

**4-METHYL-M-PHENYLENEDIAMINE
(TOLUENE-2,4-DIAMINE)**

CAS No: 95-80-7

EINECS No: 202-453-1

SUMMARY RISK ASSESSMENT REPORT

Final report 26.05.2008

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PREFACE

This report provides a summary, with conclusions, of the risk assessment report of the substance 2,4 TDA that has been prepared by Germany in the context of Council Regulation (EEC) No. 793/93 on the evaluation and control of existing substances.

For detailed information on the risk assessment principles and procedures followed, the underlying data and the literature references the reader is referred to the comprehensive Final Risk Assessment Report (Final RAR) that can be obtained from the European Chemicals Bureau¹. The Final RAR should be used for citation purposes rather than this present Summary Report.

¹ European Chemicals Bureau – Existing Chemicals – <http://ecb.jrc.it>

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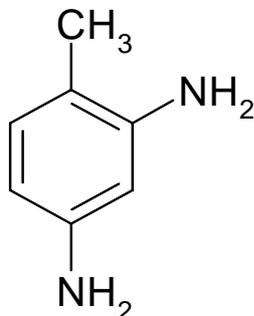
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1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS Number: 95-80-7
EINECS Number: 202-453-1
IUPAC Name: 1,3-diamino-4-methylbenzene
Synonyms: 2,4-TDA, 4-toluylenediamine, 4-methyl-m-phenylenediamine,
2,4-diamino-1-methylbenzene, toluene-2,4-diamine

Molecular weight: 122,27 g/mol
Molecular formula: C₇H₁₀N₂
Structural formula:



Commercial TDA consists of a mixture of 2,4- and 2,6-isomers. Three toluene-2,4-diamine - containing products are industrially important:

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

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

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

1.2 PURITY/IMPURITIES, ADDITIVES

2,4-TDA

Purity: 99 %

Impurities:

Water < 0.1 %

2,3-Toluylenediamine < 0.2 %

Dinitrotoluenes < 0.1 %

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

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

1.3 PHYSICO-CHEMICAL PROPERTIES

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 ¹⁾	I.I.I., 2000
Boiling point	288 °C ¹⁾	I.I.I., 2000
Relative density	1.256 at 20 °C ²⁾	I.I.I., 2000
Vapour pressure	0.017 Pa at 25 °C ³⁾	I.I.I., 2000
Surface tension	not determined	
Water solubility	38 g/l at 25 °C ⁴⁾	I.I.I., 2000
Partition coefficient	log Pow 0.074 at 25 °C ⁵⁾ 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) ⁶⁾	BAM, 2003
Flammability	not flammable ⁷⁾	I.I.I., 2000
Explosive properties	not explosive ⁸⁾	I.I.I., 2000
Oxidizing properties	no oxidizing properties ⁸⁾	I.I.I., 2000
Henry's law constant	$5.46 \cdot 10^{-5} \text{ Pa m}^3 \cdot \text{mol}^{-1}$	

¹⁾ DSC

²⁾ Pycnometer method

- 3) 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

2,4-/2,6-TDA is a clear colourless solid (at room temperature and normal pressure).

Table 1.2 Data on the physical and chemical properties of 2,4-/2,6-TDA(80/20)

Melting point	80 - 90 °C
Boiling point	283 °C at 1011 hPa
Relative density	1.2646 at 20 °C
Vapour pressure	$7.4 \cdot 10^{-3}$ Pa at 20 °C
Surface tension	72.68 mN/m at 20 °C
Water solubility	42 g/l at 38 °C
Partition coefficient	not determined
Flash point	not determined
Auto flammability	not flammable up to the melting range (80 - 90 °C)
Flammability	not flammable
Explosive properties	not explosive
Oxidizing properties	no oxidizing properties

1.4 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)

Additional classification

Mutagenic Cat 3

R 68 Possible risks of irreversible effects

Reprotoxic Cat 3

R 62 Possible risk of impaired fertility

R 48/25 Danger of serious damage to health by prolonged exposure if swallowed.

The mixture of 2,4-TDA/2,6-TDA (80/20) is additionally labeled with R41 and R20/21.

2

GENERAL INFORMATION ON EXPOSURE

Within the EU, eight companies are producers or importers of TDA. The production of TDA was stopped in 2005 by one company. Taking into account the production volumes provided by the companies for the years 1999/2000, about 280,000 t/a are produced in the EU. 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 almost exclusively used as precursor for the synthesis of 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.

From the information delivered by industry for this risk assessment, > 99% of the produced and imported TDA are used as intermediate for the production of TDI (toluylene diisocyanate). Modified or unmodified toluylene diisocyanates are processed to the following products:

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

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 ENVIRONMENTAL EXPOSURE

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. 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. 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.

Different tests on biodegradation in water showed that TDA is not readily biodegradable. Results from biodegradation simulation tests in surface water are not available. TDA has a high binding potential to soil and sediments. The microbial degradation of 2,4-TDA and 2,6-TDA in soil was investigated under aerobic and anaerobic conditions. However, it is not possible to calculate a half-life for biodegradation of TDA in soil, 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. There are no data available on biodegradation of TDA in sediments. For the oxic sediment layer, the same 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 exposure calculations a half-life of 10,000 days is assumed for the sediment compartment. Because of the binding properties of TDA onto humic substances, an accumulation of TDA derivatives in sediments cannot be excluded.

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.

Concentrations of 2,4-TDA and 2,4/2,6 TDA (80/20) in surface water, sediment and waste water treatment plants (WWTP) are estimated according to the methods in the TGD and are summarised in Table 3.1.

Table 3.1 Estimated PECs for WWTP, surface water and sediment

Site	Speciation of product	life stage	C _{eff.} [µg/l]	PEC _{local} [µg/L]	PEC _{local, sed} [µg/kg ww]
A	80/20	prod. + proc. to TDI	< 10	< 0.0026	< 0.52
B	80/20	prod. + proc. to TDI	< 70	< 0.07	< 14
C	2,4-TDA	prod.	< 20	< 0.03	< 6.4
D	80/20	prod. + proc. to TDI	9.4	0.58	116
E	80/20	prod. + proc. to TDI	35.2	0.13	26
F	80/20	prod. + proc. to TDI	300	0.30	60
G**	80/20	prod. + proc. to TDI	<3000	< 23.4	< 4,686
			140	0.87	174
H	80/20	prod. + proc. to TDI	< 6.61	< 0.065	< 13

** This site(two locations) stopped the production of TDA in 2005.

Processing to dyes yields to PECs of 0.35 and 30 µg/L for surfacewater as well as 74.5 and 6390 µg/kg ww for sediment.

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

3.2.1 Aquatic compartment (incl. sediment)

Acute and long term toxicity test results are reported for fish and invertebrates.

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. In the studies available for *Pagrus major*, the toxicity was shown to increase remarkably within the 96h exposure period. The lowest LC₅₀ of 0.161 mg/L was found for TDA 80/20 in a study performed in a semi-static system (96 h, *Pagrus major*). Although in this test the oxygen concentration at test end was slightly below the value of 60 % saturation of saturation prescribed by the OECD guideline, the study is not regarded as invalid. The lowest oxygen content was found in the control where no mortalities occurred. 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. The validity criterion of the OECD guideline may be fulfilled for this test if it is transferred to seawater. The lowest LC₅₀ values (96 h, *Pagrus major*) reported for 2,4- and 2,6-TDA are 0.2-0.4 and >1.6 mg/L, respectively.

Two long-term tests with fish are available. The studies were performed under flow-through conditions with analytical monitoring of the test substance. Holcombe et al. (1995) investigated the long-term toxicity of 2,4-TDA in a larval test with *Oryzias latipes*. No NOEC can be derived from this test. In a second study, the toxicity of 80/20 TDA to embryos and sac-fry stages of *Danio rerio* was tested for 10 days according to OECD guideline 212. The most sensitive endpoint was behaviour abnormality. A NOEC of 3.16 mg/L was derived for this parameter. 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.

Several short-term tests with invertebrates are available. The lowest effect value of 2,4-TDA was observed for *Daphnia magna* with an 48h-EC₅₀ of 1.6 mg/L. The study was performed under semi-static conditions with analytical monitoring of the test substance. From the available tests within the same species, it can be concluded that the toxicity of 2,4-TDA, TDA 80/20 and 2,6-TDA is almost the same for invertebrates.

Three long-term tests with invertebrates are available. The studies were performed under semi-static conditions with analytical monitoring of the test substance. The lowest NOEC of 0.282 mg/L was found for TDA 80/20 (21 d, *Daphnia magna*) under semi-static conditions with analytical monitoring according to OECD guideline 211. For 2,4-TDA and 2,6-TDA, tests (14 d) with *Moina macrocopa* were performed indicating similar toxicity of the two isomers to invertebrates.

The growth inhibition tests with algae were performed with two 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 2,6-TDA. The lowest EbC₅₀ (96 h) was 9.54 mg/L.

Two tests with the sediment dwelling organisms *Chironomus riparius* and *Lumbriculus variegatus* are available for TDA 80/20. The lowest NOEC was 12.3 mg/kg dw (28 d, *Lumbriculus variegates*).

Based upon all of the available data the following PNECs were derived:

PNEC_{aqua}: 1.6 µg/L (PNEC_{aqua1})

PNEC_{aqua}: 5.64 µg/L (PNEC_{aqua2})

Although the study with *Pagrus major* is regarded as valid, the Technical Meeting decided to derive alternatively a second PNEC from the *Daphnia magna* long-term study due to the uncertainties with the interpretation of the study with *Pagrus major* (influence of oxygen content on test results). Both PNECs are used for the risk characterisation.

PNEC_{microorganism}: 1 mg/L

PNEC_{sediment}: 0.24 mg/kg dw

3.2.2 Waste water treatment plant

Only one test with microorganisms is available that can be used for the derivation of the PNEC_{microorganism}. In a respiration inhibition test with activated sludge a 3h-EC₅₀ > 100 mg/L was found for TDA 80/20. A **PNEC_{microorganism} of 1 mg/L** is derived using an assessment factor of 100.

3.2.3 Terrestrial compartment

Only few data for soil dwelling organisms and terrestrial plants with 2,4/2,6-TDA 80/20 are available. The tests with *Eisenia fetida* and the terrestrial plants are regarded as short-term tests. The LC₅₀ derived from the acute test with *Eisenia fetida* was >1000 mg/kg dw (14 d). Both plant species, *Lactuca sativa* and *Avena sativa* showed an EC₅₀-value for growth between 320 and 1000 mg/kg dw. For reasons of precaution the EC₅₀-value of 320 mg/kg dw is used for the determination of the PNEC_{soil}. 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. The derived **PNEC_{soil} is 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. The derived **PNEC_{oral} is 1.97 mg/kg food**.

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_{oral}. 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

The risk characterisation is performed by comparing the PEC with the relevant PNEC for each environmental compartment. A ratio above 1 indicates concern.

PEC/PNEC ratios for surface water 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.

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

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

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

The same conclusion of the risk characterisation is obtained for both values of the PNEC_{Caqua}.

For the scenario “processing of 2,4-TDA to dyes” at site dye1 the Waste water treatment plant (WWTP) effluent concentration is above the PNEC_{microrganisms}. 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.

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

The WWTP effluent concentrations are below the PNEC_{microorganisms} for all known production and processing sites.

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

For the sediment compartment, the PEC/PNEC ratio at site dye1 processing 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.

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

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

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

There is no indication of a risk to the atmosphere, terrestrial compartment and non compartment specific effects relevant to the food chain.

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 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

Occupational exposure

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.

Detailed information on the production volumes is given in chapter 2.

Based on the available information the following relevant occupational exposure scenarios are to be expected:

- production and further processing as a chemical intermediate (scenario 1),
- production of 2,4-TDA pastilles (scenario 2),
- uses of 2,4-TDA pastilles for the production of dyes (scenario 3).

For 2,4-TDA a occupational exposure limit of 0.1 mg/m³ is established in Austria and in Switzerland, in Austria a short term limit of is also 0.4 mg/m³ valid (Ariel, 2007).

The exposure assessment is based on measured data and literature data, expert judgement and estimations according to the EASE model (Estimation and Assessment of Substance Exposure). The exposure levels should be regarded as reasonable worst case estimates representing the highly exposed workers.

The results for the different scenarios are summarised in table 4.1.1. More detailed information on inhalation and dermal exposure is given below.

Inhalation Exposure

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.

In case of inhalation exposure, for scenario 1 a sufficient number of measurement values (limits of detection) with detailed descriptions of the processes are available. There is a sufficient subset of measurement results with limits of detection below 0.0015 mg/m³. Half of this level is taken forward to the risk characterisation.

Inhalation exposure has to be assessed for the production of 2,4-TDA pastilles in the large scale chemical industry (scenario 2) and the use of 2,4-TDA pastilles (scenario 3). Exposure is possible during drumming, charging, filling, cleaning and maintenance. 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). In both cases, only one company realises the scenario.

Dermal Exposure

With regard to dermal exposure, measured results are not available. Therefore, actual dermal exposure is assessed based on the EASE model. In general, dermal exposure is assessed as exposure to part of hands and forearms.

Dermal exposure in the chemical industry (scenario 1, 2) is estimated considering that the use of gloves is highly accepted. The dyes are produced (scenario 3) in only one company which described that workers wear suitable gloves.

For the assessment of dermal exposure in scenario 1, a plant was visited. The observations revealed, that potential dermal exposure (only this was observed) on the outside of the gloves occurs daily. The workers are well instructed and trained in using personal protective equipment (gloves, glasses, respiratory protection). Suitable gloves are used at the exposure relevant activities (filling, coupling/decoupling of transfer lines and, to a less extent, sampling). 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.

Dermal exposure of scenario 2 and 3 (use of pastilles) 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 at the use of pastilles 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.

Summary of exposure data

Table 4.1: Summary of exposure data

Exposure scenario	Duration and frequency of activities relevant for exposure	Inhalation exposure Shift average [mg/m ³]	Dermal exposure Shift average [mg/p/day]
1) Production and further processing as a chemical intermediate	shift length, daily	0.00075 ⁽¹⁾	0.15 ⁽⁴⁾

Exposure scenario	Duration and frequency of activities relevant for exposure	Inhalation exposure Shift average [mg/m ³]	Dermal exposure Shift average [mg/p/day]
2) Production of 2,4-TDA pastilles	2 hours (assumed), not daily ⁽²⁾	0.025 ⁽³⁾	0.42 ^(4, 5, 6)
3) Use of 2,4-TDA pastilles for the production of dyes	2 hours (assumed), not daily	0.025 ⁽³⁾	0.42 ^(4, 6)

¹⁾ Exposure assessment based on workplace measurements, half of the detection limit of a representative sub-collective

²⁾ The frequency is limited of 1 campaign per year (4 weeks)

³⁾ Exposure assessment based on workplace measurements, half of the highest limit of detection

⁴⁾ Use of suitable gloves, protection efficiency of suitable gloves is assumed to be 90%

⁵⁾ Worst case, exposure might be considerably lower

⁶⁾ 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.

Consumer exposure

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

Humans exposed via the environment

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 %.

On the local scale, the human intake is calculated on the basis of the exposure in the vicinity of the biggest point source (site G). The calculated total intake values are 0.018 µg/kg bw/d of 2,4-TDA and 0.008 µg/kg bw/d of 2,6-TDA, resulting in 0.026 µg/kg bw/d of the TDA isomer mixture.

On the regional scale, the average intake due to exposure via the regional background concentration for each isomer is estimated. The calculated total intake values are 0.0002 µg/kg bw/d of 2,4-TDA and 0.00006 µg/kg bw/d of 2,6-TDA, resulting in 0.00026 µg/kg bw/d of the TDA isomer mixture.

The main contribution to the intake at both local and regional exposure are the $DOSE_{drw}$ and the $DOSE_{fish}$ with fractions of about 78 and 22%, respectively, to the total daily dose.

4.1.2 Effects assessment

Toxicokinetics, metabolism and distribution

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. 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 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.

Acute toxicity

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. 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). 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 approximately 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.

Irritation and corrosivity

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. Regarding corrosivity results from above Draize tests clearly demonstrate that 2,4-TDA is not a corrosive substance.

Sensitisation

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.

Repeated dose toxicity

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%.

Since 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 and lack of data for the inhalative route for 2,4-TDA as well 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.

No human data and animal data for the dermal route are available for 2,4-TDA and its 2,4-/2,6-TDA mixture.

Mutagenicity

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. 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 rather propose to classify the substance as a category-3 mutagen, R 68 (Possible risk of irreversible effects).

Carcinogenicity

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 and supportive data from other long-term studies 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. 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).

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%).

Toxicity for reproduction

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 Risk characterisation

Workers

Introduction to occupational risk assessment

For workers the exposure levels reported in table 4.1.1 are taken forward to risk characterisation. The toxicological data on 2,4-TDA are described and discussed in section 4.2. 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. For carcinogenicity which is addressed as the most significant effect in the toxicological profile of 2,4-TDA the MOE approach is used.

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. The following assumptions of systemic availability are taken forward for the calculation of MOS: 100 % after oral intake (experimental data), 25 % after dermal contact and 100 % after inhalation (default assumption). MOS values are calculated as quotient of experimental NOAEL (or LOAEL) from animal studies and workplace exposure levels. Scientifically based adjustment factors are used for the stepwise extrapolation of animal data to the worker population (e.g. adaptation of scenarios, route-to-route extrapolation, interspecies extrapolation and duration adjustment). The multiplicative combination of these different factors and an additional uncertainty factor yield the minimal MOS value as decision mark for concern. Minimal MOS values may be different for each toxicological endpoint.

In a parallel procedure, which gives identical but more direct results, a “critical exposure level” (quotient of experimental NOAEL and the according minimal MOS) is identified for each endpoint, indicating concern if occupational exposure levels exceed this value.

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, BMD05). The dose descriptor is divided by the exposure levels resulting in scenario-specific MOE values. These values are 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.

Comparing the scenario-specific MOEs with the minimal MOE only indirectly points at a „critical“ exposure level. Analogous to the MOS approach 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.

In the following risks at the workplace are considered specifically for each toxicological endpoint. Summary tables containing all scenarios are given at the end of this section.

Acute toxicity

Systemic effects (inhalation)

conclusion (ii) There is at present no need for further information and/or testing

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. This experimental value is selected as NOAEC for the risk assessment of acute inhalation toxicity. The air concentration of 1,800 mg/m³ is taken as starting point for MOS calculation. The following assessment factors are applied for the identification of the minimal MOS: an adaptation factor of 2 for exposure duration, a factor of 1.5 for physiological differences between humans at rest and workers and 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. All together the minimal MOS results in 30 (2 x 1.5 x 10). The corresponding critical exposure level is 60 mg/m³ (1,800 mg/m³ / 30).

The highest inhalation shift average values result from scenario 2 and 3 with 0.025 mg/m³. The corresponding MOS value lies in the range of 72,000. Compared to the minimal MOS this value indicates that risks due to acute inhalation toxicity are not expected under normal workplace conditions.

Systemic effects (dermal)

conclusion (ii) There is at present no need for further information and/or testing

In a rat study with dermal application, a dermal LD50 value of 1,200 mg/kg is reported. 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 and 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 as 88 mg/person (3,500 mg/person / 40).

Compared to the minimal MOS the MOS values do not indicate any concern for dermal exposure with respect to acute toxicity of 2,4-TDA.

Combined exposure

conclusion (ii) There is at present no need for further information and/or testing

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. Applying the same assessment factors as for dermal exposure 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. The corresponding MOS value is calculated as 2,500 (see table 4.1.3.2.B). These values indicate no reason for concern.

Irritation/Corrosivity

conclusion (ii) There is at present no need for further information and/or testing

Dermal, eye, and inhalative irritation of pure toluene-2,4-diamine

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. No data are available concerning the inhalation of pure 2,4-TDA. 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.

Dermal and eye irritation of the mixture of 2,4/2,6-isomers (80/20)

The result of a 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, 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.

Inhalation of the mixture of 2,4/2,6-isomers (80/20)

In cats there were microscopic visible lesions in the lung after exposure to 41.6 mg/m³ for 4 hours daily, 5 days a week for 3 weeks. Such lung effects were not reported at 9.5 mg/m³. 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/m³ (9.5 mg/m³ / 10).

The highest inhalation exposure values result from scenario 2 and 3 with 0,025 mg/m³, 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.

Sensitisation

Dermal

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

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.

Inhalation

conclusion (ii) There is at present no need for further information and/or testing

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.

Repeated dose toxicity

conclusion (ii) There is at present no need for further information and/or testing

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. 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.

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.

The key study for risk assessment concerning inhalative repeated dose toxicity is a 2-year oral gavage carcinogenicity study in rats and mice. 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 is 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/m³ (413 mg/person/day / 10 m³/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, a factor of 6 is used to extrapolate from the LOAEL to the NAEL, metabolic rate scaling from rat to human uses a factor of 4. Additionally an uncertainty factor of 5 is used. 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/m³ for inhalation (41 mg/m³ / 86).

The MOS values do not indicate any concern for inhalation with respect to repeated dose toxicity of 2,4-TDA.

Systemic effects by dermal contact, combined exposure

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 do not indicate any concern for dermal and combined exposure with respect to repeated dose toxicity of 2,4-TDA.

Mutagenicity

somatic cell mutagenicity

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

germ cell mutagenicity

conclusion (i) (on hold)

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. 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, a critical exposure level for carcinogenicity is normally assumed to cover risks for other mutagenic effects including heritable damage. 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.

Carcinogenicity

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

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. Occupational risk assessment will rely on the results of the 2-year oral gavage study from NCI (see above).

As 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 and T25-values for different tumour types are calculated. 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.

The corresponding starting point of the T25-value of 2 mg/kg/day is 140 mg/person/day (2 mg/kg x 70 kg), derived from the mammary gland tumours of the female rats. The T25-value of 14 mg/kg/day from the liver tumours of the male rats corresponds to a starting point of 980 mg/person/day (14 mg/kg x 70 kg). Expressed as airborne concentration the starting points are 14 mg/m³ (140 mg/person/day / 10 m³/day) and 98 mg/m³ (980 mg/person/day / 10 m³/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 10⁻⁵ as reference value) and 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 the Dutch Expert Committee for Occupational Standards, 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/m³ for inhalation (14 mg/m³ / 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/m³ for inhalation (98 mg/m³ / 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 tables presenting the specific data relevant for the carcinogenicity risk characterisation with the minimal MOE-concept are available in the comprehensive risk assessment report. 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).

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.

Based on the most sensitive type of tumours (the mammary gland tumours), additionally conclusions are presented 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 (summarised data are presented in tables 4.3.1.A and B, for detailed information

see comprehensive risk assessment report). 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.

Reproductive toxicity

Fertility impairment

conclusion (ii) There is at present no need for further information and/or testing

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. However, even at this dose level, diminished sperm reserves were still detected. 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/m³ (350 mg/person/day / 10 m³/day).

The following assessment factors are applied for the identification of the minimal MOS: a default value of 3 is applied because a NOAEL cannot be derived from the dose-response-relationship and 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/m³ for inhalation (35 mg/m³ / 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 workplace.

Developmental toxicity

conclusion (i) (on hold)

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. 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.

Summary tables

Tables 4.2 and 4.3 give a summary of all three exposure scenarios in the order of risk with respect to inhalation and dermal exposure, respectively. For sensitisation and mutagenicity conclusion iii applies for all scenarios (not shown).

Table 4.2: 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 ³	Carcino g. mamma ry gland tumours minMO E: 352,000	Carcino g. mamma ry gland tumours minMO E: 35,200	Carcino g. mamma ry gland tumours minMO E: 3,520	Fertility	Repeate d dose toxicity	Acute toxicity	
		Critical exposure level in mg/m ³						
		0.00004	0.0004	0.004	0.3	0.5	60	
2) Production of 2,4-TDA pastilles (4 weeks/year)	0.025(1)	concern	concern	borderli ne				
3) Use of 2,4-TDA pastilles for the production of dyes (not daily)	0.025	concern	concern	between very low concern and borderli ne				
1) Production and further processing as a chemical intermediate	0.0075	concern	borderli ne	very low concern				

(1)reduced values are taken (see table 4.1.1)

(2)blank fields: conclusion ii

Table 4.3: 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	Carcino g. mamma ry gland tumours minMO E: 352,000	Carcino g. mamma ry gland tumours minMO E: 35,200	Carcino g. mamma ry gland tumours minMO E: 3,520	Fertility	Repeate d 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)	0.42	concern	concern	between borderli ne and concern				
1) Production and further processing as a chemical intermediate	0.15	concern	concern	borderli ne				
2) Production of 2,4-TDA pastilles (4 weeks/year)	0.42(1)	concern	concern	very low concern				

(1)reduced values are taken (see table 4.1.1)

(2)blank fields: conclusion ii

Consumers

Since no consumer exposure was identified, a health risk of consumers regarding Acute toxicity, Irritation, Corrosivity, Sensitisation, Repeated dose toxicity, Mutagenicity, Carcinogenicity, and Reproductive toxicity is not expected. Conclusion (ii).

Humans exposed via the environment

Indirect exposure via the environment, resulting from oral intake of food (fish) and drinking water, is calculated as 0.026 µg/kg bw/d for the local scenario and 2.6 x 10⁻⁷ mg/kg bw/d for the regional scenario, respectively.

Repeated dose toxicity

A LOAEL of 5.9 mg/kg bw/day was derived from a 2-year study in rats. Relevant toxic effects observed at this dosage were a decreased survival rate, a delay in body weight gain, lesions of the liver and kidneys as well as tumours in the liver in high incidences. No NOAEL could be established from this study. Other repeated dose toxicity studies of 2,4-TDA using medium-term treatment periods were able to identify a NOAEL. After subchronic administration (7-weeks) the NOAEL was 250 ppm (approx. 19 mg/kg bw/day) in rats and 100 ppm (approx. 15 mg/kg bw/day) in mice . However, these studies were not entirely

according to current standards and guidelines. For establishing the MOS, the LOAEL of the most sensitive animal study (rats) has been used.

For the local scenario, 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. Conclusion (ii).

For the regional scenario, the margin of safety between the exposure level of 2.6×10^{-7} mg/kg bw/d and the oral LOAEL of 5.9 mg/kg bw/d is judged to be sufficient. Conclusion (ii).

Genotoxicity

2,4-TDA 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 (iii) for somatic cell mutagenicity; conclusion (i) for germ cell mutagenicity (on hold).

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. 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 is classified as 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 2.3×10^5 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).

Reproductive toxicity

No investigations of fertility effects are available with the 2,4-/2,6-TDA mixture (80/20). 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).

For the local scenario, 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).

For the regional scenario, the margin of safety between the exposure level of 2.6×10^{-7} mg/kg bw/d and the oral LOAEL of 5 mg/kg bw/d is judged to be sufficient. Conclusion (ii).

Reliable data for hazard assessment concerning developmental effects are not available. Thus, a risk characterisation for this endpoint cannot be performed. As 2,4-TDA is classified as a Carcinogen Category 2, risk reduction measures are required. The need for a test to evaluate developmental toxicity should be revisited when the risk reduction strategy is agreed. Conclusion (i) (on hold).

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

5 RESULTS

5.1 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.

5.2 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 limiting the risks; risk reduction measures which are already being applied shall be taken into account

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.

Consumers

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.

Humans exposed via the environment

Conclusion (i) There is a 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 limiting the risks; risk reduction measures which are already being applied shall be taken into account.

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.