> := 0.1
volume fraction of water in sediment:	F <sub>water_sed</sub> := 0.8
volume fraction of solids in sediment:	F <sub>solid_sed</sub> := 0.2
volume fraction of air in soil:	F <sub>air_soil</sub> := 0.2
volume fraction of water in soil:	F <sub>water_soil</sub> := 0.2
volume fraction of solids in soil:	F <sub>solid_soil</sub> := 0.6
aerobic fraction of the sediment comp.:	F <sub>aer_sed</sub> := 0.1
product of CONjunge and SURF <sub>air</sub> :	product := 10 <sup>-4</sup> ·Pa

### distribution air/water: Henry-constant

$$\text{HENRY} := \frac{\text{VP} \cdot \text{MOLW}}{\text{SOL}} \quad \text{HENRY} = 5.458 \cdot 10^{-5} \text{ Pa} \cdot \text{m}^3 \cdot \text{mol}^{-1}$$

$$\log \left( \frac{\text{HENRY}}{\text{Pa} \cdot \text{m}^3 \cdot \text{mol}^{-1}} \right) = -4.263$$

$$K_{\text{air\_water}} := \frac{\text{HENRY}}{R \cdot T} \quad K_{\text{air\_water}} = 2.239 \cdot 10^{-8}$$

solid/water-partition coefficient  $K_{p\_comp}$  and total compartment/water-partition coefficient  $K_{comp\_water}$

$$K_{OC} := 97631 \cdot \text{kg}^{-1} \quad (\text{measured})$$

Suspended matter

$$K_{p\_susp} := 0.1 \cdot K_{OC}$$

$$K_{p\_susp} = 976.31 \cdot \text{kg}^{-1}$$

$$K_{susp\_water} := F_{water\_susp} + F_{solid\_susp} \cdot K_{p\_susp} \cdot RHO_{solid}$$

$$K_{susp\_water} = 244.975$$

factor for the calculation of  $C_{local\_water}$ :

$$\text{faktor} := 1 + K_{p\_susp} \cdot SUSP_{water}$$

$$\text{faktor} = 1.015$$

Sediment

$$K_{p\_sed} := 0.05 \cdot K_{OC}$$

$$K_{p\_sed} = 488.155 \cdot \text{kg}^{-1}$$

$$K_{sed\_water} := F_{water\_sed} + F_{solid\_sed} \cdot K_{p\_sed} \cdot RHO_{solid}$$

$$K_{sed\_water} = 244.875$$

Soil

$$K_{p\_soil} := 0.02 \cdot K_{OC}$$

$$K_{p\_soil} = 195.262 \cdot \text{kg}^{-1}$$

$$K_{soil\_water} := Fair_{soil} \cdot K_{air\_water} + F_{water\_soil} + F_{solid\_soil} \cdot K_{p\_soil} \cdot RHO_{solid}$$

$$K_{soil\_water} = 293.09$$

Sludge

$$K_{p\_sludge} := 0.37 \cdot K_{OC}$$

$$K_{p\_sludge} = 3.612 \cdot 10^3 \cdot \text{kg}^{-1}$$

**Elimination in STPs**

rate constant in STP:  $k = 0.1 \text{ h}^{-1}$

elimination  $P = f(k, \log \text{pow}, \log H) = 41 \%$

fraction directed to surface water  $F_{\text{stp\_water}} = 59 \%$

**biodegradation in different compartments**

surface water

$k_{\text{bio\_water}} := 0 \cdot \text{d}^{-1}$  (cTGD, table 5)

soil

$DT_{50\text{bio\_soil}} := 1000 \cdot \text{d}$

$k_{\text{bio\_soil}} := \frac{\ln(2)}{DT_{50\text{bio\_soil}}}$   $k_{\text{bio\_soil}} = 6.931 \cdot 10^{-4} \text{ d}^{-1}$

sediment

$k_{\text{bio\_sed}} := \frac{\ln(2)}{DT_{50\text{bio\_soil}}} \cdot F_{\text{aer\_sed}}$   $k_{\text{bio\_sed}} = 6.931 \cdot 10^{-5} \text{ d}^{-1}$

**degradation in surface waters**

$k_{\text{hydr\_water}} := 1 \cdot 10^{-10} \cdot \text{d}^{-1}$

$k_{\text{photo\_water}} := 1 \cdot 10^{-10} \cdot \text{d}^{-1}$

$k_{\text{deg\_water}} := k_{\text{hydr\_water}} + k_{\text{photo\_water}} + k_{\text{bio\_water}}$

$k_{\text{deg\_water}} = 2 \cdot 10^{-10} \text{ d}^{-1}$

**Atmosphere**

calculation of  $CON_{\text{junge}} * SURF_{\text{aer}}$  for the OPS-model

$VPL := \frac{VP}{\exp\left[6.79 \cdot \left(1 - \frac{MP}{285 \cdot K}\right)\right]}$   $VP := \text{wenn}(MP > 285 \cdot K, VPL, VP)$   $VP = 0.109 \cdot \text{Pa}$

$F_{\text{ass\_aer}} := \frac{\text{product}}{VP + \text{product}}$

**degradation in the atmosphere**

$k_{\text{deg\_air}} = 0.433 \text{ h}^{-1}$

$F_{\text{ass\_aer}} = 9.164 \cdot 10^{-4}$

## Distribution and Fate

d := Tag

### Substance: 2,6-TDA

melting point:	MP := 377·K
vapour pressure:	VP := 0.029·Pa
water solubility:	SOL := 54000·mg·l <sup>-1</sup>
part. coefficient octanol/water:	LOGP <sub>OW</sub> := -0.137
moleculare weight:	MOLW := 0.122·kg·mol <sup>-1</sup>
gas constant:	R := 8.3143J·(mol·(K)) <sup>-1</sup>
temperature:	T := 293.15K
conc. of suspended matter in the river:	SUSP <sub>water</sub> := 15·mg·l <sup>-1</sup>
density of the solid phase:	RHO <sub>solid</sub> := 2500·kg·m <sup>-3</sup>
volume fraction water in susp. matter:	F <sub>water_susp</sub> := 0.9
volume fraction solids in susp.matter:	F <sub>solid_susp</sub> := 0.1
volume fraction of water in sediment:	F <sub>water_sed</sub> := 0.8
volume fraction of solids in sediment:	F <sub>solid_sed</sub> := 0.2
volume fraction of air in soil:	F <sub>air_soil</sub> := 0.2
volume fraction of water in soil:	F <sub>water_soil</sub> := 0.2
volume fraction of solids in soil:	F <sub>solid_soil</sub> := 0.6
aerobic fraction of the sediment comp.:	F <sub>aer_sed</sub> := 0.1
product of CONjunge and SURF <sub>air</sub> :	product := 10 <sup>-4</sup> ·Pa

### distribution air/water: Henry-constant

$$\text{HENRY} := \frac{\text{VP} \cdot \text{MOLW}}{\text{SOL}} \quad \text{HENRY} = 6.552 \cdot 10^{-5} \text{ Pa} \cdot \text{m}^3 \cdot \text{mol}^{-1}$$

$$\log \left( \frac{\text{HENRY}}{\text{Pa} \cdot \text{m}^3 \cdot \text{mol}^{-1}} \right) = -4.184$$

$$K_{\text{air\_water}} := \frac{\text{HENRY}}{R \cdot T} \quad K_{\text{air\_water}} = 2.688 \cdot 10^{-8}$$

solid/water-partition coefficient  $K_{p\_comp}$  and total compartment/water-partition coefficient  $K_{comp\_water}$

$$K_{OC} := 78051 \cdot \text{kg}^{-1} \quad (\text{measured})$$

Suspended matter

$$K_{p\_susp} := 0.1 \cdot K_{OC}$$

$$K_{p\_susp} = 780.5 \cdot \text{l} \cdot \text{kg}^{-1}$$

$$K_{susp\_water} := F_{water\_susp} + F_{solid\_susp} \cdot K_{p\_susp} \cdot RHO_{solid}$$

$$K_{susp\_water} = 196.025$$

factor for the calculation of  $C_{local\_water}$ :

$$\text{faktor} := 1 + K_{p\_susp} \cdot SUSP_{water}$$

$$\text{faktor} = 1.012$$

Sediment

$$K_{p\_sed} := 0.05 \cdot K_{OC}$$

$$K_{p\_sed} = 390.25 \cdot \text{l} \cdot \text{kg}^{-1}$$

$$K_{sed\_water} := F_{water\_sed} + F_{solid\_sed} \cdot K_{p\_sed} \cdot RHO_{solid}$$

$$K_{sed\_water} = 195.925$$

Soil

$$K_{p\_soil} := 0.02 \cdot K_{OC}$$

$$K_{p\_soil} = 156.1 \cdot \text{l} \cdot \text{kg}^{-1}$$

$$K_{soil\_water} := Fair_{soil} \cdot K_{air\_water} + F_{water\_soil} + F_{solid\_soil} \cdot K_{p\_soil} \cdot RHO_{solid}$$

$$K_{soil\_water} = 234.35$$

Sludge

$$K_{p\_sludge} := 0.37 \cdot K_{OC}$$

$$K_{p\_sludge} = 2.888 \cdot 10^3 \cdot \text{l} \cdot \text{kg}^{-1}$$

### Elimination in STPs

rate constant in STP:  $k = 0 \text{ h}^{-1}$

elimination  $P = f(k, \log \text{pow}, \log H) = 0 \%$

fraction directed to surface water  $F_{\text{stp\_water}} = 100 \%$

### biodegradation in different compartments

#### surface water

$$k_{\text{bio\_water}} := 0 \cdot \text{d}^{-1} \quad (\text{cTGD, table 5})$$

#### soil

$$DT50_{\text{bio\_soil}} := 1000 \cdot \text{d}$$

$$k_{\text{bio\_soil}} := \frac{\ln(2)}{DT50_{\text{bio\_soil}}} \quad k_{\text{bio\_soil}} = 6.931 \cdot 10^{-4} \text{ d}^{-1}$$

#### sediment

$$k_{\text{bio\_sed}} := \frac{\ln(2)}{DT50_{\text{bio\_soil}}} \cdot F_{\text{aer\_sed}} \quad k_{\text{bio\_sed}} = 6.931 \cdot 10^{-5} \text{ d}^{-1}$$

### degradation in surface waters

$$k_{\text{hydr\_water}} := 1 \cdot 10^{-10} \cdot \text{d}^{-1}$$

$$k_{\text{photo\_water}} := 1 \cdot 10^{-10} \cdot \text{d}^{-1}$$

$$k_{\text{deg\_water}} := k_{\text{hydr\_water}} + k_{\text{photo\_water}} + k_{\text{bio\_water}}$$

$$k_{\text{deg\_water}} = 2 \cdot 10^{-10} \text{ d}^{-1}$$

### Atmosphere

calculation of  $CON_{\text{junge}} * SURF_{\text{aer}}$  for the OPS-model

$$VPL := \frac{VP}{\exp\left[6.79 \cdot \left(1 - \frac{MP}{285 \cdot K}\right)\right]}$$

$$VP := \text{wenn}(MP > 285 \cdot K, VPL, VP)$$

$$VP = 0.26 \cdot \text{Pa}$$

$$F_{\text{ass\_aer}} := \frac{\text{product}}{VP + \text{product}}$$

#### degradation in the atmosphere

$$k_{\text{deg\_air}} = 0.433 \text{ h}^{-1}$$

$$F_{\text{ass\_aer}} = 3.851 \cdot 10^{-4}$$

## **Appendix A II**

of the Risk Assessment Report

TDA

Continental and Regional Exposure

**July 2003**

## Calculation for 2,4-TDA

EUSES Full report Single substance

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Study 2,4-TDA  
Assessment types 1B  
Explanation status column 'O' = Output; 'D' = Default; 'S' = Set; 'I' = Imported

### STUDY IDENTIFICATION

Study name	2,4-TDA	2,4-TDA	S
Study description	Regional	Regional	S
		concentration	concentration

### PHYSICO-CHEMICAL PROPERTIES

Molecular weight			122.17
122.17	[g.mol <sup>-1</sup> ]	S	
Melting point			99
99	[oC]	S	
Boiling point			288
288	[oC]	S	
Vapour pressure at 25 [oC]		0.017	0.017
[Pa]	S		
Octanol-water partition coefficient.		0.074	0.074
[log <sub>10</sub> ]	S		



Water solubility [mg.l-1]      S	3.8E+04	3.8E+04
-------------------------------------	---------	---------

## RELEASE ESTIMATION

### CONTINENTAL

Total continental emission to air [kg.d-1]      O	0	0
Total continental emission to wastewater [kg.yr-1]      S	0	3.392E+03
Total continental emission to surface water [kg.yr-1]      S	0	158
Total continental emission to industrial soil [kg.d-1]      O	0	0
Total continental emission to agricultural soil [kg.d-1]      O	0	0

### REGIONAL

Total regional emission to air 0 [kg.d-1]      O		0
Total regional emission to wastewater 376 [kg.yr-1]      S		0
Total regional emission to surface water 18 [kg.yr-1]      S		0
Total regional emission to industrial soil [kg.d-1]      O	0	0
Total regional emission to agricultural soil [kg.d-1]      O	0	0

## DISTRIBUTION

### PARTITION COEFFICIENTS

### SOLIDS WATER PARTITIONING

Organic carbon-water partition coefficient	1.44647	9.763E+03	[l.kg-1]
S			
Solids-water partition coefficient in soil		0.0289	
195	[l.kg-1]	O	
Solids-water partition coefficient in sediment		0.0723	
488	[l.kg-1]	O	
Solids-water partition coefficient suspended		0.145	
976	[l.kg-1]	O	
matter			
Solids-water partition coefficient in raw sewage		0.434	
2.93E+03	[l.kg-1]	O	
sludge			
Solids-water partition coefficient in settled	0.434		2.93E+03
[l.kg-1]	O		
sewage sludge			
Solids-water partition coefficient in activated		0.535	
3.61E+03	[l.kg-1]	O	
sewage sludge			
Solids-water partition coefficient in effluent		0.535	
3.61E+03	[l.kg-1]	O	
sewage sludge			
Suspended matter-water partition coefficient		0.936	
245	[m3.m-3]	O	
Soil-water partition coefficient			0.243
293	[m3.m-3]	O	
Sediment-water partition coefficient		0.836	
245	[m3.m-3]	O	

### AIR-WATER PARTITIONING AND ADSORPTION TO AEROSOL PARTICLES

Sub-cooled liquid vapour pressure		0.135	
0.135	[Pa]	O	

Fraction of chemical associated with aerosol particles	7.4E-04	7.4E-04	
	[-]	O	
Henry's law constant	5.47E-05		5.47E-05
	[Pa.m3.mol-1]	O	
Air-water partitioning coefficient		2.31E-08	2.31E-08
	[m3.m-3]	O	

#### BIOTA-WATER

Bioconcentration factor for aquatic biota	1.41		1.41
	[l.kg-1]	O	

#### DEGRADATION AND TRANSFORMATION RATES

##### ENVIRONMENTAL

Specific degradation rate constant with OH-radicals			0	0
	[cm3.molec-1.s-1]	S		
Rate constant for degradation in air			0	0
	[d-1]	S		
Rate constant for hydrolysis in surface water			6.93147E-07	0
	[d-1]	S		
Rate constant for photolysis in surface water			6.93147E-07	0
	[d-1]	S		
Rate constant for biodegradation in surface water			4.62098E-03	0
	[d-1]	S		
Total rate constant for degradation in bulk surface water	4.62E-03		0	[d-1]
		O		
Rate constant for biodegradation in bulk soil			2.31049E-03	
	[d-1]	S		
Total rate constant for degradation in bulk soil			2.31E-03	
	[d-1]	O		

Rate constant for biodegradation in aerated 2.31049E-03 6.9E-04  
[d-1] S

sediment

Total rate constant for degradation in bulk 2.31E-04 6.9E-05  
[d-1] O

sediment

### CONTINENTAL

Continental PEC in surface water (total) 0  
2.52E-06 [mg.l-1] O

Continental PEC in surface water (dissolved) 0  
2.46E-06 [mg.l-1] O

Continental PEC in air (total) 0  
2.45E-15 [mg.m-3] O

Continental PEC in agricultural soil (total) 0 8.49E-10  
[mg.kgwwt-1]O

Continental PEC in pore water of agricultural 0  
4.93E-12 [mg.l-1] O

soils

Continental PEC in natural soil (total) 0  
3.17E-09 [mg.kgwwt-1]O

Continental PEC in industrial soil (total) 0 3.17E-09  
[mg.kgwwt-1]O

Continental PEC in sediment (total) 0  
8.77E-04 [mg.kgwwt-1]O

### REGIONAL

Regional PEC in surface water (total) 0  
8.59E-06 [mg.l-1] O

Regional PEC in surface water (dissolved) 0 8.47E-06  
[mg.l-1] O

Regional PEC in air (total)			0
8.36E-15	[mg.m-3]	O	
Regional PEC in agricultural soil (total)			0
2.89E-09	[mg.kgwwt-1]	O	
Regional PEC in pore water of agricultural soils			0
1.68E-11	[mg.l-1]	O	
Regional PEC in natural soil (total)			0
1.08E-08	[mg.kgwwt-1]	O	
Regional PEC in industrial soil (total)	0	1.08E-08	[mg.kgwwt-1]
O			
Regional PEC in sediment (total)	0	2.93E-03	[mg.kgwwt-1]
O			

## Calculation for 2,6-TDA

EUSES Full report      Single substance

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Study                      2,6-TDA

Assessment types        1B

Explanation status column'O' = Output; 'D' = Default; 'S' = Set; 'I' = Imported

Name	Reference	Value	Units	Status
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### STUDY

#### STUDY IDENTIFICATION

Study name		2,6-TDA	2,6-TDA	S
Study description		Regional	Regional	S
			concentration concentration	

#### PHYSICO-CHEMICAL PROPERTIES

Molecular weight				122.17
122.17		[g.mol <sup>-1</sup> ]	S	
Melting point				106
106		[oC]	S	
Boiling point				289
289		[oC]	S	
Vapour pressure at 25 [oC]			0.029	0.029
[Pa]		S		
Octanol-water partition coefficient.			-0.137	-0.137
[log <sub>10</sub> ]		S		
Water solubility			5.4E+04	5.4E+04
[mg.l <sup>-1</sup> ]	S			

## RELEASE ESTIMATION

### CONTINENTAL

Total continental emission to air [kg.d-1] O	0	0
Total continental emission to wastewater [kg.yr-1] S	0	397
Total continental emission to surface water [kg.yr-1] S	0	40
Total continental emission to industrial soil [kg.d-1] O	0	
Total continental emission to agricultural soil [kg.d-1] O	0	0

### REGIONAL

Total regional emission to air [kg.d-1] O		0	0
Total regional emission to wastewater [kg.yr-1] S	0	45	
Total regional emission to surface water [kg.yr-1] S	0	4	
Total regional emission to industrial soil [kg.d-1] O	0	0	
Total regional emission to agricultural soil [kg.d-1] O	0	0	

## DISTRIBUTION

### PARTITION COEFFICIENTS

#### SOLIDS WATER PARTITIONING

Organic carbon-water partition coefficient	0.975889	7.805E+03	[l.kg-1]	S
Solids-water partition coefficient in soil 156		0.0195	[l.kg-1]	O
Solids-water partition coefficient in sediment 390		0.0488	[l.kg-1]	O

Solids-water partition coefficient suspended 781 [l.kg-1] O	0.0976	
matter		
Solids-water partition coefficient in raw sewage 2.34E+03 [l.kg-1] O	0.293	
sludge		
Solids-water partition coefficient in settled [l.kg-1] O	0.293	2.34E+03
sewage sludge		
Solids-water partition coefficient in activated 2.89E+03 [l.kg-1] O	0.361	
sewage sludge		
Solids-water partition coefficient in effluent 2.89E+03 [l.kg-1] O	0.361	
sewage sludge		
Suspended matter-water partition coefficient 196 [m3.m-3] O	0.924	
Soil-water partition coefficient 234 [m3.m-3] O		0.229
Sediment-water partition coefficient 196 [m3.m-3] O	0.824	

AIR-WATER PARTITIONING AND ADSORPTION TO AEROSOL PARTICLES

Sub-cooled liquid vapour pressure 0.272 [Pa] O	0.272	
Fraction of chemical associated with aerosol [-] O	3.67E-04	3.67E-04
particles		
Henry's law constant 6.56E-05 [Pa.m3.mol-1] O		6.56E-05
Air-water partitioning coefficient [m3.m-3] O	2.77E-08	2.77E-08



## BIOTA-WATER

Bioconcentration factor for aquatic biota	1.41	1.41
[l.kg <sup>-1</sup> ]	O	

## DEGRADATION AND TRANSFORMATION RATES

### ENVIRONMENTAL

Specific degradation rate constant with		0	0
[cm <sup>3</sup> .molec <sup>-1</sup> .s <sup>-1</sup> ]	S		

#### OH-radicals

Rate constant for degradation in air		0
0 [d <sup>-1</sup> ]	S	

Rate constant for hydrolysis in surface water		6.93147E-07	0
[d <sup>-1</sup> ]	S		

Rate constant for photolysis in surface water		6.93147E-07	0
[d <sup>-1</sup> ]	S		

Rate constant for biodegradation in surface water	4.62098E-03	0
[d <sup>-1</sup> ]	S	

Total rate constant for degradation in bulk	4.62E-03	0
[d <sup>-1</sup> ]	O	

#### surface water

Rate constant for biodegradation in bulk soil		2.31049E-03
6.9E-04 [d <sup>-1</sup> ]	S	

Total rate constant for degradation in bulk soil		2.31E-03
6.9E-04 [d <sup>-1</sup> ]	O	

Rate constant for biodegradation in aerated	2.31049E-03	6.9E-04
[d <sup>-1</sup> ]	S	

#### sediment

Total rate constant for degradation in bulk	2.31E-04	6.9E-05
[d <sup>-1</sup> ]	O	

#### sediment

### CONTINENTAL

Continental PEC in surface water (total) 5.3E-07 [mg.l-1] O	0	
Continental PEC in surface water (dissolved) 5.2E-07 [mg.l-1] O	0	
Continental PEC in air (total) 7.47E-16 [mg.m-3] O		0
Continental PEC in agricultural soil (total) [mg.kgwwt-1]O	0	2.14E-10
Continental PEC in pore water of agricultural 1.55E-12 [mg.l-1] O soils		0
Continental PEC in natural soil (total) 7.87E-10 [mg.kgwwt-1]O		0
Continental PEC in industrial soil (total) [mg.kgwwt-1]O	0	7.87E-10
Continental PEC in sediment (total) 1.47E-04 [mg.kgwwt-1]O		0
<b>REGIONAL</b>		
Regional PEC in surface water (total) 1.79E-06 [mg.l-1] O		0
Regional PEC in surface water (dissolved) [mg.l-1] O	0	1.77E-06
Regional PEC in air (total) 2.51E-15 [mg.m-3] O		0
Regional PEC in agricultural soil (total) 7.19E-10 [mg.kgwwt-1]O		0
Regional PEC in pore water of agricultural soils 5.22E-12 [mg.l-1] O		0
Regional PEC in natural soil (total) 2.64E-09 [mg.kgwwt-1]O		0
Regional PEC in industrial soil (total) 2.64E-09 [mg.kgwwt-1]O		0
Regional PEC in sediment (total) 4.82E-04 [mg.kgwwt-1]O		0

## **Appendix A III**

of the Risk Assessment Report

TDA

**Indirect exposure via the environment**

**July 2004**

## Calculation for 2,4-TDA

### INDIRECT EXPOSURE VIA THE ENVIRONMENT

( TGD On New and Existing Chemicals, chapter 2 )

*Parameter [Unit]*

*Symbol*

#### Definitions ( for the use in this document )

definition of the unit 'kg<sub>bw</sub>' for body weight

kg<sub>bw</sub> := 1·kg

definition of the unit 'd' for day

d := 1·Tag

scenario := 1.. 2

local := 1

regional := 2

#### Constants

gas - constant R

R := 8.314J·K<sup>-1</sup>·mol<sup>-1</sup>

#### Defaults

volume fraction air in plant tissue  
[-]

F<sub>air plant</sub> := 0.3

volume fraction water in plant tissue  
[-]

F<sub>water plant</sub> := 0.65

volume fraction lipids in plant tissue  
[-]

F<sub>lipid plant</sub> := 0.01

bulk density of plant tissue  
[kg<sub>wet plant</sub>·m<sub>plant</sub><sup>-3</sup>]

RHO<sub>plant</sub> := 700·kg·m<sup>-3</sup>

leaf surface area  
[m<sup>2</sup>]

AREA<sub>plant</sub> := 5·m<sup>2</sup>

conductance (0.001 m<sup>3</sup>·s<sup>-1</sup>)  
[m<sup>3</sup>·d<sup>-1</sup>]

g<sub>plant</sub> := 0.001·m·s<sup>-1</sup>

shoot volume  
[m<sup>3</sup>]

V<sub>leaf</sub> := 0.002·m<sup>3</sup>

transpiration stream  
[m<sup>3</sup>·d<sup>-1</sup>]

Q<sub>transp</sub> := 1·10<sup>-3</sup>·m<sup>3</sup>·d<sup>-1</sup>

correction exponent for differences  
between plant lipids and octanol  
[-]

b := 0.95

growth rate constant for dilution by growth  
[d<sup>-1</sup>]

kgrowth<sub>plant</sub> := 0.035·d<sup>-1</sup>

pseudo-first order rate constant for metabolism in plants  
[d<sup>-1</sup>]

kmetab<sub>plant</sub> := 0·d<sup>-1</sup>

pseudo-first order rate constant for photolysis in plants  
[d<sup>-1</sup>]

kphoto<sub>plant</sub> := 0·d<sup>-1</sup>



concentration in meat and milk

daily intake of grass

$[\text{kg}_{\text{wetgrass}} \cdot \text{d}^{-1}]$

$$\text{IC}_{\text{grass}} := 67.6 \cdot \text{kg} \cdot \text{d}^{-1}$$

daily intake of soil

$[\text{kg}_{\text{wet soil}} \cdot \text{d}^{-1}]$

$$\text{IC}_{\text{soil}} := 0.46 \cdot \text{kg} \cdot \text{d}^{-1}$$

daily intake of air

$[\text{m}_{\text{air}}^3 \cdot \text{d}^{-1}]$

$$\text{IC}_{\text{air}} := 122 \cdot \text{m}^3 \cdot \text{d}^{-1}$$

daily intake of drinkingwater

$[\text{l} \cdot \text{d}^{-1}]$

$$\text{IC}_{\text{drw}} := 55 \cdot \text{l} \cdot \text{d}^{-1}$$

*daily intake for human*

daily intake for the several pathways

$[\text{kg}_{\text{chem}} \cdot \text{d}^{-1}]$  or  $[\text{m}^3 \cdot \text{d}^{-1}]$

$$\text{IH}_{\text{drw}} := 2 \cdot \text{l} \cdot \text{d}^{-1}$$

$$\text{IH}_{\text{fish}} := 0.115 \cdot \text{kg} \cdot \text{d}^{-1}$$

$$\text{IH}_{\text{stem}} := 1.2 \cdot \text{kg} \cdot \text{d}^{-1}$$

$$\text{IH}_{\text{root}} := 0.384 \cdot \text{kg} \cdot \text{d}^{-1}$$

$$\text{IH}_{\text{meat}} := 0.301 \cdot \text{kg} \cdot \text{d}^{-1}$$

$$\text{IH}_{\text{milk}} := 0.561 \cdot \text{kg} \cdot \text{d}^{-1}$$

$$\text{IH}_{\text{air}} := 20 \cdot \text{m}^3 \cdot \text{d}^{-1}$$

bioavailability through route of intake  
[-]

$$\text{BIO}_{\text{inh}} := 0.75$$

$$\text{BIO}_{\text{oral}} := 1.0$$

average body weight of human  
[kg]

$$\text{BW} := 70 \cdot \text{kg}_{\text{bw}}$$

## Input

### *chemical properties*

octanol-water partitioning coefficient  
[-]

$$\log K_{OW} := 0.074$$

Henry - partitioning coefficient  
[Pa·m<sup>3</sup>·mol<sup>-1</sup>]

$$K_{OW} := 10^{\log K_{OW}}$$

$$HENRY := 5.458 \cdot 10^{-5} \cdot \text{Pa} \cdot \text{m}^3 \cdot \text{mol}^{-1}$$

air-water partitioning coefficient  
[-]

$$K_{air\_water} := 2.24 \cdot 10^{-8}$$

fraction of the chemical associated  
with aerosol particles  
[-]

$$F_{ass\_aer} := 1.0 \cdot 10^{-8}$$

half-life for biodegradation in surface water  
[d]

$$DT_{50\_bio\_water} := 0 \cdot \text{d}$$

### *environmental concentrations*

annual average local PEC in surface water (dissolved)  
[mg<sub>chem</sub> \* l<sub>water</sub><sup>-1</sup>]

$$PEC_{local\_water\_ann} := 0.0005 \cdot \text{mg} \cdot \text{l}^{-1}$$

annual average local PEC in air (total)  
[mg<sub>chem</sub> \* m<sub>air</sub><sup>-3</sup>]

$$PEC_{local\_air\_ann} := 0 \cdot \text{mg} \cdot \text{m}^{-3}$$

local PEC in grassland (total), averaged over 180 days  
[mg<sub>chem</sub> \* kg<sub>soil</sub><sup>-1</sup>]

$$PEC_{local\_grassland} := 0 \cdot \text{mg} \cdot \text{kg}^{-1}$$

local PEC in porewater of agriculture soil  
[mg<sub>chem</sub> \* l<sub>porewater</sub><sup>-1</sup>]

$$PEC_{local\_agr\_soil\_porew} := 0 \cdot \text{mg} \cdot \text{l}^{-1}$$

local PEC in porewater of grassland  
[mg<sub>chem</sub> \* l<sub>porewater</sub><sup>-1</sup>]

$$PEC_{local\_grassland\_porew} := 0 \cdot \text{mg} \cdot \text{l}^{-1}$$

local PEC in groundwater under agriculture soil  
[mg<sub>chem</sub> \* l<sub>water</sub><sup>-1</sup>]

$$PEC_{local\_grw} := 0 \cdot \text{mg} \cdot \text{l}^{-1}$$

regional PEC in surface water (dissolved)  
[mg<sub>chem</sub> \* l<sub>water</sub><sup>-1</sup>]

$$PEC_{regional\_water} := 5.46 \cdot 10^{-6} \cdot \text{mg} \cdot \text{l}^{-1}$$

regional PEC in air (total)  
[mg<sub>chem</sub> \* m<sub>air</sub><sup>-3</sup>]

$$PEC_{regional\_air} := 5.4 \cdot 10^{-15} \cdot \text{mg} \cdot \text{m}^{-3}$$

regional PEC in agriculture soil (total)  
[mg<sub>chem</sub> \* kg<sub>soil</sub><sup>-1</sup>]

$$PEC_{regional\_agr\_soil} := 1.87 \cdot 10^{-9} \cdot \text{mg} \cdot \text{kg}^{-1}$$

regional PEC in porewater of agriculture soils  
[mg<sub>chem</sub> \* l<sub>water</sub><sup>-1</sup>]

$$PEC_{regional\_agr\_soil\_porew} := 1.1 \cdot 10^{-11} \cdot \text{mg} \cdot \text{l}^{-1}$$

Definition of the concentrations used for indirect exposure

$$\begin{array}{ll}
 C_{\text{water\_local}} := \text{PEC}_{\text{local\_water\_ann}} & C_{\text{water\_regional}} := \text{PEC}_{\text{regional\_water}} \\
 C_{\text{air\_local}} := \text{PEC}_{\text{local\_air\_ann}} & C_{\text{air\_regional}} := \text{PEC}_{\text{regional\_air}} \\
 C_{\text{grassland\_local}} := \text{PEC}_{\text{local\_grassland}} & C_{\text{grassland\_regional}} := \text{PEC}_{\text{regional\_agr\_soil}} \\
 C_{\text{agr\_porew\_local}} := \text{PEC}_{\text{local\_agr\_soil\_porew}} & C_{\text{agr\_porew\_regional}} := \text{PEC}_{\text{regional\_agr\_soil\_porew}} \\
 C_{\text{grass\_porew\_local}} := \text{PEC}_{\text{local\_grassland\_porew}} & C_{\text{grass\_porew\_regional}} := \text{PEC}_{\text{regional\_agr\_soil\_porew}} \\
 C_{\text{grw\_local}} := \text{PEC}_{\text{local\_grw}} & C_{\text{grw\_regional}} := \text{PEC}_{\text{regional\_agr\_soil\_porew}}
 \end{array}$$

### bioconcentration in fish

bioconcentration factor for fish

$$[m_{\text{water}}^3 \cdot \text{kg}_{\text{chem}}^{-1}] \quad \text{BCF}_{\text{fish}} := 5 \text{ l} \cdot \text{kg}^{-1}$$

modified equation for  $\log K_{\text{OW}} > 6$

$$\text{BCF}_{\text{fish}} := \text{wenn} \left[ \log K_{\text{OW}} > 6, \left[ -0.278 \cdot (\log K_{\text{OW}})^2 + 3.38 \cdot \log K_{\text{OW}} - 5.94 \right] \cdot \text{kg}^{-1}, \text{BCF}_{\text{fish}} \right]$$

$$C_{\text{fish\_scenario}} := \text{BCF}_{\text{fish}} \cdot C_{\text{water\_scenario}}$$

### bioconcentration in plants

$$K_{\text{plant\_water}} := F_{\text{water\_plant}} + \text{Flipid}_{\text{plant}} \cdot K_{\text{OW}}^b$$

$$C_{\text{root\_agr\_plant\_scenario}} := \frac{K_{\text{plant\_water}} \cdot C_{\text{agr\_porew\_scenario}}}{\text{RHO}_{\text{plant}}}$$

$$\text{TSCF} := 0.784 \cdot e^{\frac{-(\log K_{\text{OW}} - 1.78)^2}{2.44}}$$

remark: for  $\log K_{\text{OW}}$  out of the range from -0.5 to 4.5

the TSCF is limited by the values for  $\log K_{\text{OW}} = -0.5$  resp. 4.5

$$\text{TSCF} := \text{wenn} (\log K_{\text{OW}} < -0.5, 0.903, \text{TSCF})$$

$$\text{TSCF} := \text{wenn} (\log K_{\text{OW}} > 4.5, 0.832, \text{TSCF})$$

$$K_{\text{leaf\_air}} := \text{Fair}_{\text{plant}} + \frac{K_{\text{plant\_water}}}{K_{\text{air\_water}}}$$

$$\text{kelim}_{\text{plant}} := \text{kmetab}_{\text{plant}} + \text{kphoto}_{\text{plant}}$$

$$\alpha := \frac{\text{AREA}_{\text{plant}} \cdot g_{\text{plant}}}{K_{\text{leaf\_air}} \cdot V_{\text{leaf}}} + \text{kelim}_{\text{plant}} + \text{kgrowth}_{\text{plant}}$$



$$\beta_{agr\_plant\_scenario} := C_{agr\_porew\_scenario} \cdot TSCF \cdot \frac{Q_{transp}}{V_{leaf}} + (1 - F_{ass\_aer}) \cdot C_{air\_scenario} \cdot g_{plant} \cdot \frac{AREA_{plant}}{V_{leaf}}$$

$$C_{leaf\_crops\_scenario} := \frac{\beta_{agr\_plant\_scenario}}{\alpha \cdot RHO_{plant}}$$

$$\beta_{grass\_plant\_scenario} := C_{grass\_porew\_scenario} \cdot TSCF \cdot \frac{Q_{transp}}{V_{leaf}} + (1 - F_{ass\_aer}) \cdot C_{air\_scenario} \cdot g_{plant} \cdot \frac{AREA_{plant}}{V_{leaf}}$$

$$C_{leaf\_grass\_scenario} := \frac{\beta_{grass\_plant\_scenario}}{\alpha \cdot RHO_{plant}}$$

### purification of drinking water

system may defined dependent from the aerobic biodegradation

$$system := wenn(DT_{50\_bio\_water} < 10 \cdot d, 0, 1)$$

select a column on dependence from log K<sub>OW</sub>

$$FIndex := wenn(\log K_{OW} < 4, 0, wenn(\log K_{OW} > 5, 2, 1))$$

$$Fpur_{\log Kow} := \begin{bmatrix} 1 & \frac{1}{4} & \frac{1}{16} \\ 1 & \frac{1}{2} & \frac{1}{4} \end{bmatrix}$$

$$Fpur := \frac{Fpur_{\log Kow}_{system, FIndex}}{wenn(HENRY > 100 \cdot Pa \cdot m^3 \cdot mol^{-1}, 2, 1)}$$

$$C_{drw\_scenario} := wenn\left[C_{grw\_scenario} > \left(C_{water\_scenario} \cdot Fpur\right), C_{grw\_scenario}, C_{water\_scenario} \cdot Fpur\right]$$

### Biotransfer to meat and milk

$$BTF_{meat} := 10^{-7.6 + \log K_{OW}} \cdot kg^{-1} \cdot d$$

remark: for logK<sub>OW</sub> out of the range from 1.5 to 6.5

the BTF<sub>meat</sub> is limited by the values for logK<sub>OW</sub> = 1.5 resp. 6.5

$$BTF_{meat} := wenn(\log K_{OW} < 1.5, 7.943 \cdot 10^{-7} \cdot kg^{-1} \cdot d, BTF_{meat})$$

$$BTF_{meat} := wenn(\log K_{OW} > 6.5, 0.07943 \cdot kg^{-1} \cdot d, BTF_{meat})$$

$$C_{meat\_scenario} := BTF_{meat} \cdot \left( C_{leaf\_grass\_scenario} \cdot IC_{grass} + C_{grassland\_scenario} \cdot IC_{soil} \dots \right. \\ \left. + C_{air\_scenario} \cdot IC_{air} + C_{drw\_scenario} \cdot IC_{drw} \right)$$

$$BTF_{\text{milk}} := 10^{-8.1 + \log K_{\text{OW}}} \cdot \text{kg}^{-1} \cdot \text{d}$$

remark: for  $\log K_{\text{OW}}$  out of the range from 3 to 6.5

the  $BTF_{\text{milk}}$  is limited by the values for  $\log K_{\text{OW}} = 1.5$  resp. 6.5

$$BTF_{\text{milk}} := \text{wenn} \left( \log K_{\text{OW}} < 3, 7.943 \cdot 10^{-6} \cdot \text{kg}^{-1} \cdot \text{d}, BTF_{\text{milk}} \right)$$

$$BTF_{\text{milk}} := \text{wenn} \left( \log K_{\text{OW}} > 6.5, 0.02512 \cdot \text{kg}^{-1} \cdot \text{d}, BTF_{\text{milk}} \right)$$

$$C_{\text{milk}_{\text{scenario}}} := BTF_{\text{milk}} \cdot \left( C_{\text{leaf\_grass}_{\text{scenario}}} \cdot IC_{\text{grass}} + C_{\text{grassland}_{\text{scenario}}} \cdot IC_{\text{soil}} \dots \right) \\ \left( + C_{\text{air}_{\text{scenario}}} \cdot IC_{\text{air}} + C_{\text{drw}_{\text{scenario}}} \cdot IC_{\text{drw}} \right)$$

total daily intake for human

daily dose through intake of several pathways

[kg<sub>chem</sub> \* kg<sub>bw</sub><sup>-1</sup> \* d<sup>-1</sup>]

$$\text{DOSE}_{\text{drw\_scenario}} := \frac{C_{\text{drw\_scenario}} \cdot \text{IH}_{\text{drw}}}{\text{BW}}$$

$$\text{DOSE}_{\text{air\_scenario}} := \frac{C_{\text{air\_scenario}} \cdot \text{IH}_{\text{air}} \cdot \text{BIO}_{\text{inh}}}{\text{BW} \cdot \text{BIO}_{\text{oral}}}$$

$$\text{DOSE}_{\text{stem\_scenario}} := \frac{C_{\text{leaf\_crops\_scenario}} \cdot \text{IH}_{\text{stem}}}{\text{BW}}$$

$$\text{DOSE}_{\text{root\_scenario}} := \frac{C_{\text{root\_agr\_plant\_scenario}} \cdot \text{IH}_{\text{root}}}{\text{BW}}$$

$$\text{DOSE}_{\text{meat\_scenario}} := \frac{C_{\text{meat\_scenario}} \cdot \text{IH}_{\text{meat}}}{\text{BW}}$$

$$\text{DOSE}_{\text{milk\_scenario}} := \frac{C_{\text{milk\_scenario}} \cdot \text{IH}_{\text{milk}}}{\text{BW}}$$

$$\text{DOSE}_{\text{fish\_scenario}} := \frac{C_{\text{fish\_scenario}} \cdot \text{IH}_{\text{fish}}}{\text{BW}}$$

*total daily intake for human*

total daily intake for human as sum of each pathway

[kg<sub>chem</sub> \* kg<sub>bw</sub><sup>-1</sup> \* d<sup>-1</sup>]

$$\text{DOSE}_{\text{tot\_scenario}} := \text{DOSE}_{\text{drw\_scenario}} + \text{DOSE}_{\text{fish\_scenario}} + \text{DOSE}_{\text{stem\_scenario}} + \text{DOSE}_{\text{root\_scenario}} + \dots + \text{DOSE}_{\text{meat\_scenario}} + \text{DOSE}_{\text{milk\_scenario}} + \text{DOSE}_{\text{air\_scenario}}$$

relative doses of specific different pathway (%)

$$\text{RDOSE}_{\text{drw\_scenario}} := \frac{\text{DOSE}_{\text{drw\_scenario}} \cdot 100\%}{\text{DOSE}_{\text{tot\_scenario}}}$$

$$\text{RDOSE}_{\text{air\_scenario}} := \frac{\text{DOSE}_{\text{air\_scenario}} \cdot 100\%}{\text{DOSE}_{\text{tot\_scenario}}}$$

$$\text{RDOSE}_{\text{stem\_scenario}} := \frac{\text{DOSE}_{\text{stem\_scenario}} \cdot 100\%}{\text{DOSE}_{\text{tot\_scenario}}}$$

$$\text{RDOSE}_{\text{root\_scenario}} := \frac{\text{DOSE}_{\text{root\_scenario}} \cdot 100\%}{\text{DOSE}_{\text{tot\_scenario}}}$$

$$\text{RDOSE}_{\text{meat\_scenario}} := \frac{\text{DOSE}_{\text{meat\_scenario}} \cdot 100\%}{\text{DOSE}_{\text{tot\_scenario}}}$$

$$\text{RDOSE}_{\text{milk\_scenario}} := \frac{\text{DOSE}_{\text{milk\_scenario}} \cdot 100\%}{\text{DOSE}_{\text{tot\_scenario}}}$$

$$\text{RDOSE}_{\text{fish\_scenario}} := \frac{\text{DOSE}_{\text{fish\_scenario}} \cdot 100\%}{\text{DOSE}_{\text{tot\_scenario}}}$$

## Results of calculation

$$\text{DOSE}_{\text{tot}_{\text{local}}} = 1.83947 \times 10^{-5} \frac{\text{mg}}{\text{kgbw} \cdot \text{d}}$$

$$\text{DOSE}_{\text{tot}_{\text{regional}}} = 2.008716 \times 10^{-7} \frac{\text{mg}}{\text{kgbw} \cdot \text{d}}$$

$$\text{RDOSE}_{\text{drw}_{\text{local}}} = 77.662115\%$$

$$\text{RDOSE}_{\text{drw}_{\text{regional}}} = 77.661538\%$$

$$\text{RDOSE}_{\text{air}_{\text{local}}} = 0\%$$

$$\text{RDOSE}_{\text{air}_{\text{regional}}} = 5.760609 \times 10^{-7} \%$$

$$\text{RDOSE}_{\text{stem}_{\text{local}}} = 0\%$$

$$\text{RDOSE}_{\text{stem}_{\text{regional}}} = 7.130201 \times 10^{-4} \%$$

$$\text{RDOSE}_{\text{root}_{\text{local}}} = 0\%$$

$$\text{RDOSE}_{\text{root}_{\text{regional}}} = 2.839931 \times 10^{-5} \%$$

$$\text{RDOSE}_{\text{meat}_{\text{local}}} = 5.106143 \times 10^{-4} \%$$

$$\text{RDOSE}_{\text{meat}_{\text{regional}}} = 5.106216 \times 10^{-4} \%$$

$$\text{RDOSE}_{\text{milk}_{\text{local}}} = 9.516765 \times 10^{-3} \%$$

$$\text{RDOSE}_{\text{milk}_{\text{regional}}} = 9.5169 \times 10^{-3} \%$$

$$\text{RDOSE}_{\text{fish}_{\text{local}}} = 22.327858\%$$

$$\text{RDOSE}_{\text{fish}_{\text{regional}}} = 22.327692\%$$

## **6.1            CALCULATION FOR 2,6-TDA**

**INDIRECT EXPOSURE VIA THE ENVIRONMENT**  
( TGD On New and Existing Chemicals, chapter 2 )

<i>Parameter [Unit]</i>	<i>Symbol</i>
<b>Definitions ( for the use in this document )</b>	
definition of the unit 'kg <sub>bw</sub> ' for body weight	kg <sub>bw</sub> := 1·kg
definition of the unit 'd' for day	d := 1·Tag
	scenario := 1.. 2
	local := 1
	regional := 2
<b>Constants</b>	
gas - constant R	R := 8.314·J·K <sup>-1</sup> ·mol <sup>-1</sup>
<b>Defaults</b>	
volume fraction air in plant tissue [-]	F <sub>air plant</sub> := 0.3
volume fraction water in plant tissue [-]	F <sub>water plant</sub> := 0.65
volume fraction lipids in plant tissue [-]	F <sub>lipid plant</sub> := 0.01
bulk density of plant tissue [kg <sub>wet plant</sub> · m <sub>plant</sub> <sup>-3</sup> ]	RHO <sub>plant</sub> := 700·kg·m <sup>-3</sup>
leaf surface area [m <sup>2</sup> ]	AREA <sub>plant</sub> := 5·m <sup>2</sup>
conductance (0.001 m·s <sup>-1</sup> ) [m <sup>3</sup> ·d <sup>-1</sup> ]	g <sub>plant</sub> := 0.001·m·s <sup>-1</sup>
shoot volume [m <sup>3</sup> ]	V <sub>leaf</sub> := 0.002·m <sup>3</sup>
transpiration stream [m <sup>3</sup> ·d <sup>-1</sup> ]	Q <sub>transp</sub> := 1·10 <sup>-3</sup> ·m <sup>3</sup> ·d <sup>-1</sup>
correction exponent for differences between plant lipids and octanol [-]	b := 0.95
growth rate constant for dilution by growth [d <sup>-1</sup> ]	kgrowth <sub>plant</sub> := 0.035·d <sup>-1</sup>
pseudo-first order rate constant for metabolism in plants [d <sup>-1</sup> ]	kmetab <sub>plant</sub> := 0·d <sup>-1</sup>
pseudo-first order rate constant for photolysis in plants [d <sup>-1</sup> ]	kphoto <sub>plant</sub> := 0·d <sup>-1</sup>

concentration in meat and milk

daily intake of grass

$[\text{kg}_{\text{wet grass}} \cdot \text{d}^{-1}]$

$$\text{IC}_{\text{grass}} := 67.6 \cdot \text{kg} \cdot \text{d}^{-1}$$

daily intake of soil

$[\text{kg}_{\text{wet soil}} \cdot \text{d}^{-1}]$

$$\text{IC}_{\text{soil}} := 0.46 \cdot \text{kg} \cdot \text{d}^{-1}$$

daily intake of air

$[\text{m}_{\text{air}}^3 \cdot \text{d}^{-1}]$

$$\text{IC}_{\text{air}} := 122 \cdot \text{m}^3 \cdot \text{d}^{-1}$$

daily intake of drinkingwater

$[\text{l} \cdot \text{d}^{-1}]$

$$\text{IC}_{\text{drw}} := 55 \cdot \text{l} \cdot \text{d}^{-1}$$

*daily intake for human*

daily intake for the several pathways

$[\text{kg}_{\text{chem}} \cdot \text{d}^{-1}]$  or  $[\text{m}^3 \cdot \text{d}^{-1}]$

$$\text{IH}_{\text{drw}} := 2 \cdot \text{l} \cdot \text{d}^{-1}$$

$$\text{IH}_{\text{fish}} := 0.115 \cdot \text{kg} \cdot \text{d}^{-1}$$

$$\text{IH}_{\text{stem}} := 1.2 \cdot \text{kg} \cdot \text{d}^{-1}$$

$$\text{IH}_{\text{root}} := 0.384 \cdot \text{kg} \cdot \text{d}^{-1}$$

$$\text{IH}_{\text{meat}} := 0.301 \cdot \text{kg} \cdot \text{d}^{-1}$$

$$\text{IH}_{\text{milk}} := 0.561 \cdot \text{kg} \cdot \text{d}^{-1}$$

$$\text{IH}_{\text{air}} := 20 \cdot \text{m}^3 \cdot \text{d}^{-1}$$

bioavailability through route of intake

$[-]$

$$\text{BIO}_{\text{inh}} := 0.75$$

$$\text{BIO}_{\text{oral}} := 1.0$$

average body weight of human

$[\text{kg}]$

$$\text{BW} := 70 \cdot \text{kg}_{\text{bw}}$$

## Input

### *chemical properties*

octanol-water partitioning coefficient  
[-]

$$\log K_{OW} := -0.137$$

$$K_{OW} := 10^{\log K_{OW}}$$

Henry - partitioning coefficient  
[Pa·m<sup>3</sup>·mol<sup>-1</sup>]

$$HENRY := 6.55 \cdot 10^{-5} \cdot \text{Pa} \cdot \text{m}^3 \cdot \text{mol}^{-1}$$

air-water partitioning coefficient  
[-]

$$K_{air\_water} := 2.688 \cdot 10^{-8}$$

fraction of the chemical associated  
with aerosol particles  
[-]

$$F_{ass\_aer} := 1.0 \cdot 10^{-8}$$

half-life for biodegradation in surface water  
[d]

$$DT_{50\_bio\_water} := 0 \cdot \text{d}$$

### *environmental concentrations*

annual average local PEC in surface water (dissolved)  
[mg<sub>chem</sub> \* l<sub>water</sub><sup>-1</sup>]

$$PEC_{local\_water\_ann} := 0.00021 \cdot \text{mg} \cdot \text{l}^{-1}$$

annual average local PEC in air (total)  
[mg<sub>chem</sub> \* m<sub>air</sub><sup>-3</sup>]

$$PEC_{local\_air\_ann} := 0 \cdot \text{mg} \cdot \text{m}^{-3}$$

local PEC in grassland (total), averaged over 180 days  
[mg<sub>chem</sub> \* kg<sub>soil</sub><sup>-1</sup>]

$$PEC_{local\_grassland} := 0 \cdot \text{mg} \cdot \text{kg}^{-1}$$

local PEC in porewater of agriculture soil  
[mg<sub>chem</sub> \* l<sub>porewater</sub><sup>-1</sup>]

$$PEC_{local\_agr\_soil\_porew} := 0 \cdot \text{mg} \cdot \text{l}^{-1}$$

local PEC in porewater of grassland  
[mg<sub>chem</sub> \* l<sub>porewater</sub><sup>-1</sup>]

$$PEC_{local\_grassland\_porew} := 0 \cdot \text{mg} \cdot \text{l}^{-1}$$

local PEC in groundwater under agriculture soil  
[mg<sub>chem</sub> \* l<sub>water</sub><sup>-1</sup>]

$$PEC_{local\_grw} := 0 \cdot \text{mg} \cdot \text{l}^{-1}$$

regional PEC in surface water (dissolved)  
[mg<sub>chem</sub> \* l<sub>water</sub><sup>-1</sup>]

$$PEC_{regional\_water} := 1.77 \cdot 10^{-6} \cdot \text{mg} \cdot \text{l}^{-1}$$

regional PEC in air (total)  
[mg<sub>chem</sub> \* m<sub>air</sub><sup>-3</sup>]

$$PEC_{regional\_air} := 2.51 \cdot 10^{-15} \cdot \text{mg} \cdot \text{m}^{-3}$$

regional PEC in agriculture soil (total)  
[mg<sub>chem</sub> \* kg<sub>soil</sub><sup>-1</sup>]

$$PEC_{regional\_agr\_soil} := 7.19 \cdot 10^{-10} \cdot \text{mg} \cdot \text{kg}^{-1}$$

regional PEC in porewater of agriculture soils  
[mg<sub>chem</sub> \* l<sub>water</sub><sup>-1</sup>]

$$PEC_{regional\_agr\_soil\_porew} := 5.22 \cdot 10^{-12} \cdot \text{mg} \cdot \text{l}^{-1}$$



Definition of the concentrations used for indirect exposure

$$\begin{aligned}
 C_{\text{water\_local}} &:= \text{PEC}_{\text{local\_water\_ann}} & C_{\text{water\_regional}} &:= \text{PEC}_{\text{regional\_water}} \\
 C_{\text{air\_local}} &:= \text{PEC}_{\text{local\_air\_ann}} & C_{\text{air\_regional}} &:= \text{PEC}_{\text{regional\_air}} \\
 C_{\text{grassland\_local}} &:= \text{PEC}_{\text{local\_grassland}} & C_{\text{grassland\_regional}} &:= \text{PEC}_{\text{regional\_agr\_soil}} \\
 C_{\text{agr\_porew\_local}} &:= \text{PEC}_{\text{local\_agr\_soil\_porew}} & C_{\text{agr\_porew\_regional}} &:= \text{PEC}_{\text{regional\_agr\_soil\_porew}} \\
 C_{\text{grass\_porew\_local}} &:= \text{PEC}_{\text{local\_grassland\_porew}} & C_{\text{grass\_porew\_regional}} &:= \text{PEC}_{\text{regional\_agr\_soil\_porew}} \\
 C_{\text{grw\_local}} &:= \text{PEC}_{\text{local\_grw}} & C_{\text{grw\_regional}} &:= \text{PEC}_{\text{regional\_agr\_soil\_porew}}
 \end{aligned}$$

### bioconcentration in fish

bioconcentration factor for fish

$$[m_{\text{water}}^3 \cdot \text{kg}_{\text{chem}}^{-1}] \quad \text{BCF}_{\text{fish}} := 5 \text{ l} \cdot \text{kg}^{-1}$$

modified equation for  $\log K_{\text{OW}} > 6$

$$\text{BCF}_{\text{fish}} := \text{wenn} \left[ \log K_{\text{OW}} > 6, \left[ -0.278 \cdot (\log K_{\text{OW}})^2 + 3.38 \cdot \log K_{\text{OW}} - 5.94 \right] \cdot \text{kg}^{-1}, \text{BCF}_{\text{fish}} \right]$$

$$C_{\text{fish\_scenario}} := \text{BCF}_{\text{fish}} \cdot C_{\text{water\_scenario}}$$

### bioconcentration in plants

$$K_{\text{plant\_water}} := F_{\text{water\_plant}} + \text{Flipid}_{\text{plant}} \cdot K_{\text{OW}}^b$$

$$C_{\text{root\_agr\_plant\_scenario}} := \frac{K_{\text{plant\_water}} \cdot C_{\text{agr\_porew\_scenario}}}{\text{RHO}_{\text{plant}}}$$

$$\text{TSCF} := 0.784 \cdot e^{\frac{-(\log K_{\text{OW}} - 1.78)^2}{2.44}}$$

remark: for  $\log K_{\text{OW}}$  out of the range from -0.5 to 4.5

the TSCF is limited by the values for  $\log K_{\text{OW}} = -0.5$  resp. 4.5

$$\text{TSCF} := \text{wenn} (\log K_{\text{OW}} < -0.5, 0.903, \text{TSCF})$$

$$\text{TSCF} := \text{wenn} (\log K_{\text{OW}} > 4.5, 0.832, \text{TSCF})$$

$$K_{\text{leaf\_air}} := \text{Fair}_{\text{plant}} + \frac{K_{\text{plant\_water}}}{K_{\text{air\_water}}}$$

$$\text{kelim}_{\text{plant}} := \text{kmetab}_{\text{plant}} + \text{kphoto}_{\text{plant}}$$

$$\alpha := \frac{\text{AREA}_{\text{plant}} \cdot g_{\text{plant}}}{K_{\text{leaf\_air}} \cdot V_{\text{leaf}}} + \text{kelim}_{\text{plant}} + \text{kgrowth}_{\text{plant}}$$

$$\beta_{agr\_plant\_scenario} := C_{agr\_porew\_scenario} \cdot TSCF \cdot \frac{Q_{transp}}{V_{leaf}} + (1 - F_{ass\_aer}) \cdot C_{air\_scenario} \cdot g_{plant} \cdot \frac{AREA_{plant}}{V_{leaf}}$$

$$C_{leaf\_crops\_scenario} := \frac{\beta_{agr\_plant\_scenario}}{\alpha \cdot RHO_{plant}}$$

$$\beta_{grass\_plant\_scenario} := C_{grass\_porew\_scenario} \cdot TSCF \cdot \frac{Q_{transp}}{V_{leaf}} + (1 - F_{ass\_aer}) \cdot C_{air\_scenario} \cdot g_{plant} \cdot \frac{AREA_{plant}}{V_{leaf}}$$

$$C_{leaf\_grass\_scenario} := \frac{\beta_{grass\_plant\_scenario}}{\alpha \cdot RHO_{plant}}$$

### purification of drinking water

system may defined dependent from the aerobic biodegradation

$$system := wenn(DT_{50\_bio\_water} < 10 \cdot d, 0, 1)$$

select a column on dependence from log K<sub>OW</sub>

$$FIndex := wenn(\log K_{OW} < 4, 0, wenn(\log K_{OW} > 5, 2, 1))$$

$$Fpur_{\log Kow} := \begin{bmatrix} 1 & \frac{1}{4} & \frac{1}{16} \\ 1 & \frac{1}{2} & \frac{1}{4} \end{bmatrix}$$

$$Fpur := \frac{Fpur_{\log Kow_{system, FIndex}}}{wenn(HENRY > 100 \cdot Pa \cdot m^3 \cdot mol^{-1}, 2, 1)}$$

$$C_{drw\_scenario} := wenn\left[C_{grw\_scenario} > \left(C_{water\_scenario} \cdot Fpur\right), C_{grw\_scenario}, C_{water\_scenario} \cdot Fpur\right]$$

### Biotransfer to meat and milk

$$BTF_{meat} := 10^{-7.6 + \log K_{OW}} \cdot kg^{-1} \cdot d$$

remark: for logK<sub>OW</sub> out of the range from 1.5 to 6.5

the BTF<sub>meat</sub> is limited by the values for logK<sub>OW</sub> = 1.5 resp. 6.5

$$BTF_{meat} := wenn(\log K_{OW} < 1.5, 7.943 \cdot 10^{-7} \cdot kg^{-1} \cdot d, BTF_{meat})$$

$$BTF_{meat} := wenn(\log K_{OW} > 6.5, 0.07943 \cdot kg^{-1} \cdot d, BTF_{meat})$$

$$C_{meat\_scenario} := BTF_{meat} \cdot \left( C_{leaf\_grass\_scenario} \cdot IC_{grass} + C_{grassland\_scenario} \cdot IC_{soil} \dots \right. \\ \left. + C_{air\_scenario} \cdot IC_{air} + C_{drw\_scenario} \cdot IC_{drw} \right)$$

$$BTF_{\text{milk}} := 10^{-8.1 + \log K_{\text{OW}}} \cdot \text{kg}^{-1} \cdot \text{d}$$

remark: for  $\log K_{\text{OW}}$  out of the range from 3 to 6.5

the  $BTF_{\text{milk}}$  is limited by the values for  $\log K_{\text{OW}} = 1.5$  resp. 6.5

$$BTF_{\text{milk}} := \text{wenn}(\log K_{\text{OW}} < 3, 7.943 \cdot 10^{-6} \cdot \text{kg}^{-1} \cdot \text{d}, BTF_{\text{milk}})$$

$$BTF_{\text{milk}} := \text{wenn}(\log K_{\text{OW}} > 6.5, 0.02512 \text{kg}^{-1} \cdot \text{d}, BTF_{\text{milk}})$$

$$C_{\text{milk}_{\text{scenario}}} := BTF_{\text{milk}} \left( C_{\text{leaf\_grass}_{\text{scenario}}} \cdot IC_{\text{grass}} + C_{\text{grassland}_{\text{scenario}}} \cdot IC_{\text{soil}} \dots \right. \\ \left. + C_{\text{air}_{\text{scenario}}} \cdot IC_{\text{air}} + C_{\text{drw}_{\text{scenario}}} \cdot IC_{\text{drw}} \right)$$

total daily intake for human

daily dose through intake of several pathways

[kg<sub>chem</sub> \* kg<sub>bw</sub><sup>-1</sup> \* d<sup>-1</sup>]

$$\text{DOSE}_{\text{drw\_scenario}} := \frac{C_{\text{drw\_scenario}} \cdot \text{IH}_{\text{drw}}}{\text{BW}}$$

$$\text{DOSE}_{\text{air\_scenario}} := \frac{C_{\text{air\_scenario}} \cdot \text{IH}_{\text{air}} \cdot \text{BIO}_{\text{inh}}}{\text{BW} \cdot \text{BIO}_{\text{oral}}}$$

$$\text{DOSE}_{\text{stem\_scenario}} := \frac{C_{\text{leaf\_crops\_scenario}} \cdot \text{IH}_{\text{stem}}}{\text{BW}}$$

$$\text{DOSE}_{\text{root\_scenario}} := \frac{C_{\text{root\_agr\_plant\_scenario}} \cdot \text{IH}_{\text{root}}}{\text{BW}}$$

$$\text{DOSE}_{\text{meat\_scenario}} := \frac{C_{\text{meat\_scenario}} \cdot \text{IH}_{\text{meat}}}{\text{BW}}$$

$$\text{DOSE}_{\text{milk\_scenario}} := \frac{C_{\text{milk\_scenario}} \cdot \text{IH}_{\text{milk}}}{\text{BW}}$$

$$\text{DOSE}_{\text{fish\_scenario}} := \frac{C_{\text{fish\_scenario}} \cdot \text{IH}_{\text{fish}}}{\text{BW}}$$

*total daily intake for human*

total daily intake for human as sum of each pathway

[kg<sub>chem</sub> \* kg<sub>bw</sub><sup>-1</sup> \* d<sup>-1</sup>]

$$\text{DOSE}_{\text{tot\_scenario}} := \text{DOSE}_{\text{drw\_scenario}} + \text{DOSE}_{\text{fish\_scenario}} + \text{DOSE}_{\text{stem\_scenario}} + \text{DOSE}_{\text{root\_scenario}} + \dots + \text{DOSE}_{\text{meat\_scenario}} + \text{DOSE}_{\text{milk\_scenario}} + \text{DOSE}_{\text{air\_scenario}}$$

relative doses of specific different pathway (%)

$$\text{RDOSE}_{\text{drw\_scenario}} := \frac{\text{DOSE}_{\text{drw\_scenario}} \cdot 100\%}{\text{DOSE}_{\text{tot\_scenario}}}$$

$$\text{RDOSE}_{\text{air\_scenario}} := \frac{\text{DOSE}_{\text{air\_scenario}} \cdot 100\%}{\text{DOSE}_{\text{tot\_scenario}}}$$

$$\text{RDOSE}_{\text{stem\_scenario}} := \frac{\text{DOSE}_{\text{stem\_scenario}} \cdot 100\%}{\text{DOSE}_{\text{tot\_scenario}}}$$

$$\text{RDOSE}_{\text{root\_scenario}} := \frac{\text{DOSE}_{\text{root\_scenario}} \cdot 100\%}{\text{DOSE}_{\text{tot\_scenario}}}$$

$$\text{RDOSE}_{\text{meat\_scenario}} := \frac{\text{DOSE}_{\text{meat\_scenario}} \cdot 100\%}{\text{DOSE}_{\text{tot\_scenario}}}$$

$$\text{RDOSE}_{\text{milk\_scenario}} := \frac{\text{DOSE}_{\text{milk\_scenario}} \cdot 100\%}{\text{DOSE}_{\text{tot\_scenario}}}$$

$$\text{RDOSE}_{\text{fish\_scenario}} := \frac{\text{DOSE}_{\text{fish\_scenario}} \cdot 100\%}{\text{DOSE}_{\text{tot\_scenario}}}$$

## Results of calculation

$$\text{DOSE}_{\text{tot local}} = 7.725775 \times 10^{-6} \frac{\text{mg}}{\text{kgbw} \cdot \text{d}}$$

$$\text{DOSE}_{\text{tot regional}} = 6.511783 \times 10^{-8} \frac{\text{mg}}{\text{kgbw} \cdot \text{d}}$$

$$\text{RDOSE}_{\text{drw local}} = 77.662115\%$$

$$\text{RDOSE}_{\text{drw regional}} = 77.661418\%$$

$$\text{RDOSE}_{\text{air local}} = 0\%$$

$$\text{RDOSE}_{\text{air regional}} = 8.259753 \times 10^{-7} \%$$

$$\text{RDOSE}_{\text{stem local}} = 0\%$$

$$\text{RDOSE}_{\text{stem regional}} = 8.545521 \times 10^{-4} \%$$

$$\text{RDOSE}_{\text{root local}} = 0\%$$

$$\text{RDOSE}_{\text{root regional}} = 4.129927 \times 10^{-5} \%$$

$$\text{RDOSE}_{\text{meat local}} = 5.106143 \times 10^{-4} \%$$

$$\text{RDOSE}_{\text{meat regional}} = 5.10623 \times 10^{-4} \%$$

$$\text{RDOSE}_{\text{milk local}} = 9.516765 \times 10^{-3} \%$$

$$\text{RDOSE}_{\text{milk regional}} = 9.516926 \times 10^{-3} \%$$

$$\text{RDOSE}_{\text{fish local}} = 22.327858\%$$

$$\text{RDOSE}_{\text{fish regional}} = 22.327658\%$$

The report provides the comprehensive risk assessment of the substance toluene-2,4-diamine. 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.

Further information is needed before conclusions can be drawn regarding the aquatic compartment and the functioning of waste water treatment plants in relation to the processing of the substance to dyes. Since it is no longer possible to submit this information under the Existing Substances Regulation it is proposed to consider the substance further under the REACH regulation. There is no concern for the aquatic compartment from production and other uses of the substance. There is also no concern from the production and use of the substance for the atmosphere and the terrestrial environment.

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 concern for workers and for humans exposed via the environment with regard to mutagenicity (somatic cell mutagenicity) and carcinogenicity and for workers with regard to skin sensitisation. There is a need for further information and/or testing (on hold) for mutagenicity (germ cell mutagenicity) and developmental toxicity for workers and humans exposed via the environment. There is no concern for consumers (no exposure) and for human health (physico-chemical properties).