European Chemicals Bureau

Institute for Health and Consumer Protection

European Chemicals Bureau

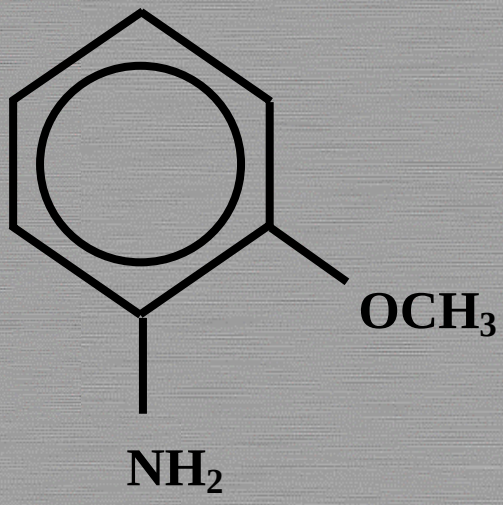
Existing Substances

European Union Risk Assessment Report

CAS No: 90-04-0

EINECS No: 201-963-1

o-anisidine



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RISK ASSESSMENT

Cataloguing data can be found at the end of this publication

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o-ANISIDINE

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RISK ASSESSMENT

Final Report, 2002

Austria

Rapporteur for the risk evaluation of o-anisidine was the Federal Ministry of the Environment, Youth and Family and the Federal Chancellery, in consultation with the Federal Environment Agency. Responsible for the risk evaluation and subsequently for the contents of this report is the Rapporteur.

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Final report:	**2002**

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[1] 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.

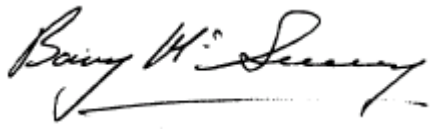
There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each 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[2], which is supported by a technical guidance document[3]. 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 Toxicity, Ecotoxicity and the Environment (CSTEE) which gives its opinion to the European Commission on the quality of the risk assessment.

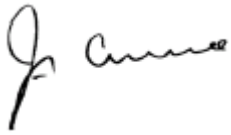
If a Risk Assessment Report concludes that measures to reduce the risks of exposure to the substances are needed, beyond any measures which may already be in place, the next step in the process is for the "Rapporteur" to develop a proposal for a strategy to limit those risks.

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.

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.



Barry Mc Sweeney
Director-General
Joint Research Centre



J. Currie
Director-General
Environment, Nuclear Safety and Civil Protection

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

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

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

0 OVERALL RESULTS OF THE RISK ASSESSMENT

CAS Number: 90-04-0
EINECS Number: 201-963-1
IUPAC Name: 1-amino-2-methoxy-benzene

Environment

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

Human health

Human health (toxicity)

o-Anisidine has not been tested adequately for sensitising properties and no test is available on developmental toxicity. Risk reduction measures are required in view of the carcinogenic properties of this substance. The need for tests to evaluate these endpoints will be revisited in the light of the risk reduction strategy.

Workers

Workers may come into contact with o-anisidine during production, processing and during the formulation and use of o-anisidine based pigments. The main possible exposure routes appear to be via inhalational and dermal contact.

Concerning production and processing, measured workplace concentrations are available for the exposure to o-anisidine via inhalation at the German reporting manufacturer. Although o-anisidine is a non-threshold carcinogen the risk for the different workplace operations at this plant concerning the uptake of the substance via inhalation can be regarded as negligible as the exposure levels are low and appropriate personal protective equipment is applied.

The dermal exposure to o-anisidine is unquantifiably low for the most workplace operations at the manufacturer. Relevant exposure concentrations estimated from EASE calculations were only determined for the possible dermal contact with the substance during the installation of gas compensation pipes. Therefore, the following conclusions can be drawn:

Conclusion (iiib) There is a need for limiting the risks; risk reduction measures that are already being applied shall be taken into account.

This conclusion applies to

- concerns for general systemic toxicity, mutagenicity and carcinogenicity, as a consequence of exposure arising from the installation of gas compensation pipes at production of the substance.

Conclusion (iiia) Risks can not be excluded for all other exposure scenarios, as the substance is identified as a non-threshold carcinogen. The adequacy of existing controls and the feasibility and practicability of further specific measures should be considered. However, the risk assessment indicates that risks are already low. This should be taken into account when considering the adequacy of existing controls and the feasibility and practicability of further specific risk reduction measures.

Consumers

The general population may come into contact with the substance during the use of consumer products coloured with pigments or dyes based on o-anisidine. From the use pattern of the substance the contact with printed packings and foils and with dyed textiles can be identified as most important. These materials may contain free o-anisidine as residues or from degradation during the printing/dyeing process or during their use. Especially in the case of dyes an unintentional release due to reductive cleavage after resorption may occur in addition. The main exposure routes appear to be dermal (skin contact with printed packings and foils and dyed textiles) and oral (young children sucking at dyed textiles). A non-negligible risk was derived from exposure estimations concerning the dermal contact with dyed textiles and the oral uptake by young children sucking at dyed textiles.

A migration of o-anisidine residues from packings into food need not be considered as the packings are superficially printed so that the substance cannot be in direct contact with the food.

From the estimation of the possible risks the following conclusions can be drawn:

Conclusion (iiib) There is a need for limiting the risks; risk reduction measures that are already being applied shall be taken into account.

This conclusion applies to

- concerns for general systemic toxicity, mutagenicity and carcinogenicity, as a consequence of dermal exposure arising from textiles coloured with dyes based on the substance,
- concerns for young children for general systemic toxicity, mutagenicity and carcinogenicity, as a consequence of oral exposure by sucking textiles coloured with dyes based on the substance.

Conclusion (iiia) Risks can not be excluded for all other exposure scenarios, as the substance is identified as a non-threshold carcinogen. The adequacy of existing controls and the feasibility and practicability of further specific measures should be considered. However, the risk assessment indicates that risks are already low. This should be taken into account when considering the adequacy of existing controls and the feasibility and practicability of further specific risk reduction measures.

Humans exposed via the environment

Indirect exposure via the environment could occur by the intake of drinking water, as the main target compartment of o-anisidine is the hydrosphere. Concentrations of o-anisidine in drinking water are not reported. Relevant intake via drinking water is not to be expected considering the use pattern of o-anisidine. Relevant intake of the substance through food consumption is also not to be expected since there is no significant potential for biomagnification along the food chain.

From calculations with EUSES very low exposure concentrations were derived for uptake by inhalation or ingestion of ambient air and water, respectively, in the vicinity of the production and processing sites.

Conclusion (iiia) Risks can not be excluded, as the substance is identified as a non-threshold carcinogen. The adequacy of existing controls and the feasibility and practicability of further specific measures should be considered. However, the risk assessment indicates that risks are already low. This should be taken into account when considering the adequacy of existing controls and the feasibility and practicability of further specific risk reduction measures.

Human health (risks from physico-chemical properties)

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

CONTENTS

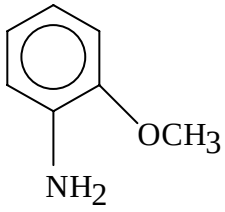
Euses Calculations can be viewed as part of the report at the website of the European Chemicals Bureau: http://ecb.jrc.it

TABLES

1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS No: 90-04-0
EINECS No: 201-963-1
IUPAC Name: 1-amino-2-methoxy-benzene
Empirical formula: C_7H_9NO
Structural formula:



Molecular weight (g/mol): 123.16
Synonyms: o-anisidine
2-methoxyaniline
2-aminoanisole
2-aminomethoxybenzene
2-methoxy-1-aminobenzene
2-methoxybenzenamine
2-methoxyphenylamine
o-aminoanisole
o-aminomethoxybenzene
o-methoxyaniline
o-methoxyphenylamine

1.2 PURITY/IMPURITIES, ADDITIVES

The purity of commercial o-anisidine is at least 99.0% and typically ≥99.4%. Possible impurities are (concentrations in brackets): aniline (≤0.4% w/w), o-chloranisol (≤0.2% w/w), o-chloraniline (≤0.4% w/w), water (≤0.1% w/w) (Hoechst AG, 1995a).

1.3 PHYSICO-CHEMICAL PROPERTIES

o-Anisidine is a light red to yellow liquid **(**20°C, 1,013 hPa**)** with a faintly aromatic odor. It becomes brownish on exposure to air and is steam volatile (Sax & Lewis, 1987; Hoechst AG, 1995b; Budavari et al., 1996). The substance is a very weak base (pK_b = 9.48; Lide, 1995/96).

Melting point (°C):	5 - >7	(Budavari et al., 1996; Lawrence & Marshall, 1985; Hoechst AG, 1995b)
Boiling point (°C):	224 - 225	(Auer, 1989; Lawrence & Marshall, 1985; Hoechst AG, 1995b)
Density (g/cm^3, 20°C):	1.0923 - 1.1	(Lawrence & Marshall, 1985; Sax & Lewis, 1987; Hoechst AG, 1995b)

Vapour pressure (hPa, 20°C):	0.02/0.05	(Hoechst AG, 1995b; Auer, 1989)
Surface tension (mN/m, 20°C):	no data	
Water solubility (g/l):	15 (20°C)	(Hoechst AG, 1995b)
Partition coefficient log K_{ow}:	1.18 (measured)	(Leo & Hansch, 1985)

1.4 CLASSIFICATION

Revision of classification was finalised in the Commission Working Groups on the Classification and Labelling of Dangerous Substances in October 1998 (human health) and in January 1999 (environment) and was published in the 26th adaptation to technical progress of Directive 67/548/EEC[4]:

Classification:	Carc.Cat. 2; R45	May cause cancer
	Muta.Cat. 3; R68[5] T; R23/24/25	Possible risk for irreversible effects Also toxic by inhalation, in contact with skin and if swallowed
	Note E	
Labelling:	T R: 45-23/24/25	S: 53-45

[4] The classification of the substance is established by Commission Directive 2001/32/EC of 19 May 2000 adapting to technical progress for the 26th time Council Directive 67/548 on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (OJ L 136, 8.6.2000, p.1).

[5] The entries were amended by replacing 'Muta.Cat. 3; R40' to 'Muta. Cat. R68' according to the Commission Directive 2001/59/EC of 6 August 2001 adapting to the technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (OJ L 225, 21.8.2001, p.1).

2 GENERAL INFORMATION ON EXPOSURE

2.1 PRODUCTION

o-Anisidine is produced from o-nitroanisol (2-methoxy-nitrobenzene) by catalytic reduction with hydrogen under pressure in an inert liquid medium (Fiege et al., 1979; Hoechst AG, 1995c). The producing and processing companies of o-anisidine in Western Europe are shown in **Table 2.1**.

Table 2.1 Producing and processing companies of o-anisidine in Western Europe [a)]

Company	Country	Production/ Processing	Quantity (t/y)	Reference
Hoechst AG (1 production and 2 processing sites)	Germany	production and processing (to acetoacet-o-anisidide, 2-oxynaphthoic o-anisidide, acet-o-anisidine [b)] and subsequent azo pigments)	>1,000 (1992) (production) <1,000 (processing past 1992)	Hoechst AG (1996a; b; 1997a; c; d)
Bayer AG	Germany	production	<1,000; production stopped in 1997	Bayer AG (1996)
Rhône-Poulenc Chimie	France	production	no data; production stopped in about 1991	Rhône-Poulenc Chimie (1996)
Ciba-Geigy PLC (number of sites not given)	UK	processing (to acetoacet-o-anisidide)	no data	SRI (1994)
Lonza AG (number of sites not given)	Switzerland	processing (to acetoacet-o-anisidide)	no data	SRI (1994)

[a)] It is not clear whether this is a complete inventory

[b)] According to Hoechst AG (1997e) the production of acet-o-anisidine was stopped meanwhile

From the available data, it can be assumed that o-anisidine is produced in the EU in amounts <1,000 t/y at present. By far the largest fraction of the produced amount is handled captively by the German reporting producing and processing company. The sold quantity represents a very small fraction of the produced amount (Hoechst AG, 1997a). The derivation of the PEC values (see Sections 3.1.2 and 3.1.3) was based on the data of this manufacturer assuming the maximal possible production capacity (15.5 t/d, 300 d/y). They, therefore represent worst-case estimations. There was no information available on import/export volumes of individual EU or other, especially OECD, countries.

For 1995, the world o-anisidine production was estimated to be about 15,000 t/y from which China alone produced about 7,000 t/y (Srour, 1996). The production in Japan is estimated to be about 100 t/y with additional 100 t/y being imported (reference year: 1993/94) (MITI, 1997).

2.2 USES

o-Anisidine is an intermediate for a number of direct yellow, red and blue azo dyes and pigments and some acid dyes (Lawrence & Marshall, 1985; Herbst & Hunger, 1995; Srour, 1996; Hoechst AG, 1997a). The most important life cycle steps of o-anisidine and the use of the resulting end products are summarized in **Table 2.2**.

Table 2.2 Primary and end products derived from o-anisidine and their use

Primary product	End product	Generic names	Use	Reference
Acetoacet-o-anisidide	yellow azo pigments	P.Y.74, P.Y.65, P.Y.17, P.Y.73 *	printing inks (e.g. books, packings, cans), colouring of polymers (e.g. PVC, polyolefines, foam material, rubber), textile printing	Herbst & Hunger (1995); Srour (1996); Hoechst AG (1997a;d;g)
Naphthol AS derivatives (e.g. 2-oxynaphthoic acid o-anisidide)	red azo pigments	P.R.15, P.R.119, P.R.188, P.R.261, P.R.9 *	printing inks (e.g. books, wallpapers), paints (automobiles, walls), alkyd laquers, crayons and coloured pencils	Herbst & Hunger (1995); Hoechst AG (1997a; d; g)
4-Nitro-o-anisidine (Fast Red B)	acid (azo) dyes	Acid Yellow 219, Acid Red 4, Acid Violet 12	textile and paper dyeing	Srour (1996); ETAD (1997a)
5-Nitro-o-anisidine (Fast Scarlet R base)	naphthol (azo) dyes	Direct Yellow 44, 117, 118, 120, 132, Direct Red 24, 26, 72	textile dyeing	Srour (1996)
Guaiacol	vanillin	-	aromatic ingredient	Srour (1996)

* Ordered by decreasing industrial importance

Worldwide, about 9,000 t o-anisidine/year were estimated to account for the dyes and pigments sector. China and India are by far the major markets for naphthol dyes. There, the production volumes are increasing. Further, vanillin is produced in China exclusively via the o-anisidine route (Srour, 1996).

The structural formulae for the industrially most important azo and naphthol pigments which are based on o-anisidine are given in **Figure 1** and **Figure 2** (according to Herbst & Hunger, 1995):

Figure 1: Structure of diaryl yellow pigments based on o-anisidine (X=Cl, CH_3,OCH_3; Y = H, Cl; R_K^n= H, CH_3, Cl, CH_3, OCH_3, OC_2H_5)

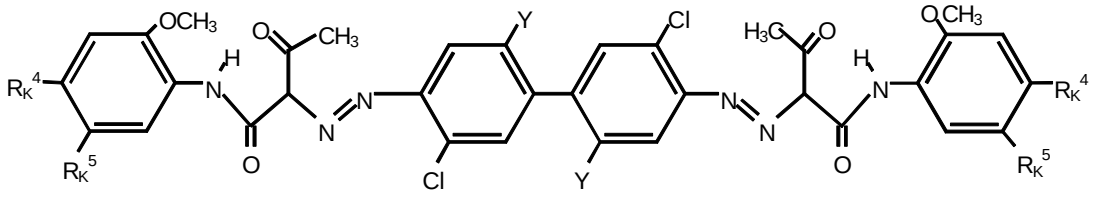
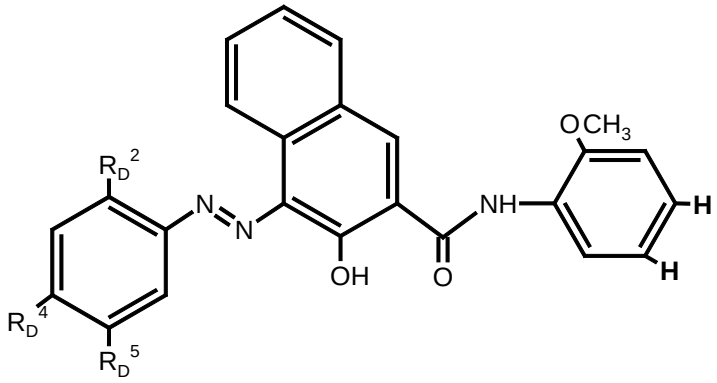


Figure 2: Structure of naphthol pigments based on o-anisidine (R_D^n/R_k^n e.g. H, Cl, CH_3, NO_2, OCH_3, OC_2H_5)



In Europe, acetoacet-o-anisidide is the main primary product derived from o-anisidine (Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers ETAD, 1997a). The quantities of o-anisidine processed in the dyes and pigments sector in the EU are given in **Table 2.3**.

Table 2.3 Quantities of o-anisidine processed in the pigments and dyes sector in the EU according to ETAD (1997a) [a)]

Pigment or dye	1995	1996 (estimated)	1997 (estimated)	1998 (estimated)
Pigments Azo pigments (i.e. diaryl yellow pigments)	750 t	700 t	no data	no data
Naphthol pigments	40 t	40 t	no data	no data
Dyes Azo dyes	118 t	74 t	60 t	49 t

[a)] The data are given with a precision of about ± 20%

From recent data it can be concluded that the use of o-anisidine for the production of azo dyes shows a decreasing tendency. About 90 % of these dyes are used for textiles (ETAD, 1998).

The use pattern of dyes and pigments based on o-anisidine in the EU is given in **Table 2.4**.

Table 2.4 Use pattern of pigments and dyes based on o-anisidine (ETAD, 1997a) [a)]

Use	1995	1996 (estimated)
Pigments Paints and printing inks	87%	91%
Dyes Textile dyes	10 %	7%
Paper dyes	3%	3%
Leather dyes	0.1%	0.1%

[a)] The data are given with a precision of about ± 20%

In Germany, the o-anisidine based pigments are used almost exclusively for the printing of packings (cardboards, polymer and aluminium foil). These pigments appear to be speciality

chemicals as their fraction of the total amount of printing inks used is estimated to be 0.5% at the most (Association of the German Printing Ink Industry, 1998). Data concerning other EU countries were not available. This use of o-anisidine based pigments in Germany therefore is assumed to be also representative for the use pattern of the respective pigments in the EU. There is some evidence that also some hair dyes are based on o-anisidine (Van Duuren, 1980; US EPA, 1996). According to summaries on potential exposure sources the US EPA and Environment Canada indicated additional uses as corrosion inhibitor for steel storage, as antioxidant for some polymercaptane resins (US EPA, 1996), in forestry products, for the pulp and paper industry and as a speciality organic chemical (no further specifications given; Environment Canada, 1997). There are no indications that these uses of o-anisidine are relevant for the EU member states.

Up to the early 80s the substance was also used in metalworking fluids as a bactericide in Western Europe (Baumann & Herberg-Liedtke, 1996; Baumann, 1996). While in Western Europe this use is discontinued now (BP Oil, 1996) it is not clear whether this applies also for other, especially other OECD countries.

From the use pattern of o-anisidine the following categories are derived for the European market according to the Technical Guidance Document (TGD) and IUCLID data set respectively:

Industrial category IC 3	Chemical industry - chemicals used in synthesis
Use category UC 33	Intermediates
Main category MC 1a	Use in closed systems, non-isolated intermediates
Main category MC 1c	Use in closed systems, isolated intermediate with controlled transport

The subsequent calculations concerning industrial releases of the substance are based on these categories.

o-Anisidine is found as a residue in the corresponding pigments in measurements of the German manufacturer in concentrations between 10 and 50 mg/kg (Hoechst AG, 1997h). Data on o-anisidine residues in dyes are not available. In addition, the substance can theoretically emerge from the corresponding pigments and dyes by reductive cleavage of the azo bond, by hydrolysis and/or metabolic degradation. These reactions are especially important for the dyes due to their significantly higher water solubility as compared to the pigments.

The pigments P.Y. 74 (CAS No. 6358-31-2), P.Y. 65 (CAS No. 6528-34-3), P.Y. 17 (CAS No. 4531-49-1), P.Y. 73 (CAS No. 13515-40-7), P.R. 9 (CAS No. 6410-38-4), P.R. 15 (CAS No. 6410-39-5), P.R. 119 (CAS No. 61968-80-7 or 72066-77-4), P.R. 188 (CAS No. 61847-48-1), P.R. 261 (CAS No. 16195-23-6) and dyes Acid Red 4 (CAS No. 5858-39-9), Acid Red 5 (CAS No. 5858-63-9), Acid Red 107 (CAS No. 6416-33-7), Acid Red 264 (CAS No. 6505-96-0), Basic Red 76 (CAS No. 68391-30-0), Acid Violet 12 (CAS No. 6625-46-3), Direct Yellow 118, Direct Yellow 120, Direct Yellow 132 (CAS No. 61968-26-1), Direct Red 24 (CAS No. 25188-08-3), Direct Red 26 (CAS No. 3687-80-7), Direct Red 72 (CAS No. 8005-64-9), Direct Red 123 (CAS No. 6470-23-1), and Food Red 16 (CAS No. 1229-55-6) have to be considered.

During spot checks of chemical companies in the course of a European inspection project on the notification of new substances the pigment P.Y. 74 was registered. Data on the used amounts were not available (NONS, 1996).

2.3 LEGISLATIVE CONTROLS

Regulations which are already in place

By Council Directive 76/769/EEC severe restrictions on the marketing and use of cat. 1 and 2 carcinogens are legally defined, and thus o-anisidine and o-anisidine containing preparations must not be sold to private consumers.

In a Resolution by the Council of Europe on the use of colorants in plastics materials and items intended likely to come into contact with food, a limit value for the sum of the aromatic amines of 500 mg/kg is given (Council of Europe, 1989).

In order to limit the risk for the consumer the use of azo dyes in textiles used for clothing, which can be degraded to aromatic amines classified as known animal carcinogens and supposed human carcinogens, is prohibited by law in Austria, France, Germany, the Netherlands and Sweden. In Germany, o-anisidine is expected to be included into the list of aromatic amines contained therein.

European legislation under preparation

European Commission has presented a "Proposal for a Directive of the Euroepan Parliament and of the Council amending for the nineteenth time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (azocolorants)". o-Anisidine is included in the list of the underlying aromatic amines on which the restricted azo dyes are based. According to the directive azodyes may not be used in textile and leather articles which have the potential of coming into direct and prolonged contact with the human skin or oral cavity. This Directive will also restrict the marketing of endproducts which are dyed with these azodyes.

3 ENVIRONMENT

The calculations concerning the Predicted Environmental Concentrations (PECs) which are necessary for the risk assessment were carried out step-by-step according to the TGD. The results which were achieved applying the EUSES model can be viewed at the website of the European Chemical Bureau[6]. There are some minor differences between the step-by-step calculations and the EUSES results which are obviously based on defaults in the EUSES model calculations which can at part not be changed by the user.

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 General discussion

3.1.1.1 Environmental releases

As far as the German producer Hoechst AG is concerned the production of o-anisidine is carried out non-continuously in a closed system with subsequent distillation of the product under reduced pressure. The air stream from the reaction vessel is condensed and cleaned by an activated carbon filter giving an emission into ambient air of about 2 g o-anisidine/t produced (Hoechst AG, 1997d). Transport vessels are filled using gas compensation pipes or sucking off volatilized materials (Hoechst AG, 1995c). Wastewater from the production is cleaned by distillation and adsorption onto activated charcoal before it is introduced into the company-owned sewage treatment plant. The emissions into the wastewater are about 1 kg o-anisidine/t produced. Wastes from the distillation of the product are incinerated (Hoechst AG, 1995c; 1997d). The release data are estimates from the analogous production process of p-anisidine (Hoechst AG, 1990a). Data from other producers are not available.

The processing of o-anisidine by the Hoechst AG, Germany, is carried out non- continuously at two facilities to the primary products acetoacetic-o-anisidide, 2-oxynaphthoic acid o-anisidide and acet-o-anisidine (Hoechst AG, 1997d). The production of acet-o-anisidine was stopped meanwhile (Hoechst AG, 1997e). The release estimates for o-anisidine into ambient air and untreated wastewater which is introduced into company-owned wastewater treatment plants according to Hoechst AG (1997d) are given in **Table 3.1**. These data were derived from the emission declarations for the local authorities (Hoechst AG, 1996c).

[6] European Chemical Bureau: http://ecb.jrc.it

Table 3.1 Release estimates for o-anisidine during processing by the German producer (Hoechst AG, 1997d)

Primary product	Release of o-anisidine into air		Release of o-anisidine into wastewater	
	kg/h	kg/t [a]	kg/h	kg/t [a]
Acetoacetic-o-anisidide	0 [b]	0 [b]	0.1	0.5
2-Oxynaphthoic acid o-anisidide Acet-o-anisidine	0.000032 [c]	0.0002 [c]	0.32 [c]	2.1 [c]

[a] Related to produced amount of primary product

[b] Production in closed system (Hoechst AG, 1995c)

[c] Production at the same processing site; according to Hoechst AG (1997e) the production of acet-o-anisidine was stopped meanwhile

As far as the German manufacturing and processing facilities are concerned emission reduction techniques such as production in a closed system, condensation and activated carbon filtering of waste air, wastewater treatment in a company-owned sewage treatment plant, controlled burning of refuse and gas compensation pipes for transport systems are applied (Hoechst AG, 1995c; 1997d). Comparable information on risk reduction techniques applied by other processing companies were not available.

Measured data concerning the emissions into surface waters are not available from the reporting company. Measured data or release estimates from other processing companies are not available. As far as the customers of the German producer are concerned it is assumed that there are at least at part effective emission control technologies available (Hoechst AG, 1997e).

The residual contents of o-anisidine in the primary products produced by the German manufacturer are given as follows (Hoechst AG, 1997e; i):

acetoacet-o-anisidide	0.001 or <0.05% (w/w) resp.
2-oxynaphthoic acid o-anisidide	<0.01% (w/w)
acet-o-anisidine *	<0.01% (w/w)

* production was stopped meanwhile

o-Anisidine can also be emitted from the printing and dyeing of paper and textiles with the corresponding printing inks and dye-products due to residual free substance. Substance concentrations of 10 – 50 mg/kg were measured in samples of some red and yellow o-anisidine based pigments (Hoechst AG, 1997h).

The substance may further be released into the environment from the corresponding pigments and dyes by decomposition. Theoretically o-anisidine can emerge from certain yellow and red azo pigments and from naphthol dyes given in Section 2, **Table 2.1**, via hydrolysis.

Tests concerning the hydrolytic stability of two industrially important pigments which are based on o-anisidine (P.Y.17 and P.R. 9; initial concentration 1 g/l) at 22°C and pH 7 showed that these pigments were hydrolytically stable during the 24 hours testing period (Hoechst AG, 1997b). The detection limit of the analytical method though was rather high (500 µg/l).

Data concerning the hydrolytic stability of dyes which are based on o-anisidine are not available. There is some evidence that such dyes (e.g. Acid Red 4, Direct Red 24) are reduced by abiotic processes in sediments releasing o-anisidine (Baughman, 1995). A photolytic or photo-oxidative

release of the substance in aqueous solutions from corresponding dyes can be excluded as the phenol derivative guaiacol is formed as a stable end product (Haag & Mill, 1987).

According to the US Toxics Release Inventory, an estimated amount of ca. 404 kg/a o-anisidine was released into the atmosphere and 78 kg/a to surface water and soils in the U.S.A. (US EPA, 1996). No information was given on emission sources. These higher emission volumes may at least partly find an explanation in the different use pattern of the substance in the U.S. compared to the European market (see Section 2).

3.1.1.2 Degradation

3.1.1.2.1 Biodegradation

In the most recent biodegradation test conducted according to OECD guideline 301 F, o-anisidine was found to be readily biodegradable. Meeting the 10-day time window criterion, 86% of the substance (initial concentration: 200 mg/l) was degraded within 28 days, as measured by manometric respirometry. The inoculum used was taken from a municipal wastewater treatment plant (Hoechst AG, 1994) which does not receive wastewater from the Hoechst AG. The lag phase until degradation started was 11 days. Although the oxygen consumption through nitrification was not considered in the test report, the test result is indicative for ready biodegradability. The calculated theoretical biochemical oxygen demand of o-anisidine is 2,079 mg O_2/mg without nitrification and 2,598 mg O_2/mg in case of complete nitrification. The ratio between these values is 1.25. In the manometric test the measured 83% biodegradation without considering nitrification would therefore correspond to 66.4% biodegradation when nitrification is considered.

Some further support for ready biodegradation can be found in an older study by Kitano (1978) were the nitrification ratio was considered. In this Japanese MITI-test the initial substance concentration was 100 mg/l, the initial activated sludge concentration was 30 mg/l. After 14 d incubation the theoretical BOD yielded 69,1% (NO_2 end product) or 81,7% (NH_3 end product). The original study report is not available any more, so the validity of the test results could not be evaluated. However it was possible to consider these data, in combination with those mentioned above, in the context of classification and labelling leading to a revised classification (January 1999, see Section 1.4).

The test results mentioned so far, supporting ready biodegradability of o-anisidine, are not consistent with the results of other biodegradation tests.

In a modified MITI test (OECD 301 C), 40 - 69% o-anisidine (initial concentration: 100 mg/l) were degraded within 14 days with an “upward trend” at the end of the test (CITI, 1992). There is no comprehensive test report available. Assuming that the range of degradation rates reported refers to the replicates tested, this test cannot be considered valid, since the difference of the replicate values is more than 20% (cf. OECD 301 C). It is also not known, whether oxygen consumption through nitrification was considered.

In another test on ready biodegradability (28 days), no or only small (less than 10%) breakdown of o-anisidine (initial concentration: 60 mg/l) was determined after inoculating with an effluent and activated sludge, respectively, obtained from a predominantly municipal wastewater treatment plant (Kool, 1984). Likewise, no ready biodegradation was found in a revised OECD

test with unadapted sewage sludge used as inoculum, whereas 50% degradation occurred within 14 days with adapted inoculum (Canton et al., 1985).

In a Zahn-Wellens test (OECD 302 B) using adapted sewage sludge as inoculum, o-anisidine was removed by 98% after 16 days (Wellens, 1990). The log phase was 11 days significantly exceeding the pass level set to 3 days in the TGD. o-Anisidine can, because of the high percentage degradation during the log phase (85%), be considered at least as inherently biodegradable.

Conclusion

Overall there is evidence that ready biodegradation of o-anisidine only takes place at certain favouring conditions most probably depending on the inoculum used, which is also true for the manometric respiratory test (OECD 301 F) described above. Thus, considering all available valid test results the biodegradability of o-anisidine in municipal wastewater treatment plants and natural waters cannot be evaluated with sufficient reliability because two tests show poor biodegradability whereas one test result indicates good biodegradability. However, in industrial sewage treatment plants where adaptation can take place ready biodegradation is assumed.

Hence, for the PEC calculations concerning the German producer and processor, the biodegradation rate constant 1 h^{-1} corresponding to the TGD category "readily biodegradable" is used. PEC calculations for other technical processes (e.g. dyeing of textiles; calculation of PEC see **Table 3.4**) are carried out using a biodegradation rate constant of 0.3 h^{-1} corresponding to the TGD category "readily biodegradable, but failing the 10-d time window" as the inoculum of sewage treatment plants which receive wastewater from those factories may not be adequately adapted to the substance.

3.1.1.2.2 Abiotic degradation

Based on the molecular structure of o-anisidine, hydrolysis is not to be expected under standard environmental conditions (Harris, 1990). In a stability experiment prior to biodegradation and ecotoxicity testing, more than 90% of the o-anisidine concentration was contained in the nonaerated standardized medium at room temperature after 8 days (Canton et al., 1985) indicating that no significant abiotic loss occurred.

There are no data available on the photolysis and photo-oxidation of o-anisidine in water. An UV spectrum of o-anisidine with acetonitrile as a solvent (substance concentration and cuvette thickness not given) showed absorption maxima at 207.2, 234.8 and 285.6 nm (Hoechst AG, 1991). According to Mill & Mabey (1985) alkoxyl substituents of aromatic compounds may shift the UV absorbance of the substance into the region of solar light so that it can be directly photolyzed by visible light. From the available data the photolytic/photo-oxidative stability of o-anisidine in aqueous solutions cannot be assessed.

There are no experimental data available on the direct or indirect photolysis of o-anisidine in the atmosphere. For the photochemical reaction of the substance with airborne OH radicals ($5 \cdot 10^5$ molecules $\cdot$ cm^{-3}) a degradation rate constant of $9.4 \cdot 10^{-11}$ $cm^3 \cdot molecule^{-1} \cdot s^{-1}$ was calculated with an increment method resulting in a half-life of approximately 4.1 hours (Hoechst AG, 1996d).

Conclusion

It can be assumed that o-anisidine is not hydrolyzed under environmental conditions. There is some evidence that the substance can be photolyzed directly in water. The calculated half-life of the reaction of o-anisidine with OH radicals in the atmosphere is significantly <1 d.

3.1.1.3 Distribution

Data concerning the volatilization of o-anisidine from aqueous surfaces are not available. From the calculated Henry´s Law constant (H) at 20°C the volatility can be estimated approximately:

H = saturation vapour pressure p_s (Pa) / saturation water solubility c_s ($mol \cdot m^{-3}$) = 0.03 Pa$\cdot$ $m^3 \cdot mol^{-1}$
with p_s = 3.5 Pa (see Section 1.3; arithmetic average) and c_s = 121.8 $mol \cdot m^{-3}$ (see Section 1.3).

This value is in the same range as the Henry's Law constants given in the IUCLID data set which were calculated from the molecular structure of the substance.

From this result a moderate to low volatility of o-anisidine from aqueous solutions can be concluded according to Thomas (1990).

There are no experimental data available on the adsorption of o-anisidine to soil or sediment. Using the QSAR equation for predominantly hydrophobic substances or anilines as suggested in the TGD (Chapter 4, Table 4), soil adsorption coefficients can be calculated from the log K_{ow} of 1.18 arriving at a K_{oc} of 11 and 38 l/kg, respectively. This value is consistent with the K_{oc} of 32.5 l/kg calculated by Hoechst AG (1996d), which is calculated according to Meylan et al. (1992) by a method basing on first-order molecular connectivity index combined with fragment contribution factors. In the following PEC calculations a K_{oc} of 38 l/kg is used which was calculated from the QSAR equation for anilines given in the TGD. Soil sorption coefficients of this magnitude are indicative for low adsorption onto soil or sediment. There is however some evidence from analogous anilines that the adsorption to organic soil constituents of this class of substances is underestimated by the commonly used calculation methods (BUA, 1994). From the dissociation constant of o-anisidine which is given as 9.48 (see Section 1.3) it can be additionally concluded that the substance is predominantly in its protonated form at pH values <4.5 and can then be adsorbed also to anorganic soil constituents.

From model calculations according to Mackay, Levels I-III, the hydrosphere can be identified as the main target compartment for o-anisidine.

Conclusion

It can be concluded that o-anisidine is slightly to moderately volatile from aqueous solutions. From QSAR calculations a low potential for adsorption onto soil or sediment is derived. There is some evidence that this method tends to underestimate the soil sorption of anilines. As far as the estimation of PEC values is concerned the calculated K_{oc} value 38 l/kg is used.

3.1.1.4 Accumulation

There are no experimental bioconcentration factors (BCF) available. Based on the log K_{ow} of 1.18 and the QSAR equation log BCF = 0.85 log K_{ow} - 0.70 given in the TGD (Chapter 4, Table 6) a BCF of 2 can be derived.

Conclusion

The calculated BCF indicates a low bioaccumulation potential of o-anisidine.

3.1.2 Aquatic compartment

3.1.2.1 Measured data

In Germany, o-anisidine was detected in the rivers Rhine, Ruhr and Emscher at a detection limit of 0.5 µg/l. A compilation of the data is given in **Table 3.2**.

Table 3.2 o-Anisidine concentrations in the German parts of the rivers Rhine and some of its tributaries (LUA, 1996; 1998)

River	Substance concentration (µg/l) (number of samples/total samples)				
	1993	1994	1995	1996	1997 *
Rhine	<0.5 (39/39)	<0.5 (26/26)	<0.5 (60/61) 0.72 (1/61)	<0.5 (30/30)	<0.5 (11/12) 1.17 (1/12)
Ruhr	<0.5 (26/26)	<0.5 (38/39) 0.72 (1/39)	<0.5 (38/38)	<0.5 (23/23)	<0.5 (6/6)
Emscher	<0.5 (11/13) mean 0.68 max 5.4 90th %ile 0.68	< 0.5 (8/13) mean 1.26 max 7.5 90th %ile 6.18	<0.5 (13/13)	<0.5 (6/6)	<0.5 (3/3)

* 1st quarter

In other tributaries of the German parts of the river Rhine the o-anisidine concentrations were below the detection limit over the 1993-1997 period (LUA, 1996; 1998). Data on the possible sources for the considerable o-anisidine concentrations in the generally highly polluted river Emscher in the years 1993/94 are not available. An investigation of the plant operator gave no result. The samples were taken downstream from the water treatment plant.

Also, in the Dutch parts of the river Rhine and the river Mass, o-anisidine concentrations were below the detection limit of 0.1 µg/l over the 1988-1990 monthly measuring period (RIZA, 1991).

The following data were reported from an environmental survey in Japan: o-anisidine was detected in 2 of 48 samples from different surface waters at levels just above the detection limit of 0.02 µg/l and in 3 of 41 different sediment samples also at concentrations slightly above the detection limit of 5 µg/kg dry weight (EPA Japan, 1990).

3.1.2.2 Estimation of PEC_{local} and $PEC_{regional}$ for aquatic systems

Estimation of PEC_{local} for production

An estimation of the PEC_{local} can be only carried out for the German reporting producer, as data from other companies are not available. Presumably, o-anisidine is presently not produced in the EU in notifiable amounts. The calculations below are based on 1995 as the reference year.

The emission $_{local, water}$ is derived from the maximal annual production capacity as follows:

Emission $_{local, water}$	=	1 kg/t ⇔ 15.5 kg/d (see Section 3.1.1.1; Hoechst AG, 1997d)

The wastewater is treated in the company-owned wastewater treatment plant with a capacity of 42500 STP inhabitants equivalents and then released into the river Main (Hoechst AG, 1997c). The dilution in the river, the local concentration of the substance in the effluent of the sewage treatment plant $C_{local, effluent}$ and subsequently the local concentration in surface water $C_{local, water}$ are calculated as follows:

Dilution	=	$(Effluent_{STP} + Flow) / Effluent_{STP} = 717$
$Effluent_{STP}$	=	$Capacity_{ST} \cdot Wastew_{inhab} = 8500$ m^3/d (Hoechst AG, 1997c) $Capacity_{STP} = 42500$ eq; $Wastew_{inhab} = 200$ l/d default value $Wastew_{inhab}$ given in the TGD, Part II, Section 2.3.7
Flow (low)	=	70.5 m^3/s $= 6.09 \cdot 10^6$ m^3/d
Concentration in untreated wastewater $C_{local, influent}$	=	$Emission_{local, water} / Effluent_{STP} = 1.82$ mg/l (from calculations above)
Fraction of emission directed to water by STP F_{STP}	=	12.6% (log H = -2; log K_{ow} = 1; d) pass levels within 28 d in a test on “ready biodegradability”; see also Section 3.1.1.2 of this document; EUSES calculation of the SIMPLETREAT model)
Concentration in the STP effluent $C_{local, effluent}$	=	*0.23* mg/l
$C_{local, water}$	=	$C_{local, effluent} / (1 + Kp_{susp} \cdot susp_{water} \cdot 10^{-6}) \cdot Dilution$ $= 3.2 \cdot 10^{-4}$ mg/l
Kp_{susp}		calculated from the estimated Koc = 38 l/kg (see Section 3.1.1.3) by multiplication with the weight fraction of organic carbon in suspended solids Foc_{susp} = 0.1 kg organic carbon/kg solid: Kp_{susp} = 3.8
$susp_{water}$		no data available. Instead, the default value of 15 mg/l given in the TGD, Part II, Section 2.3.8.3 was taken.
Dilution		see above
$C_{local, water, ann}$	=	$C_{local, water} \cdot$ (300 d/ 365 d) $= 2.6 \cdot 10^{-4}$ mg/l = *0.26* µg/l (according to TGD, Part II, Appendix 1B for non-continous production (see Section 3.1.1.1; Hoechst AG, 1997d) 300 production days/year are assumed)

The local concentration in the sediment is calculated as follows:

$C_{local, sed}$	=	$(Kp_{susp} / RHO_{susp}) \cdot C_{local, water,ann} = (3.8/1150) \cdot 2.6$ $10^{-4} \cdot 1000 \approx 8.6 \cdot 10^{-4}$ mg/kg = *0.86* µg/kg
Kp_{susp}	=	*3.8*; calculation see above
RHO_{susp}	=	1150 kg/m^3 (default value; see Section 2.3.4 of the TGD, Part II)

Estimation of PEC_{local} for processing

Data are available for two processing sites of the German reporting producer (Hoechst AG, 1997d). The results of the calculations are given in **Table 3.3**.

Table 3.3 Calculation of the local o-anisidine concentrations $C_{local, water, ann}$ in surface water for two German processing sites (Hoechst AG, 1997d)

	Site 1 Production of acetoacetic-o-anisidide	Site 2 Production of 2-oxynaphthoic o-anisidide/acet-o-anisidine [a)]
$Emission_{local, water}$ [b)]	2.4 kg/d	7.7 kg/d [c)]
	Site 1	Site 2
Capacity of the wastewater treatment plant $Capacity_{STP}$	234,045 eq (Hoechst AG, 1997c)	90, 955 eq (Hoechst AG, 1997c)
$Wastew_{inhab}$	200 l/d (according to TGD, Part II, Section 2.3.7)	
$Effluent_{STP}$	46,809 m^3/d	18,191 m^3/d
concentration in untreated wastewater $C_{local, infl}$	0.05 mg/l ($2.4 \cdot 10^6$ / $4.7 \cdot 10^7$)	0.4 mg/l ($7.7 \cdot 10^6$ / $1.8 \cdot 10^7$)
fraction of emission directed to water by STP F_{STP}	12.6% (estimation applying EUSES SIMPLETREAT [d)])	
concentration in the STP effluent $C_{local, effl}$	6.3 µg/l (0.05 · 0.13)	50 µg/l (0.4 · 0.13)
Kp_{susp}	3.8 (calculation see above)	
$SUSP_{water}$	15 mg/l (default value according to TGD, Part II, Section 2.3.8.3)	
Dilution	162 (low flow river Main: 87 m^3/s; Hoechst AG, 1997c)	367 (low flow river Main: 77 m^3/s; Hoechst AG, 1997c)
$C_{local, water}$	0.04 µg/l	0.14 µg/l
$C_{local, water, ann}$	$C_{local, water}$ · (300 d/ 365 d) = 0.03 µg/l (according to TGD, Part II, Appendix 1B for non-continous processing (see Section 3.1.1.1; Hoechst AG, 1997d) 300 processing days/year are assumed)	$C_{local, water}$ · (300 d/ 365 d) = 0.12 µg/l (according to TGD, Part II, Appendix 1B for non-continous processing (see Section 3.1.1.1; Hoechst AG, 1997d) 300 processing days/year are assumed)
$C_{local, sed}$ (calculation see above)	≈ $9.9 \cdot 10^{-5}$ mg/kg ≈ 0.1 µg/kg	≈ $3.9 \cdot 10^{-4}$ mg/kg = 0.4 µg/kg

a) According to Hoechst AG (1997e) production was stopped meanwhile.

b) See Section 3.1.1.1, Table 3.1.

c) Overall release estimation for both processing procedures; see also section 3.1.1.1, Table 3.1.

d) Basis data for the estimation with EUSES SIMPLETREAT: log H = -2; log Kow = 1; d) pass levels within 28 d in a test on "ready biodegradability".

Estimation of PEC_{local} for printing of paper

o-Anisidine can be released during the printing of paper with pigments based on this substance. o-Anisidine concentrations between 10 and 50 mg/kg in samples of some yellow and red pigments were given by an European manufacturer (Hoechst AG, 1997h).

Releases from printing inks are not covered by the TGD emission scenario document on pulp, paper and board industry. As o-anisidine based pigments apparently have a very special use in the printing of packings (cardboards, polymer and aluminium foils) and contribute only a very

small portion to the total amount of printing inks (see Section 2) a release estimation for this use seems to be of minor importance.

Estimation of PEC_{local} for printing/dyeing of textiles

o-Anisidine can be released during the printing and dyeing of textiles with pigments and dyes based on this substance. The scenario for the printing of textiles which was chosen from the TGD emission scenario document on the release of chemicals from the textile finishing industry is given in **Table 3.4**.

Table 3.4 Emission scenario for the printing of textiles with o-anisidine based pigments according to the TGD emission scenario document on the release of chemicals from the textile finishing industry

Parameter	Assumptions/calculations
Fibre	Cellulose ester [a]; 1,500 kg/d (average value related to one plant)
Printing technology	Rotary screen printing: 1.7 l wastewater/kg printed textile from printing paste + 16.7 l/kg from washing and rinsing (worst-case conditions) = 18.4 l/kg
Concentration of pigment	10 – 100 g/l (printing set equal to continuous dyeing)
o-Anisidine residue	10 – 50 mg/kg pigment (Hoechst AG, 1997h)
Fixation	0% (no retention on material)
Wastewater treatment plant	Standard conditions:10000 eq; 200 l/d x eq $\Rightarrow$ 2000 m³/d (see TGD Part II, Chapter 2.3.7) Basis data for the estimation with EUSES SIMPLETREAT: log H = -2; log K_{OW} = 1; c) pass levels within 28 d in a test on "ready biodegradability, failing 10-d time window" $\Rightarrow$ 32.5% to surface; see Section 3.1.1.2.1
o-Anisidine concentration in untreated wastewater $C_{local, infl}$	1.4 – 64 µg/l
Concentration in the STP effluent $C_{local, effluent}$	0.5 – 20 µg/l
$C_{local, water}$ (= $C_{local, water, ann}$ [b])	0.05 – 2 µg/l (basis data: Kp_{susp} = 3.8; $susp_{water}$ = 15 mg/l; D = 10 (default values, see TGD Part II Section 2.3.8.3))
$C_{local, sed}$	0.02 – 0.66 µg/kg (calculation procedure see above)

[a] The only fibre for which the printing process is listed in the TGD scenario document

[b] Assuming continous textile printing all over the year

Data on residues of o-anisidine in corresponding dyes are not available. Recent data concerning the amount of o-anisidine used for the production of textile dyes show decreasing tendency in the EU (see Section 2). Data on the import of textile dyes on the basis of o-anisidine are not available. A quantification of the emissions of o-anisidine into surface water during the dyeing of textiles is therefore not possible.

Estimation of PEC_{STP}

According to the TGD, Part II, Section 2.3.7, the concentration of a substance in the sewage treatment plant PEC_{STP} is best described by the local concentration of the substance in the untreated waste $C_{local, infl}$ as far as processes with intermittent release are considered. The production and processing of o-anisidine is carried out intermittently (Hoechst AG, 1997d). According to the above described calculations the following PEC_{STP} values can be derived:

Production		1.82 mg/l
Processing		
	acetoacet-o-anisidide	0.05 mg/l
	2-oxynaphthoic acid o-anisidide/acet-o-anisidine	0.4 mg/l
	(according to Hoechst AG (1997e) the latter was stopped meanwhile)	
Printing of paper		not quantifiable, from use
Pattern assumed to be low (see Section 3.1.2.2)		
Printing of textiles		0.0014 – 0.064 mg/l
Dyeing of textiles		not quantifiable

Calculation of $PEC_{regional}$ and $PEC_{continental}$

It was not possible to carry out generic emission scenarios for other potential processing sites in Europe (see Section 2, **Table 2.1**) as data on the processed amounts are lacking for these manufacturers. Therefore a calculation of $PEC_{regional}$ and $PEC_{continental}$ gives no additional information.

The contribution of further emissions into surface water on a regional scale from the manufacturing and use of consumer products printed or coloured with o-anisidine based pigments/dyes, like printed paper and dyed textiles cannot be quantified with the available data.

Conclusion

From the production and processing of o-anisidine at the German reporting producer $PEC_{local,water}$ values of 0.26 µg/l (production) and 0.03 and 0.12 µg/l respectively (processing) are calculated. The local concentrations in the sediment are calculated to 0.86 µg/kg (production) and 0.1 and 0.4 µg/kg respectively (processing), assuming 300 production days/y which is given as default value for intermittent production in the TGD, Part II, Appendix 1B. The PEC_{STP} is equal to the concentration of the substance in the untreated wastewater as proposed for processes with intermittent release in the TGD: 1.82 mg/l (production) and 0.05 and 0.4 mg/l respectively (processing). From the TGD emission scenario for the printing and dyeing of textiles PEC_{STP} between 1.4 and 64 µg/l and $PEC_{local, water}$ between 0.05 and 2 µg/l were estimated for the printing of cellulose fibres with o-anisidine based pigments. $PEC_{local, water}$ calculations for the printing of paper and the dyeing of textiles were not possible due to lack of data. Release estimations from the use of consumer products and their contribution to the regional and continental PECs are not possible with the available data. Due to the very special use pattern of o-anisidine based pigments and the decrease of the use of o-anisidine based textile dyes (see Section 2) an estimation of these releases is not regarded necessary as the potential contribution from these sources is considered marginal.

3.1.3 Atmosphere

3.1.3.1 Measured data

There are no data available concerning the EU.

In Japan, the substance was not detected in 51 air samples taken at various sites throughout the country at a detection limit of 500 ng/m^3 (EPA Japan, 1990).

3.1.3.2 Estimation of PEC_{local} and $PEC_{regional}$ for the atmosphere

Estimation of PEC_{local} for production

An estimation of the PEC_{local} can be only carried out for the German reporting producer as data from other companies are not available. Based on available information, o-anisidine is not produced in the EU in amounts >1,000 t/y at present.

According to the TGD the local concentration in the air $C_{local, air}$ is calculated as follows:

Emission $_{local, air}$	=	2 g o-anisidine/t produced **= 0.031 kg/d** (daily production capacity: 15.5 t; see also Section 3.1.2.2; Hoechst AG, 1997d)
$C_{local, air}$	=	max ($Emission_{local}$, $Emission_{STP}$) $\cdot$ $C_{std,air}$ **= $8.6 \cdot 10^{-6}$ mg/m^3**
max ($Emission_{local}$)		no data on the maximum emission available. The calculation was therefore carried out with the above derived $Emission_{local}$
max ($Emission_{STP}$)		can be neglected as an estimation according to the EUSES SIMPLETREAT model (log H = -2; log K_{ow} = 1; c) pass levels within 28 d in a test on "ready biodegradability", 10 d window criterion is fulfilled; see also Section 3.1.1.2) gives no significant release from o-anisidine contaminated sewage treatment plants to the atmosphere (0.01 % release to the atmosphere)
concentration in air at a source strength of 1 kg/d $C_{std,air}$	=	$2.78 \cdot 10^{-4}$ mg/m^3 (default value from Section 2.3.8.2, TGD, Part II)
$C_{local, air, ann}$	=	$C_{local, air}$ $\cdot$ (300 d/ 365 d) = **$7.1 \cdot 10^{-6}$ mg/m^3 = 7.1 ng/m^3** (according to TGD, Part II, Appendix 1B for non-continous production (see Section 3.1.1.1; Hoechst AG, 1997d) 300 production days/year are assumed)

Estimation of PEC_{local} for processing

Data are available for the German reporting producer. For the processing of o-anisidine to acetoacetic-o-anisidide no airborne emissions are to be expected because closed systems are used (see also Section 3.1.1.1, Hoechst AG, 1995c). The emissions of o-anisidine into air during the processing to 2-oxynaphthoic acid o-anisidide and acet-o-anisidide (according to Hoechst AG (1997e) the latter was stopped meanwhile) are given with $3.2 \cdot 10^{-4}$ kg/h (= $7.7 \cdot 10^{-3}$ kg/d and 0.2 g o-anisidine/t of produced primary product respectively; Hoechst AG, 1997d). With the above explained calculation procedure this leads to a local concentration of o-anisidine in ambient air at the processing site **$C_{local, air}$** of about **$2.1 \cdot 10^{-6}$ mg/m^3 (2.1 ng/m^3)**. According to the TGD, Part II, Appendix 1B for non-continuous production (see Section 3.1.1.1; Hoechst AG, 1997d) 300 production days/year are assume which leads to the annual local concentration at this processing site **$PEC_{local, air}$ = 1.7 ng/m^3**.

Estimation of PEC_{local} for other processes

From the printing and dyeing of paper and textiles residual free o-anisidine is mainly emitted to the aquatic compartment. Releases of o-anisidine from the corresponding pigments and dyes into ambient air due to residual free substance are not expected in significant amounts; thus, an estimation of the release to the atmosphere is not considered necessary.

Calculation of $PEC_{regional}$ and $PEC_{continental}$

It was not possible to carry out generic emission scenarios for other potential processing sites in Europe (see Section 2, **Table 2.1**) as data on the processed amounts are lacking for these manufacturers. Therefore a calculation of $PEC_{regional}$/$PEC_{continental}$ gives no additional information.

A significant contribution to airborne emissions from consumer products containing o-anisidine-based pigments or dyes (printed packings, dyed textiles) is not to be expected as the substance shows only a moderate to low volatility from aqueous solutions (see also Section 3.1.1.3).

Conclusion

From the available data the $PEC_{local, air, ann}$ is estimated at 7.1 ng/m^3 for the production of the substance and 1.7 ng/m^3 for its processing. $PEC_{regional}$ and $PEC_{continental}$ data were not calculated as emission data are only available from one producer and processor in Europe. A significant release from the manufacturing and use of consumer products due to residual free substance in o-anisidine based pigments and dyes is not expected as o-anisidine shows only a moderate to low volatility from aqueous solutions.

3.1.4 Terrestrial compartment

3.1.4.1 Measured data

There were no data available.

3.1.4.2 Estimation of PEC_{local} and $PEC_{regional}$ for the terrestrial compartment

From the data from one German reporting producer, no significant releases of o-anisidine into soil during production and processing are expected because both operations are carried out mainly in closed systems. The application of industrial sewage sludge would also not lead to a significant release of the substance into the soil compartment as no accumulation in sewage sludge is to be expected according to EUSES SIMPLETREAT estimations. Furthermore, the very low $PEC_{local, air}$ (see Section 3.1.3.2) shows that deposition of o-anisidine from air is of minor importance.

The substance may be discharged into soil from the deposition of dyed materials on controlled landfills by transformation of the substance from the used dyes or pigments. Data are not available. It cannot be estimated if such releases are of any significance. From the tests on biodegradability in the aquatic compartment it can be concluded that o-anisidine is also degraded by soil microorganisms.

3.1.5 Secondary poisoning

Biomagnification via the food chain is not expected because of the low n-octanol/ water partition coefficient (see Section 1.3) and the degradation characteristics of o-anisidine (see Sections 3.1.1.2 and 4.1.2.1). The substance was not detected in Japanese fish (34 samples taken at various sites throughout the country; detection limit 2 µg/kg wet wt.) (EPA Japan, 1990). The results of the EUSES model calculations for secondary poisoning and human exposure via the environment from different foods gave o-anisidine concentrations in drinking water and wet fish of significantly <1 µg/kg and for meat and milk of even < 0.01 µg/kg. With the ranking method established by Wearne et al. (1996) o-anisidine overall scored 500-1000, indicating a low potential for food contamination.

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

3.2.1 Aquatic compartment

For each trophic level required in the TGD at least one short-term toxicity test on o-anisidine is available conducted in compliance with internationally harmonized guidelines, although some tests have not been described in sufficient detail. For daphnids, the most sensitive species in the short term tests, also a test on chronic toxicity is described. As o-anisidine can be photochemically degraded in water, analytical control of the substance concentrations is an important criterion for the validity of the tests. In the publication of Canton et al. (1985) analytical control is not explicitly mentioned, but it is stated that all test results were corrected for the actual concentrations during the tests. Hence, it is assumed that analytical control was assured, although no data are given. As the substance is probably not hydrolyzed, a loss of the initial concentrations below 80% of the nominal concentrations is not expected. The test results are given in **Table 3.5**.

Table 3.5 Ecotoxicity of o-anisidine

Species	Test conditions	Effect concentration	Reference
Vertebrates			
Poecilia reticulata	static; 14 d	EC_{50} = 18 mg/l (effect: behaviour) LC_{50} = 165 mg/l	Canton et al. (1985)
Oryzias latipes	flow-through; 14 d	LC_{50} > 100 mg/l NOEC = 25 mg/l	EPA Japan (1997)
Oryzias latipes	semi-static; 96 h	LC_{50} = 196 mg/l	EPA Japan (1997)
Invertebrates			
Daphnia magna	static; 48 h	EC_{50} = 6.8 mg/l LC_{50} = 12 mg/l * (effect: immobilization)	Canton et al. (1985)
Daphnia magna	static; 24 h static; 48 h	EC_{50} = 28.5 mg/l EC_{50} = 22.5 mg/l NOEC = 6.25 mg/l	EPA Japan (1997)
Daphnia magna	flow-through; 21 d	EC_{50} > 0.206 mg/l (effect: reproduction rate) NOEC = 0.0549 mg/l **	EPA Japan (1997)
Plants			
Scenedesmus pannonicus	static; duration not given, but probably 96 h according to Slooff & Canton (1983)	EC_{50} = 12 mg/l (effect: growth inhibition)	Canton et al. (1985)
Selenastrum capricornutum	static; 72 h	EC_{50} = 21.1 mg/l (effect: reduction in biomass increase) NOEC = 7.50 mg/l	EPA Japan (1997)

* Value used as EC_{50} for classification and labelling
** NOEC from which the PNEC is derived

The substance used was commercially available o-anisidine with a purity of ≥99%.

3.2.1.1 Toxicity tests results

Microorganisms

For microorganisms, one valid toxicity test is available, which was conducted according to OECD 209 with activated sludge originating from municipal sewage (Hoechst AG, 1990b):

Activated sludge: EC_{10} = <58 mg/l (3 hours)
(effect: inhibition of respiration) EC_{50} = 800 mg/l (3 hours)
EC_{80} = <1,000 mg/l (3 hours)

The effect concentrations are nominal values. Because of the short test duration, abiotic or biotic removal processes are not expected to be of major importance.

3.2.1.2 Determination of PNEC

Determination of $PNEC_{microorganisms}$

Applying an assessment factor of 100 on the EC_{50} from the respiration inhibition test, the following PNEC is derived according to TGD Part II, Chapter 3.4:

$PNEC_{microorganisms}$ = 8,000 µg/l

Determination of $PNEC_{water}$

Short-term data from each of the three trophic levels of the base-set are available. For the most sensitive species, i.e. *Daphnia magna*, also a long-term toxicity test is described. According to the TGD Part II, Chapter 3.3.1 also the NOEC from the algae test can be used in this case as an additional long-term toxicity value. As there is sufficient evidence that *Daphnia* is the most sensitive test species and the potential of bioaccumulation is low (see Section 3.1.1.4) an assessment factor of 10 can be applied to the lowest NOEC from two species, arriving at a PNEC of:

$PNEC_{water}$ = 5.5 µg/l

This PNEC almost equals the PNEC which could also be derived from the short-term toxicity tests choosing an assessment factor of 1,000 according to the TGD Part II, Chapter 3.3.1.

Determination of $PNEC_{sed}$

There are no valid experimental data available. Therefore the equilibrium partitioning method as described in the TGD (Part II, Chapter 3.5.2) is applied and a $PNEC_{sed}$ is calculated using the following formulae:

$$PNEC_{sed} = \frac{K_{sed,\,water}}{RHO_{sed}} \cdot PNEC_{water} \cdot 1000 = 1.9 \cdot \frac{1}{1300} \cdot 0.0055 \cdot 1000$$

RHO_{sed} = 1,300 $kg \cdot m^{-3}$ (see TGD Part II, Chapter 2.3.4)

$K_{sed,\,water}$ = $Foc_{sed} \cdot K_{oc}$ (see TGD Part II, Chapter 2.3.5)

= $0.05 \cdot 38 = 1.9$

with:

$PNEC_{sed}$	Predicted No Effect Concentration in sediment [$mg \cdot kg^{-1}$]
$PNEC_{water}$	Predicted No Effect Concentration in water [$mg \cdot l^{-1}$]
$K_{sed\text{-}water}$	Partition coefficient sediment-water [$m^3 \cdot m^{-3}$]
RHO_{sed}	bulk density of wet sediment [$kg \cdot m^{-3}$]

This gives a value of:

$PNEC_{sed}$ = 8.0 µg/kg

3.2.2 Atmosphere

There are no data available.

3.2.3 Terrestrial compartment

There are no valid experimental data available. Therefore the equilibrium partitioning method as described in the TGD (Part II, Chapter 3.6.2.1) is applied and a $PNEC_{soil}$ is calculated using the following formulae:

$$PNEC_{soil} = \frac{K_{soil,\,water}}{RHO_{soil}} \cdot PNEC_{water} \cdot 1000 = 1.14 \cdot 1700^{-1} \cdot 0.0055 \cdot 1000$$

RHO_{soil} = 1,700 $kg \cdot m^{-3}$ (see TGD Part II, Chapter 2.3.4)

$K_{soil,\,water}$ = $F_{air,soil} \cdot K_{air,\,water} + F_{water,\,soil} + F_{solid,\,soil} \cdot Kp_{soil} \cdot 1000^{-1} \cdot RHO_{solid}$

= $0.2\ 46 \cdot 10^{-6} + 0.2 + (0.6 \cdot \mathit{0.76} \cdot 1000^{-1} \cdot 2500) = \mathit{1.14}$

Kp_{soil} = $Foc_{soil} \cdot K_{oc} = 0.02 \cdot \mathit{38} = \mathit{0.76} \cdot kg^{-1}$

$K_{air\text{-}water}$ = Henry / (R · Temp) = $0.11 \cdot 8.314^{-1} \cdot 285^{-1} = 46 \cdot 10^{-6}$

with:

$PNEC_{soil}$	Predicted No Effect Concentration in soil [$mg \cdot kg^{-1}$]
$PNEC_{water}$	Predicted No Effect Concentration in water [$mg \cdot l^{-1}$]
$K_{soil\text{-}water}$	Partition coefficient soil-water [$m^3 \cdot m^{-3}$]
$K_{air\text{-}water}$	Partition coefficient air-water [-]
Kp_{soil}	Partition coefficient solid-water in soil [$l \cdot kg^{-1}$]
RHO_{soil}	bulk density of wet soil [$kg \cdot m^{-3}$]
$Fsolid_{soil}$	Fraction solids in compartment soil = 0.6 $m^3 \cdot m^{-3}$ *)
RHOsolid	Density of the solid phase = 2500 $kg \cdot m^{-3}$ *)
$Fwater_{soil}$	Fraction water in compartment soil = 0.2 $m^3 \cdot m^{-3}$ *)
Foc_{soil}	Weight fraction of organic carbon in compartment soil= .02 $kg \cdot kg^{-1}$ *)
Koc	Partition coefficient organic carbon-water [$l \cdot kg^{-1}$]
$Fair_{soil}$	Fraction air in compartment soil = 0.2 $m^3 \cdot m^{-3}$ *)
HENRY	Henry´s law constant [$Pa \cdot m^{-3} \cdot mol^{-1}$]
R	gas constant = 8.314 $Pa \cdot m^3 \cdot mol^{-1} \cdot K^{-1}$
TEMP	temperature at the air-water interface = 285 K

*) Default values for standard environmental conditions

This gives a value of

$PNEC_{soil}$ = *3.7* µg/kg

3.2.4 Secondary poisoning

There is no indication for a biomagnification potential of o-anisidine via the food chain. Hence, an effects assessment is not required.

3.3 RISK CHARACTERIZATION

3.3.1 Aquatic compartment (incl. sediment)

The PEC/PNEC ratios as derived from the available data are shown in **Table 3.6**.

Table 3.6 PEC/PNEC ratios for different exposure situations concerning the hydrosphere

Site	PEC	PEC/PNEC
	Water/local ($PNEC_{water}$ = 5.5 µg/l)	
Production	*0.26* µg/l	0.047
Processing 1	0.03 µg/l	0.005
Processing 2	0.12 µg/l	0.022
Printing of textiles	0.05 – 2 µg/l	0.01 – 0.36
	STP ($PNEC_{microorganisms}$ = 8 mg/l)	
Production	1.82 mg/l	0.228
Processing 1	0.05 mg/l	0.006
Processing 2	0.4 mg/l	0.05
Printing of textiles	0.0014 – 0.064 mg/l	0.0002 – 0.008
	Sediment/local ($PNEC_{sed}$ = 8.0 µg/kg)	
Production	0.86 µg/kg	0.11
Processing 1	0.1 µg/kg	0.01
Processing 2	0.4 µg/kg	0.05
Printing of textiles	0.02 – 0.66 µg/kg	0.003 – 0.08

From the PEC/PNEC ratios for the production and processing of o-anisidine and the printing of textiles with o-anisidine based pigments there is no reason of concern for aquatic organisms. Due to the very special use pattern of o-anisidine based dyes at least in Germany (printing of packings; see Section 2) a significant contribution to the emissions into the hydrosphere from these processes is not expected although a quantification is not possible with the available data. Also, monitoring in German and Dutch rivers in the vast majority of cases yielded o-anisidine levels significantly below the PNEC value for aquatic organisms. Peak concentrations measured in 1993 and 1994 in a highly polluted German, however, gave rise to concern as they were, in isolated cases, close to or above the PNEC for aquatic organisms (see Section 3.1.2.1). Efforts to attain information concerning the possible emission sources were unsuccessful.

Furthermore there is no risk for the microorganisms of municipal wastewater treatment plants which may receive o-anisidine containing wastewaters from the above mentioned sources or from corresponding consumer products.

From the PEC and PNEC data calculated for the aquatic sediment there appears to be no risk for sediment dwelling organisms from the production and processing of o-anisidine and the use of corresponding pigments and dyes.

3.3.2 Atmosphere

For the atmosphere, PEC/PNEC ratios cannot be calculated because there are no effect data available. Emissions of o-anisidine into the atmosphere presumably play a minor role.

3.3.3 Terrestrial compartment

From the production and processing of o-anisidine at the German reporting manufacturer no significant releases of the substances into the soil compartment are to be expected because both operations are carried out mainly in closed systems. Therefore $PEC_{local,\ soil}$ data cannot be derived. The application of industrial sewage sludge and the deposition of airborne o-anisidine to soils also result in a very low contamination of soils with o-anisidine (see Section 3.1.4.2). Generic scenario calculations for other producing or processing companies within the EU cannot be carried out due to lack of data. Therefore, the calculation of a regional or continental PEC_{soil} gives no additional information. Significant releases into soils from the use of o-anisidine based pigments or dyes and consumer products containing these are not expected due to the very special use pattern (see Section 2).

3.3.4 Secondary poisoning

A risk assessment for this specific exposure route is not considered necessary because a biomagnification of the substance via the food chain is not expected (see also Section 3.1.5).

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 General discussion

In the exposure assessment of o-anisidine based products, i.e. pigments and dyes, the following distinctions have to be made:

- exposure to o-anisidine residues in pigments and dyes (see measurements by the German manufacturer concerning o-anisidine based pigments; Hoechst AG, 1997h; see Section 2);
- exposure to o-anisidine from the cleavage of the azo bonds of dyes; this reaction will not occur significantly in pigments as these are much less water soluble and thus less bioavailable.

Occupational exposure

The inhalational and the dermal route are assumed to be the most important routes of exposure whereas oral exposure is assumed to be prevented by personal hygiene measures.

Measurements are available on workplace exposure by inhalation during the **production of o-anisidine** and its **processing to pigments.** The dermal exposure concentrations were estimated.

Measurements on the o-anisidine exposure at workplaces where o-anisidine based pigments are formulated or the formulated end products are used (e.g. mainly printing of packings) are not available. The possible exposure concentrations for these situations have been estimated from the residue data of the German manufacturer (see Section 2). Also no measurements are available on workplace exposure from the **formulation and use** of o-anisidine based **dyes**. However, exposure concentrations have not been estimated for these exposure scenarios due to the low and decreasing importance of such azo dyes in the EU (see **Table 2.3**).

Consumer exposure

The general population may come into contact with residues of o-anisidine in o-anisidine based **pigments** through dermal contact with printed cardboards or foils from packings. The exposure concentrations have been estimated. Oral uptake by young children sucking at these materials was not considered relevant, because the probability of contact was considered as rather low.

The migration of o-anisidine residues from the respective printing inks into food is not to be expected as the printing is on the surface of the packings so that a direct contact is avoided.

The processing of o-anisidine to textile pigments is of minor importance within the EU. Data that allow any quantification of the exposure to o-anisidine from imported printed textiles are not available. Furthermore the bioavailability of pigments is considered low due to their low solubility and rather high stability in water. For these reasons exposure to textiles printed with pigments was not assessed.

4.1.1.2 Occupational exposure

The current standards in EU member states are given in **Table 4.1**.

Table 4.1 Occupational exposure limits for o-anisidine in the EU member states and some OECD countries

Country	Exposure limit	Limit value (ppm) [d]	Limit value (mg/m³) [d]
Austria	TRK [a] (peak limitation category 4)	0.1	0.5
Denmark	MAC [b]	0.1	0.5
France	OEL	0.1	0.5
Germany	TRK (peak limitation category 4)	0.1	0.5
Ireland	OEL	0.1	0.5
Netherlands	MAC	0.1	0.5
Norway	OEL [c]	0.1	0.5
U.S.A.	OEL	0.1	0.5

[a] TRK = Technical Guiding Concentration
[b] MAC = Maximum Accepted Concentration (workplace)
[c] OEL = Occupational Exposure Limit
[d] Conversion factor: 1 ppm = 5.12 mg/m³ for the gaseous phase at standard temperature and pressure

4.1.1.2.1 Measured exposure data

Production and processing

Personal workplace measurements at the German reporting manufacturer during the production gave exposure concentrations between 0.06 and 0.07 mg/m^3 at the reaction vessel and the distillation and filtration unit (duration of exposure 6.25 and 6.97 h). The results were in the order of magnitude of the detection limit. The production is carried out in a closed system. Additionally local mechanical exhaust ventilation is installed. During sampling special protective gloves and goggles with a breathing mask are worn. A safety shield protects against splashing. During repairs or open handling of the substance protective clothing and breathing mask are used (Hoechst AG, 1998).

During the processing of o-anisidine the following workplace concentrations (TWA values) were detected from personal air sampling of some German processing companies between 1990 and 1995 (Hoechst AG, 1996e; 1997f):

Long-term measurements (≥1 h; shift average)	0.05 - 0.15 mg/m^3 (operating; 23 data from 10 processing units with local exhaust ventilation) 0.09 mg/m^3 (cleaning, inspection and sampling; 1 data from 1 processing unit; protective clothing and breathing mask)
Short-term measurements (<1 h)	0.05 - 0.06 mg/m^3 (installation of gas compensation pipes; 2 data from 1 unit; protective clothing and breathing mask) 0.05 - 0.09 mg/m^3 (pumping; 2 data from 2 units) 0.05 mg/m^3 (operating; 1 data from 1 unit)

Formulation of pigments (especially printing inks)

Measured data for pigments are not available. Measurements from the weighing of dyes were therefore applied in a first approach to workplaces procedures in the formulation of pigments where elevated dust concentrations are to be expected (such as e.g. filling, emptying, transferring and weighing). Studies in dye-weighing houses of a number of U.S.textile dyeing factories gave concentration ranges of the colorant between 0.007 and 0.56 mg/m^3 (personal sampling at 24 textile weighers; 95th percentile 0.27 mg/m^3) for long term measurements (8-h TWA; US EPA, 1990). Assuming that the o-anisidine residues in the corresponding dyes and pigments are in the same order of magnitude (10 – 50 mg/kg for o-anisidine based pigments according to Hoechst AG, 1997h) o-anisidine levels in the air of these workplaces are estimated to range from 0.07 to 28 ng/m^3.

Printing processes

From the printing processes themselves a relevant inhalation exposure is not expected as the generation of significant quantities of dust can be excluded and the volatility of o-anisidine from aqueous solutions is moderate to low (see Section 3.1.1.3).

4.1.1.2.2 Estimated exposure data (concerning skin contact)

Production and processing

Calculations with the EASE model (see Appendix 1) showed that a dermal exposure to the substance in general appears to be so low that it is not quantified. A maximum dermal exposure concentration of 0.1 mg/cm$^2 \cdot$d was estimated for the installation of gas compensation pipes only (assumptions: direct handling of the substance, incidental contact, non-dispersive operation). For this operation, however, protective clothing is used so that the contamination of the skin is expected to be substantially reduced.

Formulation of o-anisidine based pigments (especially printing inks) / Printing processes

Dermal contact with residual free o-anisidine from corresponding pigments is possible during the formulation (e.g. by filling, emptying, transferring or weighing and mixing processes) of printing inks and during cleaning and maintenance operations at the printing machines. Usually, formulation and use of the endproducts occur in the same facilities. PPE is recommended for these activities that may reduce the dermal exposure significantly. Measured data on dermal exposure levels were not available. Instead, estimations with the EASE model were carried out. The different procedures are described best by following basic assumptions (BAuA, 1991; BG Druck & Papier, 1996):

- 20°C, liquid formulation
- non-dispersive use
- direct handling
- extensive contact

From these an exposure range of 1 to 5 mg/cm$^2 \cdot$d related to pigment/dye is derived. As residual o-anisidine in samples of some yellow and red pigment samples was measured in the range of 10 to 50 mg/kg (Hoechst AG, 1997h) the dermal exposure to o-anisidine is calculated to be 10 to 250 ng/cm$^2 \cdot$d.

4.1.1.3 Consumer exposure

Consumer products that contain o-anisidine based pigments and dyes, especially coloured paper packings, foils and textiles (see also Section 2; dyed textiles from imports), may contain free o-anisidine. In addition, reductive cleavage of the azo bond especially of dyes may occur after resorption to form free o-anisidine (see also Section 4.1.2.1). Further, the substance may emerge during the use of dyed products due to hydrolytic degradation of the dyes which have a much higher water solubility and therefore also bioavailability than pigments.

Tests concerning the hydrolytic stability of two industrially important pigments which are based on o-anisidine (P.Y.17 and P.R. 9initial concentration 1 g/l) at 22°C and pH 7 showed that these pigments were hydrolytically stable during the 24-hour testing period (Hoechst AG, 1997b), though the detection limit of the analytical method was rather high (500 µg/l).

Data concerning the hydrolytic stability of dyes that are based on o-anisidine are not available.

In the following an exposure estimation for the most relevant uses of o-anisidine based pigments and dyes (printed packing material, dyed textiles from imports) was carried out with the available literature data. The exposure scenarios which arise from minor important uses such as dyed papers and leather were not considered as they are not assumed to contribute significantly to the total estimated o-anisidine burden of the consumer. Furthermore it can be assumed that contact area, frequency of contact and contact time is much lower for dyed paper than that for dyed textiles.

Consumer exposure from printed paper

The consumer is mainly exposed to o-anisidine residues in printed packings via dermal contact of the hands during handling etc. From the residue data for some yellow and red pigments (see Section 4.1.1.2.2) an exemplary exposure estimation is carried out. The assumptions for the individual parameters are summarized in **Table 4.2**.

Table 4.2 Calculation of the dermal exposure to o-anisidine residues via printed packings according to the Association of the German Printing Ink Industry (1998)

Parameter	Paper packings	Polymer and aluminum foils
Printing ink on the packing	2% of the paper packing	-
Pigment in the coloured printing ink	10 – 20%	-
Pigment on the printed packing	0.2-0.4%	150 – 300 mg/m^2 foil
o-anisidine residues in the pigment	10-50 ppm (Hoechst AG, 1997h)	
o-anisidine residues on the printed packing	0.02 – 0.2 mg/kg paper; with an estimated paper weight of 0.06 g/cm^2: 0.012 – 0.12 mg/m^2 paper	1.5 – 15 µg/m^2 foil

As the bioavailability of o-anisidine based pigments is assumed to be low there is no concern for a significant contribution to the total exposure from metabolization or hydrolysation of absorbed pigment. Due to this and due to the very special use of paper printed with o-anisidine based pigments the contribution of children sucking at these papers to the total o-anisidine burden appears to be of minor importance and is not quantified.

Consumer exposure from the wearing of dyed textiles

In the EU, the amount of o-anisidine used in the manufacturing of textile dyes shows a low and even further decreasing tendency (see Section 2). Nevertheless, a significant amount of textiles dyed with colorants on the basis of o-anisidine may be imported from non-EU countries such as India and China. Measured data on residues of o-anisidine in dyed textiles or its emergence by the reductive cleavage of the azo bond due to metabolization or hydrolysation are not available.

In Austria, France, Germany, the Netherlands and Sweden azo dyes which can be degraded to aromatic amines which are classified as known human or animal (supposed human) carcinogens are prohibited by law for textile dyeing. For checking purposes an analytical method for these aromatic amines is laid down in Germany. Amine concentrations of <30 mg/kg are regarded as "not detected". As o-anisidine is classified as carcinogenic (carc. cat. 2; R 45) the inclusion of o-anisidine based textile dyes in the German Directive has been proposed (Amtliche Sammlung Untersuchungsverfahren, 1996; LMBG, 1997; BgVV, 1997). A European Parliament and Council Directive concerning the restrictions on the marketing and use of certain dangerous substances and preparations (azo dyes) in textile and leather products is in preparation.

o-Anisidine is included in the list of the underlying aromatic amines.

A complete risk assessment is not possible with the available data. This is especially due to the lack of information on the amount of imported textiles being dyed with o-ansidine based colorants. An estimation of the exposure situation was carried out with data on the dermal and oral exposure to azo dyes and aromatic amines which were assumed to be formed from the dyes by reductive cleavage of the azo bond due to the metabolic activity of the skin and the gastro-intestinal tract, respectively (on behalf of the European Commission Directorate General III by LGC, 1997). For direct and acid dyes for which o-anisidine is an intermediate the parameters and results of this estimate are shown in **Table 4.3**.

The calculations are presented in detail in LGC (1997). The calculated values for the potential dermal uptake of dyes are in the same order of magnitude as data from acid and alkaline perspiration experiments with some disperse and acid dyes (ETAD, 1997b).

The exposure scenario does not consider possible residues of aromatic amines in the dyes. Nevertheless, it can be assumed that the derived doses describe a worst case scenario as the estimated reduction of the azo bond by metabolism in the case of oral and dermal exposure (100% or 30% cleavage, respectively) yields much higher o-anisidine concentrations than are supposed for residual o-anisidine in the dye even if they are considerably higher than in pigments (10 to 50 ppm). Therefore, the effective doses for the dermal and the oral uptake of aromatic amines from dyed textiles in **Table 4.3** are also used for the risk characterisation of o-anisidine (see Section 4.1.3).

Table 4.3 Calculation of the dermal and the oral exposure to dyed textiles according to LGC (1997)

Parameter	Direct dye	Acid dye
Dermal exposure		
Dye weight (g/m² textile)	0.5	0.2
Weight fraction at 4% depth of shade	0.8	
Migration rate (%/h)	0.01	0.005
Duration of exposure (h/d)	10 (1 exposure event per day)	
Exposed surface area (m²) / Body weight (kg)	Adults: 1.7 / 70 Babies: 0.25 / 5 Young children: 0.4 / 10	
Percutaneous (%) penetration	1	
Extent of azo cleavage (%)	30	
Potential dermal uptake (µg/kg bw · d exposure to dye)	Adult: 9.7 Baby: 20 Young child: 16	Adult: 1.9 Baby: 4.0 Young child: 3.2
Effective dose of dye after percutaneous absorption (µg/kg bw · d exposure to dye)	Adult: 0.097 Baby: 0.200 Young child: 0.160	Adult: 0.019 Baby: 0.040 Young child: 0.032
Effective dose of aromatic amine after reductive azo cleavage in the skin (µg/kg bw · d exposure to dye)	Adult: 0.029 Baby: 0.060 Young child: 0.048	Adult: 0.006 Baby: 0.012 Young child: 0.010
Oral exposure (young children sucking; parameters as above unless otherwise stated)		
Duration of exposure (h/d)	6	
Sucked area (m²)	0.001	
Characterization of sucking activity	5 sucking bursts/minute with 3 sucks/burst	
Potential oral intake of dye/sucking event (µg/kg bw x d exposure to dye)	129.6	25.9
Effective dose of aromatic amine after reductive azo cleavage (µg/kg bw · day exposure to dye)	129.6 (100% reduction); 1.3 (1% reduction) *	25.9 (100 reduction); 0.26 (1% reduction) *

* No measured data available for the reductive cleavage of azo dyes after oral uptake

Other sources for a potential consumer exposure

There is some evidence that aromatic amines may emerge during the colouring of polymers with the corresponding pigments at temperatures >200°C (Az et al., 1991; Herbst & Hunger, 1995).

In a Resolution concerning coloured plastics materials and items intended likely to come into contact with food The Council of Europe, Committee of Ministers, recommended a limit value for the sum of aromatic amines of 500 mg/kg (Council of Europe, 1989). According to ETAD (1997a) European products containing o-anisidine-based dyes and pigments are within this limit value. At present, a quantitative exposure calculation is not possible as data concerning the migration behaviour of o-anisidine residues from dyed polymer packings into food are not available. From the very special use pattern of o-anisidine based pigments (surface printing of packings and foils) a significant migration from o-anisidine can be excluded.

Free residual o-anisidine or decomposition of vanillin to o-anisidine is not to be expected as the synthesis of vanillin via the o-anisidine route is a multi-stage process.

4.1.1.4 Humans exposed via the environment

From the available exposure data, the indirect exposure of man via the environment can only be estimated for the local releases, which result from the production and the processing of o-anisidine at the German manufacturer. By the application of EUSES the total daily intake of o-anisidine via food and drinking water is calculated to 22 ng/kg bw·d for the production and 1.4 and 7.4 ng/kg bw·d, respectively, for the two processing sites. The uptake of o-anisidine from air by inhalation for this local exposure scenario is 1.5 ng/kg bw·d for the production and 0.02 and 0.38 ng/kg bw·d, respectively, for the two processing sites.

4.1.1.5 Combined exposure

On the basis of the exposure estimates given in Sections 4.1.1.1, 4.1.1.2 and 4.1.1.3, respectively, a consumer who also works at a processing site and who uses consumer products which are coloured with o-anisidine based printing inks and dye-products will receive a maximal dose of o-anisidine which can be quantified as is shown in **Table 4.4**.

Table 4.4 Calculated exposure data concerning combined exposure

Exposure pathway	Concentration or dose	Lifetime dose [a)]
Inhalation	max. 150 µg/m³ (workplace) max. 0.0019 µg/kg bw/d (indirectly via the environment)	max. 206 mg/kg bw (workplace) max. 0.05 mg/kg bw (indirectly via the environment)
Dermal	max. 600 µg/kg bw/d (workplace, only during installation of gas compensation pipes) max. 20 µg/kg bw/d (consumer, skin contact with dyed textiles and coloured packings)	max. 5760 mg/kg bw (workplace, only during installation of gas compensation pipes) max. 548 mg/kg bw (consumer, skin contact with dyed textiles and coloured packings)
Oral	max. 0.0308 µg/kg bw/d (indirectly via the environment) max. 130 µg/kg bw/d (only small children)	max. 0.8 mg/kg bw (indirectly via the environment) max. 142 mg /kg bw (exposure from sucking clothes)

[a)] For the calculation of lifetime doses following parameters were used: 70 kg bw (adult), 75-year lifetime, 20 m³/d inhaled air (adult consumer), 10 m³/8 h workshift inhaled air, 48 weeks/y and 40 years working period (worker), 3-year exposure from sucking (child)

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

4.1.2.1 Toxicokinetics, metabolism and distribution

In vivo studies dealing specifically with the metabolism of o-anisidine were not identified in the available literature. From effects observed in toxicological studies it can be assumed that o-anisidine is resorbed after oral, dermal and inhalational exposure.

The metabolism and mode of action of o-anisidine has not yet been fully elucidated. It seems, that like other aromatic amines, o-anisidine is oxidized to a N-hydroxy derivative, which interacts with the haem group of hemoglobin resulting in the formation of methaemoglobin (McLean et al., 1969; Ashby et al., 1991; see Section 4.1.2.2).

As with other aromatic amines, N,O-acetylation may be involved in the metabolic activation of o-anisidine. In bacterial gene mutation assays with *Salmonella typhimurium* strains containing elevated levels of N- or O-acetyltransferase activity, mutagenic activity of o-anisidine could be detected (Thompson et al., 1992; Oda et al., 1995; see Section 4.1.2.7).

In vitro studies have suggested a possible role for peroxidation enzymes like prostaglandin H synthase in the metabolic activation of o-anisidine. Prostaglandin H synthase is broadly distributed in mammalian tissues including the urinary bladder (Thompson et al., 1992). Using horseradish peroxidase as a model enzyme, Thompson et al. (1991) described the formation of reactive intermediates from o-anisidine, which were covalently bound to nucleic acids and protein. The reactive intermediates formed included electrophilic diimine and quinoneimine metabolites. These metabolites were also formed with prostaglandin H synthase (Thompson et al., 1992).

Further, O-demethylation may occur in the metabolism of o-anisidine. In an *in vitro* study with microsomes from rat liver, the relative O-demethylation rate of o-anisidine as compared to the formation of formaldehyde from N,N-dimethylaniline was 13% (Schmidt et al., 1973).

When o-anisidine was incubated with thyroid peroxidase, H_2O_2 and the thyroid peroxidase substrates guaiacol and iodide, the guaiacol and iodide oxidation was effectively inhibited (IC_{50}: 1.9 µM) (Freyberger, 1994). The persistent thyroid peroxidase inhibition with concomitantly decreased thyroid hormone formation is known to induce thyroid tumours, which might be one possible explanation for the increased incidence of follicular-cell tumours observed in male F344 rats in a two-year feeding study.

In a toxicokinetic study according to OECD Guideline 417, the dye ^{14}C-FAT 92367/A was orally administered to Wistar rats (single oral administration at a target dose level of 7.4 mg/kg bw). Probably as a result of the bacterial breakdown of this compound in the gastrointestinal tract, o-anisidine could be detected in the plasma of male rats (1.5% of the administered ^{14}C-activity characterised as o-anisidine [0.017 µg/g]), in the urine of male rats (0.14% of the administered ^{14}C-activity) and in faeces of male and female rats (0.9 or 1.1% of the administered ^{14}C-activity, resp.). Within 96 hours more than 93% of the administered ^{14}C-activity were excreted mainly via urine; the major amount was excreted within the first 24 hours (Ciba-Geigy AG, 1995). The dye investigated (chrome based) does not belong to the class of the technically most important azo or naphthol pigments (see also Section 2), for which no metabolism studies are available.

Conclusion

There are no studies available dealing specifically with the resorption, distribution, or metabolism of o-anisidine in the organism. Oral, inhalational and dermal resorption can be assumed from other studies on toxicity of o-anisidine. There is only limited information concerning the mode of action. In a toxicokinetic study, the formation of o-anisidine probably as a result of the bacterial breakdown of the investigated dye in the gastrointestinal tract was shown. Due to the rapid excretion of the investigated dye and the log K_{ow} of 1.18 (see Section 1.3) o-anisidine is unlikely to bioaccumulate in the organism.

4.1.2.2 Acute toxicity

4.1.2.2.1 Animal data

In an oral study according to OECD Guideline 401 (application of 1,250 - 1,600 - 1,800 - 2,000 - 2,500 - 3,150 or 4,000 mg/kg bw via gavage), the LD_{50} for Wistar rats was 1,890 mg/kg bw. Squatting, staggering gait, reduced spontaneous activity, dizziness and respiratory depression was noted in the lower dose-groups. In addition, in the higher dose-groups, abdominal position, negative righting reflex, orange urine, pale skin and at doses of more than 2,500 mg/kg bw, in some cases respiratory sounds were reported. Gross necropsy revealed congestion of blood vessels in the gastrointestinal tract and lungs, a yellow-red foamy liquid in the intestine, and haemorrhages in stomach, intestine and urinary bladder. With exception of one animal dosed with 1,800 mg/kg bw (recovery within 11 days), in all other surviving animals no signs of toxicity were seen 4 days after application (Hoechst AG, 1984a).

In other studies, which cannot be validated due to insufficient documentation, LD_{50} values of 2,020 mg/kg bw for rats and 1,410 mg/kg bw for mice (Vasilenko & Zvezdaj, 1981) and 870 mg/kg bw for rabbits (Prosolenko, 1975) have been reported. Some haematological changes, anemia and nephrotoxicity have been described.

Similar to other aromatic amines, o-anisidine induces methaemoglobin formation. In male CBA mice or male Alpk:APfSD rats, the single oral application via gavage of 690 or 690 and 1,380 mg o-anisidine/kg bw respectively with a sampling time ranging from 3 to 48 h resulted in significantly elevated methaemoglobin levels (mice: up to 4.8% versus 0.66% in controls; rats: up to 15.4% versus 1.1% in controls) (Ashby et al., 1991). In cats, a single i.v. injection of 7.7 mg o-anisidine/kg bw with a sampling time ranging from 1 to 5 h resulted in significantly elevated methaemoglobin levels (up to 11.5% versus 1.1% in controls) (McLean et al., 1969). The increased methemoglobin level is of importance regarding human health, as cats have a comparable methaemoglobin forming capacity to humans.

In an acute inhalation study according to OECD Guideline 403, the 4-hour-LC_{50} for Wistar rats exceeded 3.87 mg/l (o-anisidine applied as aerosol), which was the highest technically feasible exposure concentration. No mortality was observed. Apart from unspecific effects, impairment of movements, respiration and reflexes was observed as well as bloody nasal discharge and cyanosis. No signs of toxicity were noted from day 8 after application. The body weight was slightly affected in only two of ten females. Gross necropsy did not indicate toxic effects (Hoechst AG, 1989b).

After dermal application according to OECD Guideline 402, the LD_{50} for Wistar rats was >2,000 mg/kg bw (Hoechst AG, 1988), which was the only dose tested. No mortality was observed. Apart from unspecific effects, ataxia, lacrimation, eyelid constriction, and orange urine were reported. Two days after application no signs of toxicity were observed and the gross pathology showed no effects.

4.1.2.2.2 Human data

Data on acute toxicity in humans are not available. At the workplace methaemoglobinaemia caused by o-anisidine has not been observed so far (Hoechst AG, 1996f).

4.1.2.2.3 Summary of acute toxicity

Based on well-documented studies with rodents, o-anisidine is harmful after oral administration (LD_{50} of 1,890 mg/kg bw). The substance caused signs of toxicity but no mortality after inhalation of the highest feasible concentration (3.87 mg/l applied as aerosol) as well as after dermal application of 2,000 mg/kg bw in a limit test. The acute signs of toxicity after oral, inhalation or dermal exposure provide evidence that the substance is resorbed via all physiological routes. After oral application to rodents or i.v. injection to cats the induction of methemoglobin-formation was noted. Due to the relevance for humans of the degree of methemoglobinemia in the cat study o-anisidine is classified as "toxic", T; R23/24/25. Classification according to Annex I of Directive 67/548/EC, see Chapter 1.

4.1.2.3 Irritation

4.1.2.3.1 Animal data

In an acute dermal irritation/corrosion study with rabbits according to OECD Guideline 404 (purity of the test substance: 99%), all observed signs of toxicity (erythema and slight edema reactions) disappeared within 72 hours after patch removal. According to the classification criteria in Guideline 83/467/EEC, o-anisidine was not classified as irritating to the skin (Hoechst AG, 1984b).

Likewise, an acute eye irritation/corrosion study with rabbits according to OECD Guideline 405 (purity of the test substance: 99%) revealed only slight irritating potential of o-anisidine. All observed signs of toxicity (chemosis, reddening of the conjunctivae, iritis and keratitis) disappeared within 7 days after application. According to the classification criteria in Guideline 83/467/EEC, o-anisidine was not classified as irritating to the eyes (Hoechst AG, 1984c).

4.1.2.3.2 Human data

Data on locally irritating effects to skin and eyes of humans are not available.

4.1.2.3.3 Summary of irritation

According to the results in both valid rabbit skin and eye irritation studies, the weak degree of irritation does not fulfil the criteria for respective classification of o-anisidine as a skin or eye irritant.

4.1.2.4 Corrosivity

Animal data

o-Anisidine has been shown to exhibit no corrosive effects on skin and eyes in rabbits (see Section 4.1.2.3.1).

Human data

Data on corrosive effects to skin and eyes of humans are not available.

Summary of corrosivity

According to the result in the valid rabbit skin irritation/corrosivity study o-anisidine is not corrosive to the skin.

4.1.2.5 Sensitisation

4.1.2.5.1 Animal data

o-Anisidine was a weak sensitizer in the guinea pig after intra- and epicutaneous application of 0.5 or 2.5 mg/kg bw resp. (no further information available) (Ilichkina, 1985). Due to the insufficient documentation of the study, the validity of this test result cannot be judged.

In a mouse local lymph node assay (Ashby et al., 1995), where several aromatic amines were tested using a standard test protocol in order to explore structure activity relationships (SAR) based on the electrophilic theory of skin sensitization, o-, m-, and p-aminophenols were among those substances with a dose related unequivocal positive response. o-Aminophenol, which may be formed in the metabolism of o-anisidine by o-demethylation (as was shown in *in vitro* studies; Schmidt et al., 1973; see Section 4.1.2.1), also gave a positive result in a modified single injection adjuvant test (Basketter & Goodwin, 1988).

In addition skin sensitizing properties of o-aminophenol, p-toluidine and aniline using dermal test protocols are reported in the respective IUCLID data sheets. Contrasting to this positive evidence examination of the structural analogue o-phenetidine (1-amino-2-ethoxy-benzene) in a valid and well-documented Magnusson and Kligman guinea pig maximization test yielded a negative result (Bayer AG, 1991).

4.1.2.5.2 Human data

Data on sensitizing effects in humans are not available. A mixture of chemicals - containing 0.7 % o-anisidine – liberated in a chemical accident in Germany led to a higher frequency of atopic dermatitis among exposed children (Traupe et al., 1997). However, due to the presence of more than twenty substances in this mixture, it is not possible to assess a possible role of o-anisidine in the elicitation of dermatitis.

4.1.2.5.3 Summary of sensitisation

Information of skin sensitisation potential of o-anisidine is limited to animal studies. Although a weak positive result was obtained in a study which used intra- and epicutaneous application in guinea pigs, there are uncertainties about its adequacy due to insufficient documentation. A number of structurally related substances, including the metabolite o-aminophenol have also produced positive results in other tests. However, the structural analogue o-phenetidine was negative in a well conducted and documented maximisation test. Overall, uncertainty remains as

to whether or not o-anisidine possesses skin sensitizing potential and further information would be required to resolve this issue.

4.1.2.6 Repeated dose toxicity

4.1.2.6.1 Animal data

In a 28-day oral study according to OECD guideline 407 with male and female Wistar rats (daily application of 0, 16, 80 or 400 mg o-anisidine/kg bw via gavage), no substance-related (i.e. unspecific) effects were observed at a dose level of 16 mg/(kg bw·d). In animals dosed with ≥80 mg/kg bw, yellow urine and a slight haemolytic anemia were noted, more pronounced at 400 mg/kg bw. In females, the bilirubin levels in blood and the relative liver weights were increased. In both sexes, the histopathologic examination gave morphological changes of the spleen (haemosiderosis, hyperaemia, and increased haematopoiesis). The 400 mg/kg group showed salivation, squatting and inflated abdomen at day 15. In males reduced body weights and an increase in relative liver and kidney weights was seen, while in females the glutamic pyruvic transaminase (GPT) levels were increased. In animals of both sexes, increased drinking water consumption, an increase of bilirubin and urea-nitrogen levels in blood, and increased relative spleen weights were observed. From this study, a NO(A)EL of 16 mg/(kg bw·d) and a LOAEL of 80 mg/kg bw was derived.

In a range finding study with F344 rats and $B_6C_3F_1$ mice (application of 0 - 1,000 - 3,000 - 10,000 or 30,000 ppm o-anisidine hydrochloride (CAS no. 134-29-2) via diet [rats: ca. 75, 225, 750 or 2,250 mg/kg bw and day; mice: ca. 150, 450, 1,500 or 4,500 mg/kg bw and day] for 7 weeks; 5 animals/sex/dose), doses of ≥10,000 ppm in rats resulted in dose-dependent weight depression of more than 10% and moderately enlarged spleens, which were black and granular; spleens of male rats administered 1,000 or 3,000 ppm were granular. In mice doses of ≥3,000 ppm resulted in dose-dependent weight depression of more than 10%; at doses of ≥10,000 ppm the spleens were also black and enlarged (NCI, 1978,).

In the 2-year study (NCI, 1978), o-anisidine hydrochloride was administered in the diet at doses of 0, 5,000 or 10,000 ppm to F344 rats. $B_6C_3F_1$ mice received doses of 0, 2,500 or 5,000 ppm of o-anisidine hydrochloride. All surviving rats were killed at 103 - 107 weeks, and all surviving mice at 104 or 105 weeks. On all animals found dead and those killed at the end of the bioassay histopathologic but no hematological or biochemical examinations were performed routinely. The carcinogenic effects are described in Section 4.1.2.8.1. For both species dose-related depression of body weight was recorded. None of the non-neoplastic lesions found in histopathological examinations were considered to be compound related for both species.

No inhalation or dermal studies with repeated application are available for o-anisidine.

4.1.2.6.2 Human data

Data on effects in humans after repeated exposure are not available.

4.1.2.6.3 Summary of repeated dose toxicity

From a 28-day oral study in rats performed according to OECD guideline 407, a NO(A)EL of 16 mg/(kg bw·d) and a LOAEL of 80 mg/(kg bw·d) was derived. The effects observed at 80 mg/(kg bw·d) are considered to occur consequently to the acute methemoglobin formation; for this effect a classification as toxic is already foreseen. On this basis, a classification as "harmful", Xn; R 48/22 for systemic toxicity is not proposed by the rapporteur. There are no studies available concerning repeated inhalation or dermal exposure in experimental animals.

4.1.2.7 Mutagenicity

The results of the *in vitro* and *in vivo* mutagenicity studies are described in detail in the following **Tables 4.5** and **4.6**.

Bacterial and yeast systems

In several of the routinely used *in vitro* test systems with procaryotes (Ames test or umu-test with *Salmonella typhimurium*, reverse mutation assay in *Escherichia coli*), o-anisidine gave negative results with *or* without metabolic activation.

However, positive results were obtained in some tests in the presence of metabolic activation with the *Salmonella typhimurium* strains TA 98, TA 100, TA 1537, and TA 1538 even if very low concentrations were tested as well as in one *Salmonella typhimurium* strain containing elevated levels of N-acetyltransferase activity (see also Section 4.1.2.1). One interlaboratory study gave considerable differences in response. A very strong genotoxic response was shown in the *Salmonella typhimurium* strain TA 98 in the presence of S9 mix after addition of Norharman.

Furthermore, o-anisidine was able to induce deletions, due to intrachromosomal recombination, in *Saccharomyces cerevisiae*.

In vitro systems with mammalian cells

With or without metabolic activation, positive results were obtained in a chromosome aberration assay and a sister chromatid exchange assay with CHO cells, as well as in a mouse lymphoma assay. Only in the presence of metabolic activation, a positive result was shown in an alkaline elution assay with mouse lymphoma cells, while an unscheduled DNA synthesis assay with rat hepatocytes was negative.

In vivo systems with mammalian cells

In most of the *in vivo* test systems with mammalian cells (covalent binding of o-anisidine to DNA using ^{14}C-labelled material or the ^{32}P-post-labelling assay [bladder, liver], DNA single strand break assay [liver, kidney, spleen, bladder, thymus, testes], host-mediated assay [oral application], micronucleus assay after oral or i.p. application [bone marrow or liver] and UDS assay [kidney, liver]) or in the sex-linked recessive lethal assay with *Drosophila melanogaster*, o-anisidine gave negative results.

Positive results were obtained in the host-mediated assay (i.p. application), in the "The entries were amended by replacing 'Muta.Cat. 3; R40' to 'Muta. Cat. R68' according to the Commission Directive 2001/59/EC of 6 August 2001 adapting to the technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous

substances (OJ L 225, 21.8.2001, p.1).Friedman-Staub assay" (inhibition of testicular DNA synthesis) and in a somatic mutation and recombination test with *Drosophila melanogaster*.

In a recently established Big Blue™ transgenic mouse mutation assay, which is a standardized method, but not formally validated, a weak induction of lac I^+ mutations was observed in the bladder, the main target tissue for carcinogenicity in both mice and rats but not in the liver.

Up to now based on experiments on genotoxicity the mechanism of action of o-anisidine is unclear. A possible role for peroxidation enzymes or N-acetyltransferase enzymes has been suggested (see Section 4.1.2.1). This may indicate that radical species are formed from o-anisidine or its metabolites in the bladder, which would be consistent with mutations observed in the absence of o-anisidine adducts.

Table 4.5 Mutagenicity of o-anisidine *in vitro*

Assay	Strain / Type	Metabolic activation	Concentration range	Results	Comments	Reference
Procaryotes						
Escherichia coli	WP2uvrA	+ / -	0.3 – 10,000 µg/plate	ambiguous with S9 mix from mouse and hamster; considerable differences in results	four-laboratory study; o-Anisidine HCl was tested; study well documented and described in sufficient detail	Dunkel et al. (1985)
Escherichia coli	WP2, WP2uvrA$^-$	+ / -	up to 1,000 µg/ml (only highest tested concentration given)	negative	study well documented and described in sufficient detail	Thompson et al. (1983)
Escherichia coli	WP2/pKM101, WP2uvrA/pKM 101	-	313 – 5,000 µg/plate	negative	two-laboratory study; study well documented and described in sufficient detail	Watanabe et al. (1996)
Escherichia coli	WP2uvrA	+ / -	0.004 - 10 µl/plate	negative	study well documented and described in sufficient detail	Hoechst AG (1984)
Escherichia coli	I. K12/343/636 II. K12/343/591	+ / -	up to 117 mg/ml (only highest tested concentration given)	both strains negative with S9 mix; positive in K12/343/591 without S9 mix	study well documented and described in sufficient detail	Hellmér & Bolcsfoldi (1992a)
Salmonella typhimurium	TA 98, TA 100, TA 1535, TA 1537, TA 1538	+ / -	0.004 - 10 µl/plate	negative	study well documented and described in sufficient detail	Hoechst AG (1984)
Salmonella typhimurium	TA 98, TA 100, TA 1535, TA 1537, TA 1538, G46, C 3076, D 3052	+ / -	up to 1,000 µg/ml (only highest tested concentration given)	negative	study well documented and described in sufficient detail	Thompson et al. (1983)
Salmonella typhimurium	TA 98, TA 100, TA 102	+ / -	100 µg/plate	negative	in Italian (brief English summary)	Vito et al. (1985)

Table 4.5 continued overleaf

Table 4.5 Mutagenicity of o-anisidine *in vitro* continued

Assay	Strain / Type	Metabolic activation	Concentration range	Results	Comments	Reference
Salmonella typhimurium	TA 98, TA 100	+ / -	1 – 5,000 µg/plate	TA 98: dose-dependent increase from about 1-3,000 µg with S9 mix (also positive when tested with S9 mix and norharman); TA 100: dose-dependent increase from about 1-100 µg with S9 mix	study well documented and described in sufficient detail	Shimizu & Takemura (1983)
Salmonella typhimurium	TA 98, TA 100, TA 1535, TA 1537	+ / -	10 – 10,810 µg/plate	ambiguous in TA 100 without S9 mix at about ≥ 1,000 µg	study well documented and described in sufficient detail	Haworth et al. (1983)
Salmonella typhimurium	TA 98, TA 100, TA 1535, TA 1537	+ / -	100 – 5,000 µg/plate	TA 98: positive with S9 mix and norharman	study well documented and described in sufficient detail	Wagner & Pugh (1995)
Salmonella typhimurium	YG 1012, YG 1029	+ / -	1 – 1,230 µg/plate	positive in YG 1029 with S9 mix at about ≥ 12 µg	hamster S9 fraction; the strain YG 1029 has an elevated N-acetyltransferase level; study well documented and described in sufficient detail	Thompson et al. (1992)
Salmonella typhimurium	TA 98, TA 100, TA 1535, TA 1537, TA 1538	+ / -	0.3 – 10,000 µg/plate	TA 98, TA 100, TA 1537, and TA 1538 with S9 mix: judged as positive, although there were considerable differences in results	four-laboratory study; o-Anisidine HCl was tested; study well documented and described in sufficient detail	Dunkel et al. (1985)
Salmonella typhimurium	TA 1538	+ / -	50 or 100 µg/plate	negative	study well documented and described in sufficient detail	Garner & Nutman (1977)
Salmonella typhimurium	TA 98, TA 100	+ / -	3 – 10,000 µg/plate	TA 98: positive with S9 mix at about ≥ 100 µg; TA 100: positive with S9 mix at about ≥ 33 µg	study well documented and described in sufficient detail	Zeiger et al. (1992)
Salmonella typhimurium	TA 1538	+ / -	100 µg/plate	negative	study well documented and described in sufficient detail	Ferretti et al. (1977)

Table 4.5 continued overleaf

Table 4.5 Mutagenicity of o-anisidine *in vitro* continued

Assay	Strain / Type	Metabolic activation	Concentration range	Results	Comments	Reference
Salmonella typhimurium	TA 102, TA 2638	-	313 – 5,000 µg/plate	negative	two-laboratory study; study well documented and described in sufficient detail	Watanabe et al. (1996)
umu-test *Salmonella typhimurium*	TA 1535, NM 2009, NM 2000	+	125 – 1,000 µg/ml	weak increase for umuC gene expression in NM 2009 at ≤ 500 µg	the strain NM 2009 has an elevated O-acetyltransferase level; study well documented and described in sufficient detail	Oda et al. (1995)
Yeast						
DEL assay *Saccharomyces cerevisiae*	RS112	-	5 – 7.5 mg/ml	positive significant increases in recombination at 5 mg/ml and a 7-fold increase at 7.5 mg/ml recombination was reduced by co-incubation with antioxidant N-acetyl-cysteine	study well documented and described in sufficient detail	Brennan &Schiestl (1999)
Mammalian cell mutation						
Alkaline elution assay	Mouse lymphoma L5178Y/TK$^{+/-}$ cells	+ / -	without S9 mix: 0.12 - 1.85 mg/ml; with S9 mix: 0.1 - 0.5 mg/ml	negative without S9 mix; positive with S9 mix at about ≥ 0.17 mg	study well documented and described in sufficient detail	Garberg et al. (1988)
Chromosome aberration assay	CHO cells	+ / -	without S9 mix: 1200-1400 µg/ml; with S9 mix: 2,400-2,800 µg/ml	without S9 mix: positive at 1,200-1,300 µg; with S9 mix: positive at ≥ 2,400-2,800 µg	precipitation of the substance at ≥ 1,200 µg; study well documented and described in sufficient detail	Galloway et al. (1987)

Table 4.5 continued overleaf

Table 4.5 Mutagenicity of o-anisidine *in vitro* continued

Assay	Strain / Type	Metabolic activation	Concentration range	Results	Comments	Reference
Chromosome aberration assay	human lymphocytes	+ / -	0.1 or 25 mg/m^3	positive	no further information available (English translation from Russian); documentation insufficient for assessment	Ilichkina (1985)
Mouse lymphoma assay	L5178Y TK$^{+/-}$ cells	+ / -	without S9 mix: 246 - 1230 µg/ml; with S9 mix: 123 - 370 µg/ml	without S9 mix: positive at ≥ 246 µg; with S9 mix: positive at ≥ 23 µg	study well documented and described in sufficient detail	Wangenheim & Bolcsfoldi (1988)
Sister chromatid exchange assay	CHO cells	+ / -	without S9 mix: 38-377 µg/ml; with S9 mix: 2500-3000 µg/ml	without S9 mix: positive at ≥38 µg; with S9 mix: positive at ≥2,500 µg; positive mainly at doses that induced marked cell cycle delay	precipitation of the substance at ≥ 2,500 µg/ml; study well documented and described in sufficient detail	Galloway et al. (1987)
Unscheduled DNA synthesis assay	rat hepatocytes	-	0.04 – 1 mg/ml	negative	study well documented and described in sufficient detail	San & Sly (1995)
Unscheduled DNA synthesis assay	rat hepatocytes	-	0.06 - 123 ng/ml	negative	study well documented and described in sufficient detail	Thompson et al. (1983)
Unscheduled DNA synthesis assay	rat hepatocytes	-	0.123 - 123 mg/ml	negative	study well documented and described in sufficient detail	Yoshimi et al. (1988)

Table 4.6 Mutagenicity of o-anisidine *in vivo*

Assay	Sex / Strain / Animal	Route of administration	Exposure concentration and duration	Results	Comments	Reference
DNA adduct assay (^{32}P-post-labelling) (bladder, liver)	female $B_6C_3F_1$ mice or female transgenic $B_6C_3F_1$ (Big Blue™) mice	single oral application via gavage	750 mg o-anisidine HCl/kg	negative	sampling time: 24 h	Ashby et al. (1994)
DNA adduct assay (^{14}C-labelling) (bladder, liver)	female $B_6C_3F_1$ mice	single oral application via gavage	750 mg ^{14}C-o-anisidine HCl/kg	negative	sampling time: 6, 12 or 24 h	Ashby et al. (1994
Big Blue™ transgenic mouse mutation assay (bladder, liver)	female transgenic $B_6C_3F_1$ (Big Blue™) mice	1, 3 or 10 applications via gavage	750 mg o-anisidine HCl/kg	small increase in mutation frequency in the bladder (increased mutation frequencies were observed following 1, 3, or 10 daily doses with sampling times 1 or 2 weeks after the final dose); statistical significance was only reached 2 weeks after either 3 or 10 daily administrations		Glickman et al. (1993); Ashby et al. (1994; Morrison & Ashby (1994)
DNA single strand break assay (liver, kidney, spleen, bladder)	male Wistar rats	I. single oral application via gavage; II. single i.p. application	I. 500 mg/kg; II. 500 or 750 mg/kg	negative	sampling time: I. 4 h; II. 500 mg/kg: 1 or 4 h, 750 mg/kg: 4 h	Ashby et al. (1991)
DNA single strand break assay (liver, kidney, spleen, bladder)	male Wistar rats	6 i.p. applications (no further data)	200 mg/kg	negative		Ashby et al. (1991)
DNA single strand break assay (liver, kidney, spleen, bladder)	male Wistar rats	single i.p. application 5 days after tissue enzyme induction with Aroclor 1254	200 mg/kg	negative	sampling time: 4 h	Ashby et al. (1991)
DNA single strand break assay (liver, thymus, testes)	male Sprague-Dawley rats	single oral application via gavage	700 mg/kg	negative	hepatocytes were isolated at 3 or 16 h; cells from thymus and testes were isolated at 16 h	Ashby et al. (1991)

Table 4.6 continued overleaf

Table 4.6 Mutagenicity of o-anisidine *in vivo* continued

Assay	Sex / Strain / Animal	Route of administration	Exposure concentration and duration	Results	Comments	Reference
Host-mediated assay	male NMRI mice	oral unspecified	430 or 1,300 mg/kg	negative	test with *E. coli* K12; bacterial samples were collected after a 2-h exposure from blood, liver, lung, kidney and testes	Hellmér & Bolcsfoldi (1992b)
Host-mediated assay	male NMRI mice	i.p.	310 or 920 mg/kg	positive in blood, liver and kidney	test with *E. coli* K12; bacterial samples were collected after a 2-h exposure from blood, liver, lung, kidney and testes	Hellmér & Bolcsfoldi (1992b)
Inhibition of testicular DNA synthesis	male mice	oral unspecified	200 mg/kg	positive	"Friedman-Staub assay" (test system not sensitive enough for this investigated parameter)	Seiler (1977)
Micronucleus assay (bone marrow)	male CBA mice	3 oral applications via gavage (no further data)	345 or 690 mg/kg	negative	sampling time: 48 h	Ashby et al. (1991)
Micronucleus assay (bone marrow)	male $B_6C_3F_1$ mice	3 i.p. applications (no further data)	125, 250 or 500 mg/kg	negative	sampling time: 24 h	Ashby et al. (1991)
Micronucleus assay (bone marrow)	male Alpk:APfSD rats	single oral application via gavage	690 or 1380 mg/kg	negative	sampling time: 24 h	Ashby et al. (1991)
Micronucleus assay (bone marrow)	male & female NMRI mice	single oral application via gavage	1000 mg/kg	negative	sampling time: 24 - 72 h; the ratio of polychromatic to normochromatic erythrocytes was statistically different from control values	Hoechst AG (1989)

Table 4.6 continued overleaf

Table 4.6 Mutagenicity of o-anisidine *in vivo* continued

Assay	Sex / Strain / Animal	Route of administration	Exposure concentration and duration	Results	Comments	Reference
Micronucleus assay (bone marrow)	male & female ICR mice	single oral application via gavage	225, 450 or 900 mg/kg (m); 275, 550 or 1100 mg/kg (f)	negative	sampling time:24 or 48 h	Putman et al. (1998)
Micronucleus assay (bone marrow)	male CBA mice	single oral application via gavage	690 mg/kg	negative	sampling time:24 or 48 h	Ashby et al. (1991)
Micronucleus assay (liver)	male Alpk:APfSD rats	single oral application via gavage	690 or 1104 mg/kg	negative	hepatocytes were isolated on day 5	Ashby et al. (1991)
Micronucleus assay (liver)	male F344 rats	single oral application via gavage	150, 350 or 690 mg/kg	negative	hepatocytes were isolated on day 5	Ashby et al. (1991)
UDS assay (kidney)	male F344 rats	i.p.	200 or 500 mg/kg	negative	sampling time: 12 h	Tyson & Mirsalis (1985)
UDS assay (liver)	male Alpk:APfSD rats	single oral application via gavage	I. 100, 200, 400, 690 or 1104 mg/kg II. 50, 100, 200 or 400, 690 or 1104 mg/kg	negative	sampling time: I. 2 h; II. 12 h	Ashby et al. (1991)
Sex-linked recessive lethal assay	*Drosophila melanogaster*	I. via diet II. injection	I. 500 ppm II. 2000 ppm	negative		Yoon et al. (1985)
w/w+ somatic assay (somatic mutation and recombination test)	Drosophila melanogaster	chronic exposure	62 - 616 mg	positive	flies were permitted to lay eggs for 3 days on standard medium prepared with the test substance	Rodriguez-Arnaiz & Aranda (1994)

Summary of mutagenicity

From the established short-term tests, there is sufficient evidence that o-anisidine is genotoxic *in vitro*, while the *in vivo* assays gave contradictory results. Although the *in vivo* tests in which o-anisidine was tested positive are not explicitly included in the EU classification criteria for mutagenicity the evidence from the transgenic mouse mutation assay and supporting *in vitro*, *in vivo* and SAR evidence is considered reliable enough by the rapporteur to justify a proposal for classification as mutagenic, cat. 3, R 68. Classification according to Annex I of Directive 67/548/EC, see Chapter 1.

4.1.2.8 Carcinogenicity

4.1.2.8.1 Animal data

In a two year study (NCI, 1978), o-anisidine hydrochloride was administered in the diet at doses of 0, 5,000 or 10,000 ppm to F344 rats (males ca. 333 or 666 mg/(kg bw.d) corresponding to 256 or 512 mg/(kg bw.d) for the free base; females ca. 500 or 1,000 mg/(kg bw.d) corresponding to 385 or 770 mg/(kg bw.d) for the free base). $B_6C_3F_1$ mice received doses of 0, 2,500 or 5,000 ppm of o-anisidine hydrochloride (males ca. 214 or 428 mg/(kg bw.d) corresponding to 164 or 328 mg/kg bw/d for the free base; females ca. 250 or 500 mg/(kg bw.d) corresponding to 192 or 384 mg/(kg bw.d) for the free base)[7]. All surviving rats were killed at 103 - 107 weeks, and all surviving mice at 104 or 105 weeks. On all animals found dead and those killed at the end of the bioassay histopathologic but not hematological or biochemical examinations were performed routinely.

o-Anisidine hydrochloride was carcinogenic in both species.

In male and female rats, transitional-cell carcinomas or papillomas of the urinary bladder and in male rats transitional-cell carcinomas of the pelvis of the kidney and follicular-cell tumors of the thyroid were observed (see **Table 4.7**). At the high dose group also a leiomyosarcoma occurred in the bladder, a tumour observed extremely seldom in rats (Jonkinen, 1990). In the high-dose group, all rats died from cancer within 83 - 88 weeks. Also in the low dose survival was significantly reduced.

The thyroid tumors may be caused by thyroid-pituitary imbalance, leading to increases of size and proliferation of certain thyroid cells, in order to be able to produce enough hormone (Thomas & Williams, 1991; Andrae & Greim, 1992).

Mice were less sensitive: survival was not different from controls. Only in high-dosed mice the incidence of transitional-cell carcinomas or papillomas of the urinary bladder was increased in a significant manner. No others tumours were observed (see **Table 4.8**).

[7] Mean body weight in rats: males 300 g and females 200g; estimated food consumption per day: 20 g;
Mean body weight in mice: males 35 g and females 30g; estimated food consumption per day: 3 g.

Table 4.7 Results of the carcinogenicity study with rats (NCI, 1978)

	Dose (ppm)		
	0	5,000	10,000
Survival (at week 52)	m: 55/55 f: 55/55	m: 55/55 f: 55/55	m: 49/55 f: 44/55
Kidney or kidney-pelvis			
Transitional-cell carcinoma	m: 0/53 f: 0/52	m: 3/55 f: 0/52	m: 7/53* f: 1/54
Urinary bladder			
Transitional-cell carcinoma or papilloma	m: 0/51 f: 0/49	m: 52/54* f: 46/49*	m: 52/52* f: 50/51*
only papilloma	m: 0/51 f: 0/49	m: 1/54 f: 5/49	m: 2/52 f: 0/51
Thyroid			
C-cell carcinoma	m: 0/53 f: 3/49	m: 2/40 f: 1/45	m: 0/40 f: 0/46
C-cell adenoma or carcinoma	m: 3/53 f: 4/49	m: 3/40 f: 1/45	m: 0/40 f: 0/46
Follicular-cell carcinoma	m: 0/53 f: 0/49	m: 2/40 f: 3/45	m: 2/40 f: 0/46
All follicular-cell tumors**	m: 0/53 f: 1/49	m: 7/40* f: 4/45	m: 6/40* f: 3/46

* Statistically significant increase
** Carcinomas, cystadenocarcinomas, adenomas, cystadenomas and papillary cystadenomas

Table 4.8 Results of the carcinogenicity study with mice (NCI, 1978)

	Dose (ppm)		
	0	2,500	5,000
Survival (at week 103)	m: 44/55 f: 44/55	m: 43/55 f: 38/55	m: 43/55 f: 42/55
Urinary bladder			
Transitional-cell carcinoma or papilloma	m: 0/48 f: 0/50	m: 2/55 f: 1/51	m: 22/53* f: 22/50*
Hyperplasia	m: 1/48 f: 0/50	m: 2/55 f: 1/51	m: 21/53 f: 12/50

* Statistically significant increase

To investigate o-anisidine for a tumor-promoting potential, two groups of 13 - 16 male rats each (F344) received drinking water containing N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN; 0.05%) as initiator for an initial 4 weeks. A third group of 10 males received untreated water during the same period. One of the groups pretreated with BBN and the group receiving untreated drinking water were then given 1,700 ppm o-anisidine in feed (about 85 mg/(kg bw·d)) during the following 2 weeks and then 425 ppm (about 21.3 mg/(kg bw·d)) for 30 weeks. All rats were sacrificed after 36 weeks. In rats treated with BBN and o-anisidine

the final average body weights were significantly reduced and the histological examination of the urinary bladder showed a significant rise in the incidence of papillary and nodular hyperplasias and an increase in papillomas and carcinomas of the urinary bladder compared to those treated only with BBN, while the findings in the case of the animals that received only o-anisidine were negative (see **Table 4.9**) (Ono et al., 1992).

Table 4.9 Result of the tumor-promoting potential of o-anisidine (Ono et al., 1992)

	Hyperplasia n (%)	Papilloma n (%)	Carcinoma n (%)
o-anisidine only (10 rats)	0 (0)	0 (0)	0 (0)
BBN only (13 rats)	2 (15)	0 (0)	0 (0)
BBN and o-anisidine (16 rats)	13 (81)	3 (19)	2 (13)

An indirect, non-genotoxic mechanism of carcinogenicity has been discussed (Ashby et al., 1991). On the other hand, the aggressive nature of the bladder carcinogenicity (short latency period and high incidence of tumours) as well as some positive results from tests on genotoxicity is inconsistent with a non-genotoxic mechanism of carcinogenicity.

4.1.2.8.2 Human data

Data on carcinogenic effects in humans are not available.

4.1.2.8.3 Summary of carcinogenicity

o-Anisidine hydrochloride was carcinogenic in rats and mice. In both species, the main target organ is the urinary bladder, while in male rats in addition an increased incidence of tumors of the thyroid was observed. It can be assumed that the carcinogenic effect of o-anisidine hydrochloride after oral administration is due to o-anisidine itself. From the established short-term tests, there is sufficient evidence that o-anisidine is mutagenic *in vitro*, while *in vivo* assays gave contradictory results. Positive results were observed in test systems measuring gene mutations. Therefore, o-anisidine has to be considered a genotoxic carcinogen. However, since no indications were found for DNA adduction both in liver and bladder, whereas in transgenic mice an increase in the mutation frequency was observed in the bladder, an indirect mechanism for o-anisidine cannot be entirely excluded.

The European Commission (Annex I of Dir. 67/548/EEC), the International Agency for Research on Cancer (IARC, 1999) and the German MAK Committee (DFG, 1996) have rated o-anisidine as carcinogenic. In agreement with the presently valid classification under Directive 67/548/EEC o-anisidine has been confirmed as category 2 carcinogen with the label R 45 (May cause cancer). Classification according to Annex I of Directive 67/548/EC, see Chapter 1.

4.1.2.9 Toxicity for reproduction

4.1.2.9.1 Animal data

Data on fertility impairment or developmental toxicity/teratogenicity are not available.

In a two-year carcinogenicity study with rats and mice (NCI, 1978), the histopathologic examination of seminal vesicles, prostate, and testis in males or ovary and uterus in females gave no indication for a damaging effect of o-anisidine hydrochloride on the male and female reproductive organs. In addition in a subacute study with rats no effects were observed in the testis (the female reproductive organs were not examined; Hoechst AG, 1990c).

In contrast to o-anisidine, information on developmental toxicity and teratogenic effects can be found in the IUCLID data sheets on structural analogues. Limited evidence points at embryotoxic and teratogenic properties of o-aminophenol after i.p. application of 100 mg/kg bw. in the Syrian Hamster in the absence of maternal toxicity. This information has to be considered as o-aminophenol may be formed in the metabolism of o-anisidine by o-demethylation (as was shown in *in vitro* studies; Schmidt et al., 1973; see section 4.1.2.1), it is, however, noted that the substance was not applied via a physiological route and, therefore, the relevance is questionable. On p-aminophenol, a structure less closely related, information is abundant and clearly shows that the substance is teratogenic and toxic to development after oral application in the rat in several studies (no statement given whether the concentrations applied were toxic to the dams), and after i.p. or i.v. application in the hamster in the absence of maternal toxicity. For aniline, there are also indications of developmental toxicity and terato-genicity. As for o-anisidine, methemoglobinemia is a common initial response observed following acute application of these aromatic amines. It can be speculated that this effect is equally or even more detrimental to the developing organism than for the dams. Damaging effects to the early stages of development could also be caused by genotoxicity (both o- and p-aminophenol are cat.3 mutagens) but it is noted that the picture for o-anisidine from the *in vivo* genotoxicity tests is not entirely convincing.

4.1.2.9.2 Human data

Data on reproductive effects in humans are not available.

4.1.2.9.3 Summary of toxicity for reproduction

The lack of observed microscopic effects on the reproductive organs in the two-year carcinogenicity study indicates that o-anisidine does not impair reproduction.

Due to lack of data, at present no statement concerning developmental toxicity/teratogenicity of o-anisidine can be given. In view of the positive evidence from related substances, o-anisidine may be suspected to possess teratogenic properties.

4.1.3 Risk characterisation

4.1.3.1 General aspects

In the EU >90% of the produced o-anisidine are processed to pigments which are used mainly for the printing of packings.

Workers can be exposed at workplaces in the production and processing industry as well as in the formulation of printing inks and their use in technical printing operations. The processing to o-anisidine based dye-products and their use for e.g. textile dyeing is in the EU of low and decreasing relevance and therefore not discussed further in the subsequent risk characterisation.

The main exposure paths are inhalation and skin contact. Oral exposure is presumed to be prevented by personal hygiene measures (see **Table 4.10**).

The consumer may be exposed to the substance by dermal contact with products printed with o-anisidine based pigments (such as printed cardboard or foils) and with imported textiles which may be dyed with a significant but not quantifiable amount of o-anisidine based dyes. For small children oral exposure via the sucking of dyed textiles is possible (see **Table 4.13**).

Indirect exposure via the environment may occur via the air or water in the vicinity of production or processing sites.

Studies dealing specifically with o-anisidine absorption or providing detailed information on the distribution and metabolism in the body are not available. From studies with experimental animals it can be assumed that o-anisidine is absorbed through the skin, in the gastrointestinal and the respiratory tract. There is no evidence for accumulation in the body.

In rodents, o-anisidine is harmful after acute oral uptake, and produces signs of toxicity after inhalation of the highest feasible concentration as well as after dermal application in a limit dose study. o-Anisidine leads to methaemoglobin formation in the rat and mouse and at a lower dose in the cat, a species with comparable methaemoglobin forming capacity to humans. The LOAEL in the cat after i.v. application is 7.7 mg/kg bw causing methaemoglobin levels of up to 11.5%.

In valid rabbit skin and eye irritation studies only a weak irritation potential was found, clearly below the classification threshold. Therefore, for this specific endpoint a risk assessment was not performed.

o-anisidine is not adequately tested for sensitising properties. There are indications of sensitizing properties in a study with guinea pigs with intra- and epicutaneous application (study insufficiently documented). The information from available data on structural analogues has to be regarded as inconclusive. Data on sensitizing effects in humans are not available.

In rats and mice, the repeated oral administration resulted in haemolytic anemia and changes in enzyme parameters or organ weights (liver, kidney, spleen). From a valid oral subacute study with rats performed according to OECD guideline 407, a NO(A)EL of 16 mg/(kg bw·d) was derived. This value was used for calculating the Margin of Safety. From a subchronic and a chronic oral study, where higher doses of o-anisidine were given, a NOEL cannot be derived. However, these studies show that the toxicity of o-anisidine does not increase significantly with duration of exposure. An acute i.v. study with cats (methaemoglobin formation at a single dose of 7.7 mg o-anisidine/kg bw) was not used as much higher plasma peak levels can be expected than after physiological routes of exposure.

No inhalation and dermal studies with repeated application are available for o-anisidine. In the risk assessment for non-carcinogenic effects for these routes of exposure a route to route extrapolation from the oral subacute study is performed. This is possible as in this study and the carcinogenicity study systemic effects but no local effects were observed.

The critical effect of o-anisidine for the assessment of human health is its carcinogenicity. In rats as well as in mice, tumours of the urinary system, especially of the bladder, occurred at high incidences. In addition, an increased incidence of tumours in the thyroid was observed in male rats. The bladder is also the main target organ in humans for this substance class of aromatic amines. Interindividual differences due to different activities of N-acetyltransferases and other toxifying and detoxifying enzyme activities may occur also for o-anisidine. For the calculation of the T25 in the case of o-anisidine in addition to the usual uncertainties concerning linear

extrapolation, additional uncertainties have to be taken into consideration. In the low dose group, the tumour incidence of papillomas or carcinomas of the urinary bladder was approximately 100%. Even if the proportion of carcinomas to papillomas is considered, which should be higher in the high dose group (Jonkinen, 1990), there is no clear difference between the dosages. Finally there was high mortality in the high dose group but in the low dose group to a lower extent as well. All these findings indicate saturation of the dose-response curve. It can therefore be assumed, that even much lower concentrations would give high tumour yields. Therefore in this extrapolation the tumour risk may be underestimated. On the other hand, an overestimation of the tumour rate cannot be excluded because usually due to the shape of the dose-response curve, the tumour risk obtained from linear extrapolation for a given carcinogen is much lower if the extrapolation starts from lower tumour incidences.

The mutagenic effects of o-anisidine have to be considered in combination with its carcinogenicity. From the established short-term tests, there is sufficient evidence that o-anisidine is mutagenic *in vitro*, while the *in vivo* assays gave contradictory results. Based on all the evidence o-anisidine is considered a genotoxic carcinogen. However, since no indications were found for DNA adduction both in liver and bladder, whereas in transgenic mice an increase in the mutation frequency was observed in the bladder, the main target tissue for carcinogenicity in mice and rats, an indirect mechanism for o-anisidine cannot be entirely excluded.

Specific data regarding effects on fertility in humans and animals are not available. However, as the histopathological examinations of the reproductive organs in the longterm studies did not yield any changes, effects on fertility are not considered as very probable.

Developmental effects cannot be evaluated due to lack of data on o-anisidine. There is a concern for embryotoxicity and teratogenicity from evidence on related substances.

4.1.3.2 Workers

The selection of the exposure scenarios, for which a risk characterisation was carried out and the figures used for the risk characterisation are shown in **Table 4.10**. Details on the risk assessment for repeated dose toxicity and carcinogenicity are given in **Table 4.11**, all endpoints are covered in **Table 4.12**.

Table 4.10 Selection of workplace exposure scenarios and values used for the risk assessment

Workplace	Further information	Exposure by inhalation	Dermal exposure	Exposure by ingestion
Production of o-anisidine	data available for one producer in Europe	0.06-0.07 mg/m³	unquantifiably low	prevented by personal hygiene
Processing of o-anisidine	data available for two processing sites, only production of pigments			
	operating (shift average)	0.05-0.15 mg/m³	unquantifiably low c)	prevented by personal hygiene
	cleaning, inspection, sampling (shift average)	0.09 mg/m³	unquantifiably low c)	prevented by personal hygiene
	installation of gas compensation pipes (< 1h) pumping operating	0.05—0.06 mg/m³ 0.05-0.09 mg/m³ 0.05 mg/m³	0.1 mg/cm² · d c) (600 µg/kg bw · d) *	prevented by personal hygiene
Formulation of pigments	No measurements Calculation according to the following assumptions: 1) Exposure to pigments during pigment formulation = exposure to dust in US textile dyeing factories measured concentrations during weighing processes: 0.007-0.56 mg/m³	0.07-28 ng/m³	10 – 250 ng/cm² · d c) (0.06 – 1.5 µg/kg · d) *	prevented by personal hygiene
	2) o-anisidine residues in pigments: 10 – 50 mg/kg according to measurements by the German manufacturer			
	Internal exposure due to reductive cleavage of the azo bond not relevant due to lacking bioavailability of pigments	not relevant	not relevant	not relevant
Formulation of dyes	Minor importance, <10% of o-anisidine production for dye-products with decreasing tendency	not relevant	not relevant	not relevant
Printing (use of pigments)	Inhalation: no relevant exposure: no significant quantities of dust, volatility of o-anisidine from aqueous solutions moderately to low Dermal exposure assumed to be comparable to formulation of pigments	not relevant	10 – 250 ng/cm² · d c) (0.06 – 1.5 µg/kg · d)*	not relevant
Dyeing	Minor importance, <10% of o-anisidine production for dye-products with decreasing tendency	not relevant	not relevant	not relevant

c) = calculated with EASE (see Chapter 4.1.1.1.2)

* Assuming 100% absorption, exposure of palms (420 cm²), 70 kg bw

4.1.3.2.1 Acute toxicity

Oral exposure

As oral exposure is considered highly unlikely under conditions of normal handling and use a risk for workers is not anticipated.

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

Exposure by inhalation

In an acute rat study, o-anisidine caused signs of toxicity but no mortality after inhalation of the highest feasible concentration (3,870 mg/m^3 applied as aerosol). The calculated Margin of Safety is between 25,800 and 77,400 for the production and processing of o-anisidine and $1.3 \cdot 10^8$ and $5.5 \cdot 10^{10}$ for the formulation of printing inks. In addition, the classification of o-anisidine for its acute toxicity by inhalation warrants an effective warning measure. Therefore, an acute inhalation risk is not considered of concern.

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

Dermal exposure

Assuming a mean body weight for workers of 70 kg, an exposure of the whole palms, and a dermal absorption of 100%, the calculated body burden is at a maximum of 0.6 mg/(kg bw·d) (0.1 mg/$cm^2 \cdot 420$ cm^2 [palms]/70 kg bw for the installation of gas compensation pipes and 0.00006 -0.0015 mg/(kg bw·d) (10 or 250 ng/$cm^2 \cdot 420$ cm^2 [palms] / 70 kg bw) for the formulation and use of o-anisidine based printing inks. However, the use of protective clothing (e.g. suitable gloves) is recommended, so that this exposure will be mitigated significantly.

In a limit test with rats, o-anisidine caused signs of toxicity but no mortality after dermal application of 2,000 mg/kg bw. The calculated Margin of Safety is ≥3,333 for the installation of gas compensation pipes and between $1.3 \cdot 10^6$ to $3.3 \cdot 10^7$ for the formulation and use of o-anisidine based printing inks. In addition, the classification of o-anisidine for its acute toxicity by the dermal route warrants an effective warning measure. Therefore, there is no concern for an acute dermal risk at the workplace.

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

4.1.3.2.2 Irritation / Corrosion

Dermal exposure

According to the results from valid studies in experimental animals, o-anisidine is not classified as irritating to skin or eyes. Therefore, dermal exposure of workers is not anticipated to result in irritant effects.

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

Exposure by inhalation

There are no data available concerning respiratory tract irritation. Based on the results from acute irritation/corrosion studies in experimental animals, o-anisidine is not suspected to be a respiratory tract irritant.

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

4.1.3.2.3 Sensitisation

o-Anisidine has not been tested adequately for sensitising properties.

The need for a test to evaluate the sensitising properties will be revisited in the light of the risk reduction strategy due to its carcinogenic properties.

4.1.3.2.4 Repeated dose toxicity

Oral exposure

As oral exposure is considered highly unlikely to occur under conditions of normal handling and use a risk for workers by this route is not anticipated.

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

Exposure by inhalation

Inhalation studies with repeated application of o-anisidine are not available. Therefore, a valid NOAEL of 16 mg/(kg bw·d) (rat, subacute, oral) was used for route to route extrapolation (oral → inhalation) concerning the risk assessment for human health.

For the calculation of the anticipated human NAEC the following assessment factor is applied:

- 2.6 for route-to-route extrapolation (oral → inhalation). This factor is based on a respiratory volume for the rat of 0.8 l/min/kg bw, on exposure by the inhalation route of 8 hours/day, and on the assumptions of a 100% resorption after inhalational uptake and, that o-anisidine uptake by the inhalation route results in the same degree of systemic toxicity as by the oral route.

By multiplying the NOAEL with this adjustment factor a human NAEC of about 42 mg/m3 is obtained. Using the respiratory volume of the rat involves species-species extrapolation by metabolic scaling since it is assumed that the respiratory volume corresponds to the energy consumption of the different species.

The calculated Margins of Safety are in the range of 280 – 840 for production and processing workplaces and $1.5 \cdot 10^6 – 6 \cdot 10^8$ for the formulation pigments (**Table 4.11**).

The symptoms observed in the oral subacute rat study are suggestive of acute methemoglobinemia to be the causative basic effect. Symptoms of methemoglobin formation have not been reported to occur in workers. As the rat does not respond with the same high sensitivity as humans to methemoglobin forming aromatic amines, a tentative factor for species

to species extrapolation of 10 is suggested to cover this difference, resulting in an estimated effective MOS of 28 – 84 and 1.5.105 – 6.107, respectively (**Table 4.11**).

Further, the merely subacute duration of the oral study on which this MOS is based has to be taken into consideration. Here a factor of 6 could be applied for extrapolating from subacute to chronic duration, which would result in a MOS of 5 – 14 and 25,000 – 1.107, respectively. However this would be a very conservative approach since o-anisidine, at least during production and processing where the higher exposure levels occur, is released intermittently in batches. In addition, the classification of o-anisidine for its acute toxicity and carcinogenicity represents an effective hazard warning. Therefore, the above calculated MOSs of 28 -84 and 1.5.105 – 6.107 are considered to reflect reasonably the exposure situation, and health risks due to repeated inhalation exposure also taking into consideration interindividual differences are not anticipated.

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

Dermal exposure

Body burdens are calculated from unquantifiably low to a maximum of 0.6 mg/(kg bw.d) for production and processing (installation of gas compensation pipes) and in the range of 0.00006 - 0.0015 mg/(kg bw.d) for the formulation and use of o-anisidine based printing inks.

Studies with repeated dermal application of o-anisidine are not available. Therefore, a valid NO(A)EL of 16 mg/(kg bw·d) (rat, subacute, oral) was used for route to route extrapolation (oral → dermal).

For the calculation of the anticipated human NAEL the following assessment factors were taken into account:

- 1 / 4 for metabolic rate scaling (rat → human; ECETOC, 1995);
- 1 for estimation of oral → dermal uptake (for a conservative estimation it is assumed that the dermal uptake of o-anisidine is about 100% of the oral uptake [based on the oral or dermal LD_{50} values in rats of 1,890 or >2,000 mg/kg bw respectively]).

By multiplying the NO(A)EL with these adjustment factors a human NAEL of about 4 mg/kg bw is obtained which is used for the risk assessment.

The calculated Margins of Safety are between infinitely high and maximal 6.7 (installation of gas compensation pipes) and from 2,667 – 66,667 (formulation and use of o-anisidine based printing inks), respectively (**Table 4.11**). Considering further that observations in the rat do not adequately reflect the higher human sensitivity to methemoglobin forming agents, a tentative factor for species to species extrapolation of 10 yields a MOS of <1 for the installation of gas compensation pipes which is clearly insufficient for worker health protection, so that for this specific workplace situation risk reduction measures are necessary.

The merely subacute duration of the oral study on which the MOS is based could be taken into consideration by applying a tentative factor of 6 for the extrapolation from subacute to chronic exposure. The latter, however, is not considered necessary for this scenario from the assumed exposure situation (batch production and processing).

For the formulation and use of pigments (especially printing inks) a chronic dermal exposure situation is also not anticipated. Still, in case of applying both extrapolation factors (interspecies,

subacute to chronic) a MOS in the range of 44 to 1,111 results which does not lead to concern for an elevated risk.

Conclusion (ii) (all workplaces except installation of gas compensation pipes).
There is at present no need for further information and/or testing and no need for risk reduction measures beyond those that are being applied already.

Conclusion (iii) (installation of gas compensation pipes).
There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Combined exposure

As can be seen from the MOS in **Table 4.11**, either dermal exposure or exposure by inhalation significantly contributes to the risk for each of the different exposure scenarios. Therefore it is not necessary to assess combined exposure.

4.1.3.2.5 Mutagenicity

Exposure at the workplace, as measured and calculated, occurs by inhalation and by the dermal route. From the established short-term tests, there is sufficient evidence that o-anisidine is mutagenic in vitro, while in vivo assays gave contradictory results. Supportive SAR evidence and tumorigenicity in more than one organ in rodents justify the suspicion that o-anisidine exerts genotoxic effects in humans. Overall, o-anisidine is considered a genotoxic carcinogen, which implies far-reaching risk reduction measures at the workplace.

Conclusion (iii) (for all workplaces; to be considered along with the individual conclusions on carcinogenic effects).
There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

4.1.3.2.6 Carcinogenicity

Data on carcinogenic effects in humans were not identified in the available literature. o-anisidine (tested as hydrochloride) was carcinogenic in rats and mice, rats being more susceptible. It can be assumed that the carcinogenic effect of o-anisidine hydrochloride after oral administration is due to o-anisidine itself. For continuous life time exposure, the T25 value for o-anisidine hydrochloride for rats was calculated to be 51.6 mg/(kg bw·d) (Dybing et al., 1997). Multiplying this value with a correction factor for the molecular weight of o-anisidine (0.77), a T25 value of 39.7 mg/(kg bw·d) is obtained.

Exposure by inhalation

Inhalation studies with repeated application for o-anisidine are not available. Therefore, the above calculated T25 value of 39.7 mg/(kg bw·d) was used for route to route extrapolation (oral → inhalation) concerning the risk assessment for human health.

For the calculation the following assessment factors were taken into account (see Section 4.1.3.1.4):

- 2.6 for route to route extrapolation (oral → inhalation) based on a respiratory volume of the rat of 0.8 l/min · kg bw, exposure of 8 hours/day and 100% resorption in the lung;
- 1 for metabolic scaling.

By multiplying the T25 value of 39.7 mg/(kg bw · d) with this adjustment factors a value of about 103 mg/m^3 is obtained. For adjustment from lifetime exposure to occupational exposure, additional adjustment factors were taken into account, resulting in a T25 value for workplace conditions of about 294 mg/m^3:

$$103\ \text{mg/m}^3 = \frac{75\ \text{years} \cdot 52\ \text{weeks} \cdot 7\ \text{days}}{40\ \text{years} \cdot 48\ \text{weeks} \cdot 5\ \text{days}} = 294\ \text{mg/m}^3$$

For production and processing workplaces Margins of Exposure (MOEs) of 1,960 – 5,880 are calculated and during the formulation of printing inks of $1.1 \cdot 10^7$ - $4.2 \cdot 10^9$ (**Table 4.11**). However, the carcinogenic risk is extrapolated with several uncertainties, i.e. possible interindividual differences due to different N-acetyl-transferase and other toxifying and detoxifying enzyme activities should also be taken into consideration.

Conclusion (iiia) Risks can not be excluded for all exposure scenarios, as the substance is identified as a non-threshold carcinogen. The adequacy of existing controls and the feasibility and practicability of further specific measures should be considered. However, the risk assessment indicates that risks are already low. This should be taken into account when considering the adequacy of existing controls and the feasibility and practicability of further specific risk reduction measures.

Dermal exposure

Studies with repeated dermal application of o-anisidine are not available. Therefore, the above calculated T25 value of 39.7 mg/(kg bw · d) was used for route to route extrapolation (oral → dermal) concerning the risk assessment for human health.

For the calculation the following assessment factors were taken into account (see Section 4.1.3.1.4):

- 1 / 4 for metabolic rate scaling (rat → human);
- 1 for estimation of oral → dermal uptake.

By multiplying the T25 value of 39.7 mg/(kg bw · d) with this adjustment factor a value of about 9.9 mg/(kg bw · d) is obtained. For adjustment from lifetime exposure to occupational exposure, additional adjustment were taken into account, resulting in a T25 value for workplace conditions of about 28.2 (mg/kg bw · d):

$$9.9\ \text{mg/m}^3\ (\text{kg bw} \cdot \text{d}) = \frac{75\ \text{years} \cdot 52\ \text{weeks} \cdot 7\ \text{days}}{40\ \text{years} \cdot 48\ \text{weeks} \cdot 5\ \text{days}} = 28.2\ \text{mg}\ (\text{kg bw} \cdot \text{d})$$

From the body burdens given in Section 4.1.3.1, MOEs of a maximum of 47 for the installation of gas compensation pipes and $1.9 \cdot 10^4$ to $4.7 \cdot 10^5$ for the formulation and use of printing inks can be calculated (**Table 4.11**).

Conclusion (iiib) (installation of gas compensation pipes).
There is a need for specific measures to limit the risks for carcinogenicity.

Conclusion (iiia) (all workplaces except installation of gas compensation pipes).
Risks can not be excluded for all other exposure scenarios, as the substance is identified as a non-threshold carcinogen. The adequacy of existing controls and the feasibility and practicability of further specific measures should be considered. However, the risk assessment indicates that risks are already low. This should be taken into account when considering the adequacy of existing controls and the feasibility and practicability of further specific risk reduction measures.

Combined exposure

As can be seen from the MOE in **Table 4.11**, either dermal exposure or exposure by inhalation significantly contributes to the risk for each of the different exposure scenarios. Therefore it is not necessary to assess combined exposure in addition to the assessment of the single exposure paths.

4.1.3.2.7 Toxicity for reproduction

Specific data concerning fertility impairment or developmental toxicity/teratogenicity in humans or experimental animals were not identified in the available literature.

Fertility impairment

As described in Section 4.1.2.9, the lack of observed effects in histopathological examinations of the reproductive organs in a two-year carcinogenicity study (o-anisidine given as hydrochloride) as well as the results from a subacute study indicate that o-anisidine does not impair reproduction.

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

Developmental toxicity/teratogenicity

Dermal exposure

o-Anisidine has not been tested for developmental toxicity/teratogenicity. The need for a test to evaluate developmental toxicity will be revisited in the light of the risk reduction strategy due to its carcinogenic properties.

Table 4.11 Occupational risk assessment (relevant workplace exposure scenarios) for repeated dose toxicity and carcinogenicity

Workplace operation	Exposure path	Dose (origin of exposure levels)	Duration, PPE	Repeated dose toxicity			Carcinogenicity		
				NAEC(L)	MOS (with safety factor *)	Conclusion	T25	MOE	Conclusion
Production									
Reaction vessel, distillation and filtration unit	Inhalation	0.06-0.07 mg/m3 (measured [a)])	Long term (6-7 h), batch production; protective gloves, goggles with a breathing mask, safety shield	42 mg/m3	600-700 (60-70)	ii	294 mg/m3	4,200-4,900	iiia
	Skin contact	unquantifiably low (EASE)	batch production; protective gloves	4 mg/kg bw · d	∝	ii	28.2 mg/kg bw · d	-	iiia
Processing									
Operating	Inhalation	0.05-0.15 mg/m3 (measured [a)])	long term, batch processing; LEV, protective clothing and breathing mask	42 mg/m3	280-840 (28-84)	ii	294 mg/m3	1,960-5,880	iiia
	Skin contact	unquantifiably low (EASE)	long term, batch processing; LEV, protective clothing	4 mg/kg bw · d	∝	ii	28.2 mg/kg bw · d	-	iiia
Cleaning, inspection, sampling	Inhalation	0.09 mg/m^3 (measured [a)])	long term, batch processing; protective clothes and breathing mask	42 mg/m^3	470 (47)	ii	294 mg/m^3	3267	iiia
	Skin contact	unquantifiably low (EASE)	long term; protective clothes and breathing mask; batch processing	4 mg/kg bw · d	∝	ii	28.2 mg/kg bw · d	-	iiia

Table 4.11 continued overleaf

Table 4.11 Occupational risk assessment (relevant workplace exposure scenarios) for repeated dose toxicity and carcinogenicity continued

Workplace operation	Exposure path	Dose (origin of exposure levels)	Duration, PPE	Repeated dose toxicity			Carcinogenicity		
				NAEC(L)	MOS (with safety factor *)	Conclusion	T25	MOE	Conclusion
Installation of gas compensation pipes	Inhalation	0.05-0.06 mg/m³ (measured [a)])	short term; protective clothes and breathing mask	42 mg/m³	700-840 (70-84)	ii	294 mg/m³	4,900-5,880	iiia
	Skin contact	unquant. low-600 µg/kg bw · d (EASE)	protective clothes and breathing mask	4 mg/kg bw · d	≥7 (≥0.7)	iii	28.2 mg/kg bw · d	47	iiib
Other workplaces (printing industry)									
Formulation of pigments (especially printing inks; e.g. weighing processes)	Inhalation	0.07-28 ng/m3 (calculated from measured data for colorants)	long term, at part LEV	42 mg/m3	1.5.105-6.107 (2.5.104 - 1.107) **	ii	294 mg/m3	1.1.107-4.2.109	iiia
	Skin contact	0.06-1.5 µg/kg bw.d (EASE)	extensive, safety gloves	4 mg/kg bw · d	2.7.103-6.7.104 (44-1,111)**	ii	28.2 mg/kg bw · d	1.9.104-4.7.105	iiia
Use of pigments (printing)	Inhalation	generation of dusts is not to be expected		42 mg/m3	∝	ii	294 mg/m3	∝	ii
	Skin contact	calculations for the formulation of printing inks can be transferred 0.06-1.5 µg/kg bw.d (EASE)	extensive, safety gloves	4 mg/kg bw x d	2.7.103-6.7.104 (44-1,111) **	ii	28.2 mg/kg bw x d	1.9.104-4.7.105	iiia

[a)] Data from the German manufacturer.

* Safety factor of 10 for the possibly higher sensitivity of humans to the formation of methemoglobin included.

** For the assumed continous exposure to o-anisidine at these workplaces inclusion of an additional safety factor of 6 for the extrapolation from subacute to chronic duration.

Table 4.12 Overview of the conclusions on the relevant workplace exposure scenarios for all toxicological endpoints

Endpoint	Acute Toxicity[a]		Irritation/ Corrosion		Sensitization		Repeated Dose Toxicity [a)]		Muta-genicity [b)]	Carcino-genicity		Fertility	Developm. Toxicity
Exposure scenario/route	Inh.	Der.	Inh.	Der.	Inh.	Der.	Inh.	Der.	Inh. Der.	Inh.	Der.	Inh. Der.	Inh. Der.
Production													
- Reaction vessel, distillation and filtration unit	ii	ii	ii	ii	[-]	[-]	ii	ii	iiia	iiia	iiia	ii	[-]
Processing													
- Operating - Cleaning, inspection, sampling	ii	ii	ii	ii	[-]	[-]	ii	ii	iiia	iiia	iiia	ii	[-]
- Installation of gas compensation pipes	ii	ii	ii	ii	[-]	[-]	ii	iii	iiia	iiia	iiib	ii	[-]
Formulation of printing inks	ii	ii	ii	ii	[-]	[-]	ii	ii	iiia	iiia	iiia	ii	[-]
Printing	ii	ii	ii	ii	[-]	[-]	ii	ii	Inh: ii Der.: iiia	ii	iiia	ii	[-]

a) The oral uptake of o-anisidine is assumed to be prevented by personal hygienic measures.

b) o-Anisidine is considered a genotoxic carcinogen. Mutagenicity and carcinogenicity have to be evaluated together. For the development of risk reduction strategies the classification concerning the carcinogenicity is relevant.

[-] Not (adequately) tested, need for testing will be revisited in the light of the risk reduction strategy due to its carcinogenic properties.

4.1.3.3 Consumers

The general population is exposed to o-anisidine in consumer products in the form of residual free substance in consumer goods which contain o-ansidine based pigments (e.g. printed cardboards and foils) or from metabolic/hydrolytic degradation of o-anisidine based dyes during the wearing of textiles. Therefore a significant exposure and uptake by inhalation is not to be expected. The relevant routes of exposure are oral (small children sucking clothes which were coloured with dyes based on o-anisidine) and dermal from direct skin contact with textiles. The uptake of o-anisidine via food from the migration of the substance from printed packings and foils and the dermal contact with these packings appear to be of minor importance. For children sucking at packing material and foils coloured with o-anisidine based pigments and dyes (low-level use of dyes for packing material) no attempt is made to quantify a potential exposure to o-anisidine due to lack of data and the fact that mainly biologically inert pigments are used. This scenario is, moreover, not anticipated to occur at a frequency and duration giving rise to concern.

The selection of the exposure scenarios, for which a risk characterisation was carried out and the values used for risk characterisation are summarized in **Table 4.13**. Details on the risk assessment for repeated dose toxicity and carcinogenicity are given in **Table 4.14**, all endpoints are covered in **Table 4.15**.

Table 4.13 Selection of consumer exposure scenarios for risk assessment

Source of exposure	Exposure scenario	Oral exposure µg/kg bw /d	Dermal exposure µg/kg bw/d	Exposure by inhalation
Pigments (especially printing inks)				
Printed cardboard from packings	skin contact: assumptions and calculation see Table 4.2 young children sucking probability of contact is considered of minor importance; bioavailability of pigments low inhalation: negligible due to physicochemical properties	not relevant	0.00009 - 0.0009	not relevant
Printed foils from packings	skin contact: assumptions and calculation see Table 4.2 young children sucking: probability of contact is considered of minor importance; bioavailability of pigments low inhalation: negligible due to physicochemical properties	not relevant	0.00001 -0.00011	not relevant
Food contaminated by migration of pigments into food	regarded as negligible due to surface printing of packings	not relevant		
Textiles printed with pigments	industrial importance in the EU very low; imported amount of textiles printed with o-anisidine based pigments possibly significant but not quantifiable; uptake of o-anisidine from residues possible; uptake via cleavage of the azo bond due to low bioavailability not expected	not relevant	not relevant	not relevant
Dyes				
Dyed clothes	minor importance for european production (<10% of o-anisidine production) exposure scenario considered, because textiles with dyes based on o-anisidine may be imported from non-EU countries			
	skin contact: assumptions and calculation see Table 4.3, lowest value adults, highest value: babies		0.006-20	
	young children sucking: assumptions and calculation see Table 4.3	0.3 - 130		
	inhalation: negligible due to physicochemical properties			not relevant
Dyed paper or leather	use of o-anisidine based dyes for paper or leather dyeing of minor importance (<5% of o-anisidine production); contact area, frequency of contact and contact time assumed to be significantly lower than for dyed textiles	not relevant	not relevant	not relevant
Vanilline	residues of o-ansidine in vanilline are not to be expected due to the multi-stage manufacturing process	not relevant	not relevant	not relevant

4.1.3.3.1 Acute toxicity

Oral exposure

Starting with a valid oral LD_{50} for rats of 1,890 mg/kg bw, the calculated Margin of Safety is in the range of $1.5 \cdot 10^4$ to $6.3 \cdot 10^6$ for young children sucking at clothes. Therefore, an acute oral risk is not considered of concern.

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

Dermal exposure

In a limit test with rats, o-anisidine caused signs of toxicity but no mortality after dermal application of 2,000 mg/kg bw. The calculated Margins of Safety in the case of skin contact must be higher than the following figures:

- skin contact with printed paper from packings: $2.2 \cdot 10^9$ to $2.2 \cdot 10^{10}$
- skin contact with printed foils from packings: $1.8 \cdot 10^{10}$ to $2 \cdot 10^{11}$
- skin contact with dyed textiles: $1.0 \cdot 10^5$ to $3.3 \cdot 10^8$
- combined exposure: $1 \cdot 10^5$ to $1.5 \cdot 10^8$

Therefore, acute dermal exposure is not considered of concern.

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

4.1.3.3.2 Irritation / Corrosion

According to the results from valid studies in experimental animals, o-anisidine is not classified as irritating to skin or eyes. Therefore, dermal exposure of consumers is not anticipated to result in irritant or corrosive effects.

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

4.1.3.3.3 Sensitisation

o-anisidine has not been tested adequately for sensitising properties. The issue of testing o-anisidine for sensitising properties has to be revisited under the light of the risk reduction strategy due to its carcinogenic properties.

4.1.3.3.4 Repeated dose toxicity

Oral exposure

For a first approximation of the risk of young children sucking at textiles coloured with o-anisidine based dyes calculated oral doses of 0.3 to 130 µg o-anisidine per kg bw per day and a valid NOAEL of 16 mg/kg bw/d (rat, subacute, oral) were used. This was adjusted by1/4 for

metabolic rate scaling (rat → human; ECETOC, 1995). Thus a NAEL of 4 mg/kg bw/d was obtained. The MOS for young children sucking at textiles is in the range of 31 – 13,333.

Due to the chronic exposure situation in addition an assessment factor of 1/6 for subacute to chronic threshold level may be taken into account. By multiplying the NOAEL with this adjustment factor, a human NAEL of about 0.7 mg/kg bw/d is obtained. The symptoms observed in the oral subacute rat study are further suggestive of acute methemoglobinemia to be the causative basic effect. As the rat does not respond with the same high sensitivity as humans to methemoglobin forming aromatic amines, an additional tentative factor for species to species extrapolation of 10 is suggested to cover this difference. This yields a calculated effective NAEL of 0.07 mg/kg bw/d for this exposure scenario.

By applying these considerations for the oral uptake of o-anisidine by small children while sucking at dyed clothes a worst-case Margin of Safety in the range of 0.5 to 223 is estimated. This MOS range is insufficient for the protection of the health of children, resulting in

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

Dermal exposure

If the estimation of risk is based on the above derived dermal NAEL of 4 mg/kg bw/d (see Section 4.1.3.1.4) MOS values are obtained as shown for the different dermal consumer exposure scenarios below (for the exposure levels see Section 4.1.3.2).

Further, in brackets, the MOS values are listed which would result if a tentative factor of 10 is applied for the higher human sensivity to methemoglobin forming agents compared with the rat (species to species extrapolation) and if chronic exposure situations (assessment factor of 1/6 for subacute to chronic threshold level) are taken as a basis. It is assumed that the latter is an approach realistic only to a certain degree for skin contact with dyed textiles:

- skin contact with printed paper: $4.4 \cdot 10^6$ to $4.4 \cdot 10^7$ ($7.3 \cdot 10^4$ to $7.3 \cdot 10^5$)
- skin contact with printed foils: $4 \cdot 10^7$ to $4 \cdot 10^8$ ($7 \cdot 10^5$ to $7 \cdot 10^6$)
- skin contact with dyed textiles: 200 to $6.7 \cdot 10^5$ (3.5 to $1.2 \cdot 10^4$)
- combined exposure: 200 to $6.5 \cdot 10^5$

Due to uncertainties in the exposure estimation the MOS ranges are very large. A safety factor of 3.5 from a worst case approach in the case of skin contact with dyed textiles appears to be insufficient for human health protection, therefore, for this specific exposure scenario a health risk cannot be excluded.

Conclusion (ii) (for dermal contact with printed paper or foils).
There is at present no need for further information and/or testing and no need for risk reduction measures beyond those that are being applied already.

Conclusion (iii) (for dermal contact with dyed textiles).
There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

4.1.3.3.5 Mutagenicity

Consumer exposure may occur by the oral and the dermal route. From the established short-term tests and other supportive evidence, like SAR, o-anisidine is suspected to be a mutagen. Tumorigenicity in more than one organ in rodents further justifies the suspicion that o-anisidine exerts genotoxic effects in humans. This gives rise to concern for an unquantifiable, not negligible risk for consumers.

Conclusion (iii) (for all exposure scenarios concerning the general public; to be considered along with the individual conclusions on carcinogenic effects).
There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

4.1.3.3.6 Carcinogenicity

Oral exposure

For assessing the risk of young children sucking textiles coloured on the basis of o-anisidine dyes calculated oral doses of 0.3 to 130 µg o-anisidine per kg bw per day and a calculated T25 value of 9.9 mg/kg bw/d were used (see Section 4.1.3.1.6). The calculated Margin of Exposure is in the range of about 305 to $1.3 \cdot 10^5$. A MOE of 305 is insufficient for the protection of the health of children.

Conclusion (iiib) (for small children during sucking at dyed clothes).
There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Dermal exposure

For risk assessment purposes a calculated T25 value of 9.9 mg/kg bw/d was used (see Section 4.1.3.1.6, dermal exposure). MOE values are obtained as shown for the different dermal consumer exposure scenarios below (for the exposure levels see Section 4.1.3.2):

- skin contact with printed paper: $1.1 \cdot 10^7$ to $1.1 \cdot 10^8$
- skin contact with printed foils: $9 \cdot 10^7$ to 9.9 10^8
- skin contact with dyed textiles: 495 to $1.7 \cdot 10^6$
- combined exposure: 495 to $1.6 \cdot 10^6$

A margin of exposure of 495 in the case of skin contact with dyed textiles is insufficient for the protection of consumers from the carcinogenicity of o-anisidine. Therefore, in this specific case of exposure further risk reduction measures are considered necessary.

Conclusion (iiib) There is a need for specific measures to limit the risks (for dermal contact with dyed textiles).

Conclusion (iiia) (for dermal contact with printed packings or foils):
Risks can not be excluded for all other exposure scenarios, as the substance is identified as a non-threshold carcinogen. The adequacy of existing controls and the feasibility and practicability of further specific measures should be considered. However, the risk assessment indicates that risks are already low. This should be taken into account when considering the adequacy of existing

controls and the feasibility and practicability of further specific risk reduction measures.

4.1.3.3.7 Toxicity for reproduction

Specific data concerning fertility impairment or developmental toxicity/teratogenicity in humans or experimental animals were not identified in the available literature.

Fertility impairment

As described in Section 4.1.2.9 reproductive toxicity testing has not been carried out (fertility impairment or developmental toxicity/teratogenicity) and there are no valid literature data available. Founded on the lack of changes observed in the reproductive organs in long-term studies with o-anisidine, effects on fertility are unlikely.

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

Developmental toxicity/teratogenicity

o-anisidine has not been tested for developmental toxicity. The issue of testing o-anisidine for developmental toxicity has to be revisited in the light of the risk reduction strategy due to its carcinogenic properties.

Table 4.14 Risk assessment for the relevant consumer exposure scenarios for repeated dose toxicity and carcinogenicity

Source of exposure	Exposure path	Dose (origin of exposure levels)	Repeated dose toxicity			Carcinogenicity		
			NAEL	MOS (with safety factors*)	Conclusion	T25	MOE	Conclusion
Dyed textiles	Ingestion	$3 \cdot 10^{-4}$- 0.13 mg/kg bw · d (young children sucking textiles; calculated)	4 mg/kg bw · d	31 - 13333 (0.5-223)	iii	9.9 mg/kg bw · d	$76\text{-}3.3 \cdot 10^{4}$	iiib
	Skin contact	$6 \cdot 10^{-6}$- 0.02 mg/kg bw · d (calculated)	4 mg/kg bw · d	$200\text{-}6.7 \cdot 10^{5}$ ($3.5\text{-}1.2 \cdot 10^{4}$)	iii	9.9 mg/kg bw · d	$495\text{-}1.7 \cdot 10^{6}$	iiib
Multicoloured packings (cardboard and foils)	Skin contact	$1 \cdot 10^{-8}$- $9 \cdot 10^{-7}$ mg/kg bw · d (calculated)	4 mg/kg bw · d	$4.4 \cdot 10^{6}$-$4 \cdot 10^{8}$ ($7.4 \cdot 10^{4}$- $7 \cdot 10^{6}$)	ii	9.9 mg/kg bw · d	$1.1 \cdot 10^{7}$ - $9.9 \cdot 10^{8}$	iiia

Safety factor of 10 for possibe higher sensivity of humans to the formation of methemoglobin with additional safety factor of 6 for the extrapolation from subacute to chronic duration included

Table 4.15 Overview of the conclusions for the relevant consumer exposure scenarios for all toxicological endpoints

Endpoint / Exposure scenario	Route of exposure	Acute Toxicity	Irritation / Corrosion	Sensitization	Repeated Dose Toxicity	Mutagenicity[a)]	Carcinogenicity	Fertility	Developmental toxicity
Skin contact with packings printed with pigments	Dermal	ii	ii	[-]	ii	iiia	iiia	ii	[-]
Young children sucking textiles coloured with dyes	Oral	ii	ii	not applicable	iii	iiib	iiib	ii	[-]
Skin contact with dyed textiles	Dermal	ii	ii	[-]	iii	iiib	iiib	ii	[-]

[a)] o-Anisidine is considered a genotoxic carcinogen. Mutagenicity and carcinogenicity have to be evaluated together. For the development of risk reduction strategies the classification concerning the carcinogenicity is relevant

[-] Not (adequately) tested, need for testing will be revisited in the light of the risk reduction strategy due to its carcinogenic properties

4.1.3.4 Humans exposed via the environment

There are no experimental data available concerning indirect exposure of man via the environment. By the application of EUSES the total daily intake of o-anisidine via food and drinking water is calculated to amount to 22 ng/kg bw/d for the production and 1.4 and 7.4 ng/kg bw/d, respectively, for the two processing sites. The inhalation uptake of o-anisidine from air for this local exposure scenario is 1.5 ng/kg bw/d for the production and 0.02 and 0.38 ng/kg bw/d, respectively, for the two processing sites (see **Table 4.16**).

As a biomagnification of o-anisidine via the food chain is not to be expected (see Section 3.1.5) there should be no significant uptake of the substance from food.

For risk assessment purposes concerning repeated oral or inhalation uptake, a calculated human NAEL of 4 mg/kg bw/d was used (see Section 4.1.3.1.4). Considering a total body burden of 23.5 ng/kg bw/d for the production site and 1.4 and 7.8 ng/kg bw/d for each processing site (sum of oral and inhalation uptake), the calculated Margins of Safety are $1.7 \cdot 10^5$ for the production site and $2.9 \cdot 10^6$ and $5.1 \cdot 10^5$, respectively, for each processing site (**Table 4.16**).

In addition, the MOS values are given which would result if a tentative factor of 10 is applied for the higher human sensivity to methemoglobin forming agents compared with the rat (species to species extrapolation) and if chronic exposure situations (assessment factor of 1/6 for subacute to chronic threshold level) are taken as a basis: $3 \cdot 10^3$ (production site) and $9 \cdot 10^3$ and $4.8 \cdot 10^4$ (two processing sites). For man exposed indirectly via the environment to apply these factors is considered to yield a worst-case approach. The MOSs do not give rise to concern of an increased risk of consumers.

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

For risk assessment purposes concerning carcinogenic effects a calculated T25 value of 39.7 mg/kg bw/d was used (see Section 4.1.3.1.6). The calculated Margin of Exposure for the production site is $1.7 \cdot 10^6$ and $2.8 \cdot 10^7$ and $5.1 \cdot 10^6$, respectively, for each processing site. Although the MOEs seem sufficiently high a residual risk cannot be ruled out.

Conclusion (iiia) Risks can not be excluded for all other exposure scenarios, as the substance is identified as a non-threshold carcinogen. The adequacy of existing controls and the feasibility and practicability of further specific measures should be considered. However, the risk assessment indicates that risks are already low. This should be taken into account when considering the adequacy of existing controls and the feasibility and practicability of further specific risk reduction measures.

From the data on mutagenicity of o-anisidine **conclusion** (**iii**) would have to be applied. However, this endpoint has to be considered in relation to the carcinogenicity of o-anisidine as the substance is a genotoxic carcinogen.

Table 4.16 Risk assessment for man exposed indirectly via the environment for repeated dose toxicity and carcinogenicity

Source of exposure	Exposure path	Dose	Repeated dose toxicity			Carcinogenicity		
			NOAEC(L)	MOS (with safety factors *)	Conclusion	T25	MOE	Conclusion
Emissions from production site	Inhalation [a)]	$1.5 \cdot 10^{-6}$ mg/kg bw · d (calculated)	4 mg/kg bw · d	$2.7 \cdot 10^{6}$ ($4.4 \cdot 10^{4}$)	ii	9.9 mg/kg bw · d	$6.6 \cdot 10^{6}$	iiia
	Ingestion [b)]	$22 \cdot 10^{-5}$ mg/kg bw · d (calculated)	4 mg/kg bw · d	$1.8 \cdot 10^{5}$ (3,000)	ii	9.9 mg/kg bw · d	$4.5 \cdot 10^{4}$	iiia
	Combined	$23.5 \cdot 10^{-5}$ mg/kg bw·d (calculated)	4 mg/kg bw · d	$1.7 \cdot 10^{5}$ (3,000)	ii	9.9 mg/kg bw · d	$4.2 \cdot 10^{4}$	iiia
Emissions from processing sites	Inhalation	$2 \cdot 10^{-8}$-$3.8 \cdot 10^{-7}$ mg/kg bw · d (calculated)	4 mg/kg bw · d	$1 \cdot 10^{7}$-$2 \cdot 10^{8}$ ($2 \cdot 10^{5}$-$3 \cdot 10^{6}$)	ii	9.9 mg/kg bw · d	$2.6 \cdot 10^{7}$ - $5.0 \cdot 10^{8}$	iiia
	Ingestion [b)]	$1.4 \cdot 10^{-6}$-$7.4 \cdot 10^{-6}$ mg/kg bw · d (calculated)	4 mg/kg bw · d	$2.9 \cdot 10^{6}$-$5.4 \cdot 10^{5}$ ($4.8 \cdot 10^{4}$-9000)	ii	9.9 mg/kg bw · d	$7.0 \cdot 10^{6}$-$1.3 \cdot 10^{6}$	iiia
	Combined	$1.4 \cdot 10^{-6}$-$7.8 \cdot 10^{-6}$ mg/kg bw·d (calculated)	4 mg/kg bw · d	$2.9 \cdot 10^{6}$-$5.1 \cdot 10^{5}$ ($4.8 \cdot 10^{4}$-9000)	ii	9.9 mg/kg bw · d	$7.3 \cdot 10^{6}$-$1.3 \cdot 10^{6}$	iiia

[a)] Due to releases from production and processing contaminated air

[b)] Food and drinking water contaminated via emissions from production and processing

* Safety factor of 10 for possibly higher sensivity of humans to the formation of methemoglobin with additional safety factor of 6 for the extrapolation from subacute to chronic duration included

Table 4.17 Overview of the conclusions for man exposed indirectly via the environment for all toxicological endpoints

Endpoint / Exposure scenario	Route of exposure	Acute Toxicity	Irritation / Corrosion	Sensitization	Repeated Dose Toxicity	Mutagenicity [a)]	Carcinogenicity	Fertility	Developmental toxicity
Emissions from production and processing	Oral	ii	ii	not applicable	ii	iiia	iiia	ii	[-]
	Inhalation	ii	ii	[-]	ii	iiia	iiia	ii	[-]

[a)] o-Anisidine is considered a genotoxic carcinogen. Mutagenicity and carcinogenicity have to be evaluated together. For the development of risk reduction strategies the classification concerning the carcinogenicity is relevant

[-] not (adequately) tested, need for testing will be revisited in the light of the risk reduction strategy

4.1.3.5 Combined exposure

From the compilation of data given in Section 4.1.1.4, it is obvious that the consumer exposure is about a factor of 10 lower than the occupational exposure related to the lifetime doses. For this, the additional health risk of workers using consumer products, which are coloured on the basis of o-anisidine, appears to be low. The conclusions for the workplace exposure scenarios therefore also apply for combined exposure (see **Table 4.12**).

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

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

5 CONCLUSIONS

5.1 ENVIRONMENT

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

5.2 HUMAN HEALTH

5.2.1 Human health (toxicity)

o-Anisidine has not been tested adequately for sensitising properties and no test is available on developmental toxicity. Risk reduction measures are required in view of the carcinogenic properties of this substance. The need for tests to evaluate these endpoints will be revisited in the light of the risk reduction strategy.

5.2.1.1 Workers

Workers may come into contact with o-anisidine during production, processing and during the formulation and use of o-anisidine based pigments. The main possible exposure routes appear to be via inhalational and dermal contact.

Concerning production and processing, measured workplace concentrations are available for the exposure to o-anisidine via inhalation at the German reporting manufacturer. Although o-anisidine is a non-threshold carcinogen the risk for the different workplace operations at this plant concerning the uptake of the substance via inhalation can be regarded as negligible as the exposure levels are low and appropriate personal protective equipment is applied.

The dermal exposure to o-anisidine is unquantifiably low for the most workplace operations at the manufacturer. Relevant exposure concentrations estimated from EASE calculations were only determined for the possible dermal contact with the substance during the installation of gas compensation pipes. Therefore, the following conclusions can be drawn:

Conclusion (iiib) There is a need for limiting the risks; risk reduction measures that are already being applied shall be taken into account.

This conclusion applies to

- concerns for general systemic toxicity, mutagenicity and carcinogenicity, as a consequence of exposure arising from the installation of gas compensation pipes at production of the substance.

Conclusion (iiia) Risks can not be excluded for all other exposure scenarios, as the substance is identified as a non-threshold carcinogen. The adequacy of existing controls and the feasibility and practicability of further specific measures should be considered. However, the risk assessment indicates that risks are already low. This should be taken into account when considering the adequacy of existing controls and the feasibility and practicability of further specific risk reduction measures.

5.2.1.2 Consumers

The general population may come into contact with the substance during the use of consumer products coloured with pigments or dyes based on o-anisidine. From the use pattern of the substance the contact with printed packings and foils and with dyed textiles can be identified as most important. These materials may contain free o-anisidine as residues or from degradation during the printing/dyeing process or during their use. Especially in the case of dyes an unintentional release due to reductive cleavage after resorption may occur in addition. The main exposure routes appear to be dermal (skin contact with printed packings and foils and dyed textiles) and oral (young children sucking at dyed textiles). A non-negligible risk was derived from exposure estimations concerning the dermal contact with dyed textiles and the oral uptake by young children sucking at dyed textiles.

A migration of o-anisidine residues from packings into food need not be considered as the packings are superficially printed so that the substance cannot be in direct contact with the food.

From the estimation of the possible risks the following conclusions can be drawn:

Conclusion (iiib) There is a need for limiting the risks; risk reduction measures that are already being applied shall be taken into account.

This conclusion applies to

- concerns for general systemic toxicity, mutagenicity and carcinogenicity, as a consequence of dermal exposure arising from textiles coloured with dyes based on the substance,
- concerns for young children for general systemic toxicity, mutagenicity and carcinogenicity, as a consequence of oral exposure by sucking textiles coloured with dyes based on the substance.

Conclusion (iiia) Risks can not be excluded for all other exposure scenarios, as the substance is identified as a non-threshold carcinogen. The adequacy of existing controls and the feasibility and practicability of further specific measures should be considered. However, the risk assessment indicates that risks are already low. This should be taken into account when considering the adequacy of existing controls and the feasibility and practicability of further specific risk reduction measures.

5.2.1.3 Humans exposed via the environment

Indirect exposure via the environment could occur by the intake of drinking water, as the main target compartment of o-anisidine is the hydrosphere. Concentrations of o-anisidine in drinking water are not reported. Relevant intake via drinking water is not to be expected considering the use pattern of o-anisidine. Relevant intake of the substance through food consumption is also not to be expected since there is no significant potential for biomagnification along the food chain. From calculations with EUSES very low exposure concentrations were derived for uptake by inhalation or ingestion of ambient air and water, respectively, in the vicinity of the production and processing sites.

Conclusion (iiia) Risks can not be excluded, as the substance is identified as a non-threshold carcinogen. The adequacy of existing controls and the feasibility and practicability of further specific measures should be considered. However, the risk assessment indicates that risks are already low. This should be taken into account when considering the adequacy of existing controls and the feasibility and practicability of further specific risk reduction measures.

5.2.2 Human health (risks from physico-chemical properties)

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

6 REFERENCES

Amtliche Sammlung Untersuchungsverfahren. Untersuchung von Bedarfsgegenständen. Nachweis der Verwendung verbotener Azofarbstoffe auf gefärbten textilen Bedarfsgegenständen. Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG 1996; 82.02/2.

Andrae U, Greim H. (1992). Initiation and promotion in thyroid carcinogenesis. **In**: Tissue Specific Toxicity: Biochemical Mechanisms. Academic Press Ltd. pp71-93.

Ashby J, Lefevre PA, Tinwell H, Brunborg G, Schmezer P, Pool-Zobel B, Shanu-Wilson R, Holme JA, Soderlund EJ, Gulati D, Wojciechowski JP (1991). The non-genotoxicity to rodents of the potent rodent bladder carcinogens o-anisidine and p-cresidine. Mutat. Res.; **250**: 115-133.

Ashby J, Short JM, Jones NJ, Lefevre PA, Provost GS, Rogers BJ, Martin EA, Parry JM, Burnette K, Glickman BW, Tinwell H (1994). Mutagenicity of o-anisidine to the bladder of lacI transgenic B6C3F1 mice: absence of 14C or 32P bladder DNA adduction. Carcinogenesis; **15**: 2291-2296.

Ashby J, Basketter, DA, Paton, D, Kimber, I (1995). Structure activity relationships in skin sensitization using the murine local lymph node assay. Toxicology. **103**: 177-194.

Association of the German Printing Ink Industry (1998). Use of o-anisidine based printing inks. Association of the German Printing Ink Industry, Frankfurt/M., 07.05.1998.

Auer. Auer Technikum (1989). Ausgabe 12. Bestell.-Nr. 0999-322.

Az R, Dewald B, Schnaitmann D (1991). Pigment decomposition in polymers in applications at elevated temperatures. Dyes Pigments. **15**: 1-14.

Basketter DA, Goodwin BFJ (1988). Investigation of the prohapten concept. Cross reactions between 1,4-substituted benzene derivatives in the guinea pig. Contact Dermatitis. **19**: 248-253.

Baughman GL. Fate of dyes in aquatic systems. Part 3: The role of suspended sediments in adsorption and reaction of acid and direct dyes. Dyes Pigments 1995; **27**: 197-210.

Baumann W, Herberg-Liedtke B (1995). Chemikalien in der Metallbearbeitung - Daten und Fakten zum Umweltschutz. Springer Verlag. Berlin.

Baumann W (1996). Personal communication. INFU Institut für Umweltschutz, Universität Dortmund, Dortmunt.

Bayer AG (1991). o-Phenetidine–Untersuchungen auf hautsensibilisierende Wirkung bei Meerschweinchen (Maximierungstest nach Magnusson und Kligman). Unpublished report; Bayer AG, Wuppertal.

Bayer AG (1996). Personal communication. Bayer AG, Leverkusen.

BG Druck & Papier (1996). Sicheres Arbeiten in der Siebdruckerei. BG Druck und Papierverarbeitung. Wiesbaden. 47 pp.

BgVV. (1997). Personal communication. BgVV. Berlin.

BP Oil (1996). o-Anisidine in metalworking fluids. Unpublished data. BP Oil Europe; Brussels/Belgium.

Brennan RJ, Schiestl RH (1999). The aromatic amine carcinogens o-toluidine and o-anisidine induce free radicals and intrachromosomal recombination in Saccharomyces cerevisiae. Mutat. Res. **430**: 37-45.

BUA. (1994). 2,4-Dichloroaniline, 2,5-Dichloroaniline, 3,4-Dichloroaniline. BUA Report 140. German Advisory Committee on Existing Chemicals of Environmental Relevance (BUA). Hirzel Wissenschaftliche Verlagsgesellschaft. Stuttgart.

Budavari S, O'Neil MJ, Smith A, Heckelman PE, Kinneary JF (1996). The Merck Index. An encyclopedia of chemicals, drugs, and biologicals, 12th ed.. Whitehouse Station, NJ USA, Merck & Co., Inc., 3 p.

BAuA. (1991). Stoffbelastungen in der Textilundustrie. Schriftenreihe Gefährliche Arbeitsstofe. GA 37. Bundesanstalt für Arbeitsschutz, Dortmund. 65 pp.

BAuA. Bulun SE, Economos K, Miller D & Simpson ER (1994) CYP 19 (aromatase cytochrome P 450) gene expression in human malignant endometrial tumors. J. Clin. Endocrinol. Metab. **79**: 1831-1834.

Amtliche Mitteilungen der Bundesanstalt für Arbeitsschutz (1994). Neue Stoffe am Arbeitsplatz: Ein Bewertungskonzept. Risikoermittlung, Risikobewertung, Maßnahmen zur Risikoverminderung. Sonderdruck 1994, Bundesanstalt für Arbeitsschutz und Arbeitsmedizin, Dortmund. 143 pp.

Canton JH, Sloof W, Kool HJ, Struys J, Pouw TJM, Wegman RCC, Piet GJ (1985). Toxicity, biodegradability, and accumulation of a number of CI/N-containing compounds for classification and establishing water quality criteria. Reg. Toxicol. Pharmacol. **5**: 123-131.

Ciba-Geigy AG (1995). Absorption, distribution, metabolism and excretion after single oral administration to male and female rats. 14C-FAT 92367/A. Unpublished report 381903. RCC, Itingen, Switzerland..

CITI (1992). Biodegradation and bioaccumulation. Data of Existing Chemicals based on the CSCL Japan. Chemicals Inspection & Testing Institute (ed.). Japan.

Council of Europe (1989). Resolution on the use of colourants in plastics materials coming into contact with food. Adopted by the Committee of Ministers on the 13th of September 1989, AP (89)I.

DFG (1996). MAK- und BAT-Werte-Liste 1996. Verlag Chemie, Weinheim.

Dunkel VC, Zeiger E, Brusick D, McCoy E, McGregor D, Mortelmans K, Rosenkranz HS, Simmon F (1985). Reproducibility of microbial mutagenicity assays: II. testing of carcinogens and noncarcinogens in salmonella typhimurium and escherichia coli. Environ. Mutagen. **7**: 1-248.

Dybing E, Sanner T, Roelfzelma H, Kroese D, Tennnant RW (1997). T25: A simplified carcinogenic potency index: Description of the system and study of correlations between carcinogenic potency and species/site specificity and mutagenicity. Pharmacol. Toxicol. **80**: 272-279.

ECETOC (1995). Assessment factors in human health risk assessment. Technical Report No. 68. ECETOC; Brussels, 57 pp.

Environment Canada (1997). OECD-SIDS exposure information: Canadian exposure data on o-anisidine. Environment Canada, Canada. 10.01.1997 (electronic mail).

EPA Japan (1990). Summary of the fiscal year 1990 general inspection survey of chemical substances on environmental safety. Environment Agency of Japan, Tokyo, Japan.

EPA Japan (1997). Toxicity of o-anisidine on aquatic invertebrates, plants and fish. Unpublished results. Environment Agency of Japan, Tokyo, Japan.

ETAD (1997a). (Ecological and toxicological association of dyes and organic pigments manufacturers). Use of o-anisidine. ETAD, Basel/Switzerland.

ETAD (1997b). (Ecological and toxicological association of dyes and organic pigments manufacturers). Extractability of dyestuffs from textiles over a normal life time of use. ETAD Project No. G 1033. ETAD, Basel/Switzerland.

ETAD (1998). (Ecological and toxicological association of dyes and organic pigments manufacturers). Production and use of o-anisidine based azo dyes. ETAD, Basel/Switzerland.

Ferretti JJ, Lu W, Liu M (1977) Mutagenicity of benzidine and related compounds employed in the detection of hemoglobin. Am. J. Clin. Pathol. **67**: 526-527.

Fiege H, Buysch HJ, Köller H, Waldmann H, Garbe D, Kaiser R, Lindner O, Paulus W. Phenol-Derivate (1979). **In**: Bartholomé E, Biekert E, Hellmann H, Ley H, Weigert WM, Weise E (ed.) Ullmanns Encyklopädie der technischen Chemie 4. rev. ed. Verlag Chemie, Weinheim.

Freyberger A (1994). Irreversible inhibition of thyroid peroxidase (tpo) by thyreotoxic aromatic amines in vitro.Naunyn-Schmiedeberg's Arch. Pharmacol. **349**: R110.

Galloway SM, Armstrong MJ, Reuben C, Colman S, Brown B, Cannon C, Bloom AD, Nakamura F, Ahmed M, Duk S, Rimpo J, Mergolin BH, Resnik MA, Anderson B, Zeiger E (1987). Chromosome aberration and sister chromatid exchanges in chinese hamster ovary cells: Evalutations of 108 chemicals. Environ. Mol. Mutagen. **10**: 1-175.

Garberg P, Akerblom EL & Bolcsfoldi G (1988). Evaluation of a genotoxicity test measuring DNA-strand breaks in mouse lymphoma cells by alkaline unwinding and hydroxyapatite elution. Mutat. Res. **203**: 155-176.

Garner RC, Nutman CA (1977). Testing of some azo dyes and their reduction products for mutagenicity using Salmonella Thyphimurium TA 1538. Mutat. Res. **44**: 9-19.

Glickman BW, Ahmed A, Roger BJ, Hamner R, Short JM, Tinwell H, Lefevre PA, Asby J (1993). Mutational specificity of o-anisidine in the bladder of the big blue. Environ. Mol. Mutagen. **21**: 1.

Haag, WR, Mill T (1987). Direct and indirect photolysis of water-soluble azodyes: Kinetic measurements and structure-activity relationships. Environ. Toxicol. Chem. **6**: 359-369.

Harris JC (1990). Rate of hydrolysis. In: Handbook of Chemical Property Estimation Methods. Lyman WJ, Reehl WF, Rosenblatt DH (eds.). American Chemical Society. Washington, DC. 48 pp.

Haworth S, Lawlor T, Mortelmans K, Speck W, Zeiger E (1983). Salmonella mutagenicity test results for 250 chemicals. Environ. Mutagenesis Suppl. Suppl. **1**: 3-142.

Health Council of the Netherlands (1995). Dutch Expert Committee on Occupational Standards (DECOS): Calculating cancer risk. The Hague: Health Council of the Netherlands, Publ.-No. 1995/06WGD.

Hellmér L, Bolcsfoldi G (1992a). An evaluation of the E.coli K-12 uvrB/recA DNA repair host-mediated assay. I. In vitro sensitivity of the bacteria to 61 compounds. Mutat. Res. **272**: 145-160.

Hellmér L, Bolcsfoldi G (1992b). An evaluation of the *E.coli K*-12 uvrB/recA DNA repair host-mediated assay. II. In vivo results for 36 compounds tested in the mouse. Mutat. Res. **272**: 161-173.

Herbst W, Hunger K (1995). Industrielle Organische Pigmente. Herstellung, Eigenschaften, Anwendung. 2nd rev.ed.. VCH Verlagsgesellschaft gmbH; Weinheim.

Hoechst AG (1984a). Echtrot BB Base flüssig, Prüfung der akuten oralen Toxizität an der männlichen und weiblichen Wistar-Ratte. Unpublished report. Hoechst AG, Frankfurt.

Hoechst AG (1984b). Echtrot BB Base flüssig, Prüfung auf Hautreizung am Kaninchen. Unpublished report. Hoechst AG, Frankfurt.

Hoechst AG (1984c). Echtrot BB Base flüssig, Prüfung auf Augenreizung am Kaninchen. Unpublished report. Hoechst AG, Frankfurt.

Hoechst AG (1984d). A mutagenicity screening of echtrot bb base in bacteria. Unpublished report. Hoechst AG, Frankfurt.

Hoechst AG (1988). o-Anisidin/ Prüfung der akuten dermalen Toxitität an der Wistar-Ratte. Unpublished report. Hoechst AG, Frankfurt.

Hoechst AG (1989a). Produktinformation o-Anisidine. Abteilung Verkauf Feinchemikalien (03/89). Hoechst AG, Frankfurt.

Hoechst AG (1989b). o-Anisidin D. Prüfung der akuten Aerosolinhalation an männlichen und weiblichen SPF-Wistar-Ratten (4-Stunden LC_{50}). Unpublished report. Hoechst AG, Frankfurt.

Hoechst AG (1989c). o-Anisidin D. Micronucleus test in male and female NMRI mice after oral administration. Unpublished report. Hoechst AG, Frankfurt.

Hoechst AG (1990a). Emissions of o-anisidine during production. Unpublished data. Hoechst AG, Frankfurt.

Hoechst AG (1990b). o-Anisidin: Respirationshemmtest nach OECD-Guideline 209. Dr. U. Noack, Laboratorium für angewandte Biologie, Hildesheim. Journal-Nr. 1481. Unpublished report. Hoechst AG, Frankfurt.

Hoechst AG (1990c). o-Anisidin. Subakute orale Toxizität (28 Applikationen in 29 Tagen) an SPF-Ratten. Unpublished report. Hoechst AG, Frankfurt.

Hoechst AG (1991). UV-spectrum of o-anisidine. Unpublished data. Hoechst AG, Frankfurt.

Hoechst AG (1994). Prüfung der leichten biologischen Abbaubarkeit von o-Anisidin D. Unpublished report. Hoechst AG, Frankfurt.

Hoechst AG(1995a). Purity of the commercial product. Unpublished data. Hoechst AG, Frankfurt.

Hoechst AG (1995b). EG-Sicherheitsdatenblatt o-Anisidine. Hoechst AG, Frankfurt.

Hoechst AG (1995c). Emissions of o-anisidine during production and processing. Unpublished data. Werk Griesheim, Katalytische Reduktion, Frankfurt. Hoechst AG, Frankfurt.

Hoechst AG (1996a). Production levels of o-anisidine. Unpublished data. Abt. Produktsicherheit/Ökologie (PSE), Frankfurt. Hoechst AG, Frankfurt.

Hoechst AG (1996b). IUCLID Datensatz o-Anisidin. Hoechst AG, Frankfurt.

Hoechst AG (1996c). Emissions of o-anisidine during processing. Unpublished data. Werk Offenbach, Acet-Betrieb. Hoechst AG, Frankfurt.

Hoechst AG (1996d). Interne Berechnung. Abt. SU Umwelt/Produktsicherheit, Frankfurt. Unpublished data Hoechst AG, Frankfurt.

Hoechst AG (1996e). o-Anisidin. Exposition am Arbeitsplatz. Unpublished data. Hoechst AG, Frankfurt.

Hoechst AG (1996f). Personal communication. Hoechst AG, Frankfurt.

Hoechst AG (1997a). Use of o-anisidine. Unpublished data. Hoechst AG, Frankfurt.

Hoechst AG (1997b). Hydrolytic stability of two pigments on the basis of o-anisidine. Unpublished data. Hoechst AG, Frankfurt.

Hoechst AG (1997c). Production and environmental release of o-anisidine during production and processing. Unpublished data. Hoechst AG, Frankfurt.

Hoechst AG (1997d). Draft environmental risk assessment. Unpublished data. Hoechst AG, Frankfurt.

Hoechst AG (1997e). Customers of o-anisidine and residues in primary products. Unpublished data. Hoechst AG, Frankfurt.

Hoechst AG (1997f). Workplace exposure during production. Unpublished data. Hoechst AG, Frankfurt.

Hoechst AG (1997g). Commercial importance of certain yellow and red pigments on the basis of o-anisidine. Unpublished data. Hoechst AG, Frankfurt.

Hoechst AG (1997h). Residual o-anisidine in coloured consumer products. Unpublished data. Hoechst AG, Frankfurt.

Hoechst AG (1997i). Purity of acet-o-anisidide. Unpublished data. Hoechst AG, Frankfurt.

Hoechst AG (1998). Workplace measurements during the production of o-anisidine Unpublished data. Hoechst AG, Frankfurt.

IARC (1999). Some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances.IARC Monogr Eval. Carcinog. Risk. Chem. Hum. **73**: 49-58.

Ichikawa Y, Yamano T, Fujishima H (1979). Relationship between the interconversion of cytochrome P450 and P420 and its activities in hydroxylations and demethylations by P450 oxidase system. Biochim Biophys Acta 1969; **171**: 32-46; cited in: Hansch C, Leo A. Substituent constants for correlation analysis in chemistry and biology. John Wiley & Sons, New York.

Ilichkina AG (1985). Toxikologisch-Hygienische Eigenschaften von Ortho- und Paraanisidinen. Gig. Sanit. **6**: 77-78.

Jonkinen MP (1990). Urinary bladder, ureter, and urethra. **In**: Boorman GA, Eustit SL, Elwell MR, Montgomery CA, MacKenzie WF (eds.) Pathology of the fischer rat. Reference and atlas. Academic Press, San Diego, pp. 109-126.

Kitano M. OECD Tokyo Meeting (May, 8-13, 1978). Ref Book Tsu-No. 3; cited in: HSDB (1998) Hazardous Substances Data Bank. National Library of Medicine, Rockville Pike, USA.

Kool HJ (1984). Influence of microbial biomass on the biodegradability of organic compounds. Chemosphere **13**: 751-761.

Lawrence FR, Marshall WJ (1985). Aniline. **In**: Gerhartz W, Yamamoto YS, Campbell FT, Pfefferkorn R, Rounasaville JF (eds.). Ullmann's encyclopedia of industrial chemistry, 5th ed., Volume A2: Amines, aliphatic to antibiotic. VCH Verlagsgesellschaft mbH, Weinheim, pp. 303-312.

LMBG (1997). Gesetz über den Verkehr mit Lebensmitteln, Tabakerzeugnissen, kosmetischen Mitteln und sonstigen Bedarfsgegenständen (Lebensmittel- und Bedarfsgegenständegesetz. BGBl. Teil I Nr. 63. 17.09.1997.

Leo A, Hansch C (1985). Pomona College. Unpublished analysis; cited **in**: Hoechst AG. IUCLID Datensatz o-Anisidin. Hoechst AG 1996c; Frankfurt.

LGC (1997). Best estimate risk assessment calculations for dermal and oral exposure. LGC Ltd, Teddington/UK.

Lide DR, Frederikse HPR (1995). CRC Handbook of Chemistry and Physics. A ready-reference book of chemical and physical data. 76th ed.. CRC Press, Boca Raton, USA, 2 p.

LUA (Landesumweltamt Nordrhein-Westfalen) (1996). o-Anisidin im Rheineinzugsgebiet. Landesumweltamt Nordrhein-Westfalen, Essen. 29.10.1996.

LUA (Landesumweltamt Nordrhein-Westfalen) (1998). o-Anisidin im Rheineinzugsgebiet. Landesumweltamt Nordrhein-Westfalen, Essen. 31.03.1998.

McLean S, Starmer GA, Thomas J (1969). Methaemoglobin formation by aromatic amines. J. Pharm. Pharmacol. . **21**: 441-450.

Meylan W, Howard PH, Boethling RS (1992). Molecular topology/fragment contribution method for predicting soil sorption coefficients. Environ. Sci. Technol. **26**: 1560-1567.

Mill T, Mabey W (1985). Photochemical transformations. **In**: Neely WB, Blau GE (eds.) Environmental exposure from chemicals Volume 1. CRC Press, Boca Raton, Florida, pp. 175-216.

MITI (1997). Exposure information on o-anisidine. Unpublished data. Tokyo, Japan.

Morrison V, Ashby J (1994). A preliminary evaluation of the performance of the Muta Mouse (lacZ) and Big Blue (lacI) transgenic mouse mutation assays. Mutagenesis **9**: 367-375.

National Cancer Institute (1978). Bioassay of o-Anisidine hydrochloride for possible carcinogenicity. Technical Rpt Series No. 89, DHEW Pub No. (NIH) 78-1339. U.S. Department of Health Education and Welfare, National Cancer Institute, Bethesda, MD.

NONS (1995). European inspection project on the notification of new substances. Final report. Inspected substances (confidential list). Oda Y, Yamazaki H, Watanabe M, Nohmi T, Shimada T. Development of high sensitive umu test system: rapid detection of genotoxicity of promutagenic aromatic amines by Salmonella typhimurium strain NM2009 possessing high O-acetyltransferase activity. Mutat. Res. **334**: 145-156.

Ono S, Kurata Y, Shichino Y, Sano M, Fukushima S (1992). Synergism of environmental carcinogens and promoters on bladder cancer development initiated by N-butyl-N-(4hydroxybutyl)nitrosamine in F344 rats. Jpn. J. Cancer Res. **83**: 955-963.

Prosolenko NV (1975). Comparative toxocological evaluation of methoxyanilines (o- and p-anisidines). Tr Khar'k Gos. Med. Inst. **124**: 11-14.

Putman DL, Gudi R, Young RR (1998). Micronucleus cytogenetic assay with mice. Unpublished report. Microbiological Associates, Inc., Rockville. Sponsored by: BG Chemie, Heidelberg.

RIZA (1991). Organische Micro Verontreinigingen in Rijn en Maas 1988-1990. Rijksinstituut voor integral zoerwaterbeheer en afvalwaterbehandling, The Netherlands.

Rhone-Poulenc Chimie (1996). Production of o-anisidine. Unpublished data. Rhone-Poulenc Chimie, Saint-Fons.

Rodriguez-Arnaiz R, Hernández Aranda J (1994). Activity of aromatic amines in the eye: w/w+ somatic assay of drosophila melanogaster. Environ. Mol. Mutagen. **24**: 75-79.

San RHC & Sly JE (1995). Unscheduled DNA synthesis assay in rat primary hepatocytes. Unpublished report. Microbiological Associates, Inc., Rockville. Sponsored by: BG Chemie, Heidelberg.

Sax NI, Lewis RJ (1987). o-Anisidine. **In**: Sax IN, Lewis RJ (eds.) Hawley's Condensed Chemical Dictionary, 11th ed.. Van Nostrand Reinhold Co., New York, p. 82.

Schmidt HL, Moeller MR, Weber N (1973). Über den Einfluß von Substituenten auf die mikrosomale Entalkylierung aromatischer N-, O- und S-Alkylverbindungen. Biochem. Pharmacol. **22**: 2989-2996.

Seiler JP (1977). Inhibition of testicular DNA synthesis by chemical mutagens and carcinogens. Preliminary results in the validation of a novel short term test. Mutat. Res. **46**: 305-310.

Shimizu H, Takemura N (1983). Mutagenicity of some aniline derivatives. In: Orford RR (ed.) Proceedings of the 11th international congress on occupational health in the chemical industry. Calgary, Canada, pp. 497-506.

Sloof W, Canton JH (1983). Comparison of the susceptibility of 11 freshwater species to 8 chemical compounds. II. (Semi)chronic toxicity tests. Aquat Toxicol. **4**: 271-282.

SRI (1994). Directory of chemical producers Western Europe. SRI International, Menlo Park, USA.

Srour R (1996). The BEICIP Aromatic intermediates report.Section B: chlorobenzenes and derivates. BEICIP (Bureau d'Etudes Industrielles et le Coopération de l'Institut Francais du Pétrole), Paris.

Thomas RG (1990). Volatilization from water. **In**: Lyman WJ, Reehl WF, Rosenblatt DH (eds.) Handbook of chemical property estimation methods. Environmental behavior of organic compounds. American Chemical Society. New York, pp. 15.1-15.34.

Thomas GA & Williams ED (1991). Evidence for and possible memchanisms of non-genotoxic carcinogenesis in the rodent thyroid. Mutat. Res. **248**: 357-370.

Thompson CZ, Hill LE, Epp JK, Probst GS (1983). The induction of bacterial mutation and hepatocyte unscheduled DNA synthesis by monosubstituted anilines. Environ. Mutagen. **5**: 803-811.

Thompson DC, Eling TE (1991). Reactive intermediates formed during the peroxidative oxidation of anisidine isomers. Chem. Res. Toxicol. **4**: 474-481.

Thompson DC, Josephy PD, Chu JWK, Eling TE (1992). Enhanced mutagenicity of anisidine isomers in bacterial strains containing elevated N-acetyltransferase activity. Mutat. Res. **279**: 83-89.

Traupe H, Menge G, Kandt I, Karmaus W (1997). Higher frequency of atopic dermatitis and decrease in viral warts among children exposed to chemicals liberated in a chemical accident in Frankfurt, Germany. Dermatology **1995**: 112-118.

Tyson CK, Mirsalis JC (1985). Measurement of unscheduled DNA synthesis in rat kidney cells following in vivo treatment with genotoxic agents. Environ. Mutagen. **7**: 889-899.

US EPA (1990). Textile dye weighing monitoring study (EPA 560/5-90-009). US EPA Office of Toxic Substances, Washington, USA.

US EPA (1996). Exposure information for SIDS initial assessment in non-sponsor countries. US EPA, Washington, USA.

Van Duuren BL (1980). Carcinogenicity of hair dye components. J. Environ. Pathol. Toxicol. **3**: 237-251.

Vasilenko NM, Zvezdaj VI (1981). Die Möglichkeit mathematischer Prognostizierung einiger Kriterien der Toxizität bei den Nitro- und Aminoverbindungen der aromatischen Reihe. Gig. Tr. Prof. Zabol. **25**: 50-52.

Vito M, Esposito G, Vicari L, Lembo S, Imperatrice ML, De Marinis E (1985). Attivita' mutagena del clorodimetilsolfuro e di alcuni suoi derivati anilinici. Boll. Soc. It. Biol. Sper. **61**: 917-923.

VSI (Verband Schmierstoff-Industrie e.V.) (1996). o-Anisidin in metal-working fluids. Unpublished data. Verband Schmierstoff-Industrie e.V. Schenefeld.

Wagner VO, Pugh DL (1995). Salmonella plate incorporation mutagenicity assay (Ames test) with a confirmatory assay. Unpublished report. Microbiological Associates, Inc., Rockville. Sponsored by: BG Chemie, Heidelberg.

Wangenheim J, Bolcsfoldi G (1988). Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds. Mutagenesis **3**: 193-205.

Watanabe K, Sakamoto K, Sasaki T (1996). Comparisons on chemically-induced mutagenicity among four bacterial strains, Salmonella typhimurium TA 102 and TA 2638, and Escherichia coli WP2/pKM101 and WP2uvrA/pKM101: Collaborative study I. Mutat. Res. **361**: 143-155.

Wearne SJ, Gem MGM, Harrison N, Collier PP, Fairweather F, Fielding M, Franklin A, Startin JR, Tregunno RJ, Walton H (1996). Contaminants of Food: Prioritisation scheme to identify manufactured organic chemicals as potential contaminants of food. Environ. Sci. Poll. Res. Int. **3**: 83-88.

Wellens H (1990). Zur biologischen Abbaubarkeit mono- und disubstituierter Benzolderivate. Z Wasser Abwasser Forsch. **23**: 85-98.

WHO (1994). Assessing human health risks of chemicals: derivation of guidance value for health-based exposure limits. Environmental Health Criteria 170, WHO, Genf.

Yoon JS, Mason JM, Valencia R, Woodruff RC, Zimmering S (1985). Chemical mutagenesis testing in drosophila. IV. results of 45 coded compounds tested for the national toxicology program. Environ. Mutagen. **7**: 349-367.

Yoshimi N, Sugie S, Iwata H, Niwa K, Mori H, Hashida C, Shimizu H (1988). The gentoxicity of a variety of aniline derivatives in a DNA repair test with primary cultured rat hepatocytes. Mutat. Res. **206**: 183-191.

Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K (1992). Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. Environ. Mol. Mutagen. Suppl. **19**: 2-141.

ABBREVIATIONS

ADI	Acceptable Daily Intake
AF	Assessment Factor
ASTM	American Society for Testing and Materials
ATP	Adaptation to Technical Progress
AUC	Area Under The Curve
B	Bioaccumulation
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft
BCF	Bioconcentration Factor
BMC	Benchmark Concentration
BMD	Benchmark Dose
BMF	Biomagnification Factor
bw	body weight / *Bw*, *b.w.*
C	Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
CA	Chromosome Aberration
CA	Competent Authority
CAS	Chemical Abstract Services
CEC	Commission of the European Communities
CEN	European Standards Organisation / European Committee for Normalisation
CMR	Carcinogenic, Mutagenic and toxic to Reproduction
CNS	Central Nervous System
COD	Chemical Oxygen Demand
CSTEE	Scientific Committee for Toxicity, Ecotoxicity and the Environment (DG SANCO)
CT_{50}	Clearance Time, elimination or depuration expressed as half-life
d.wt	dry weight / dw
dfi	daily food intake
DG	Directorate General
DIN	Deutsche Industrie Norm (German norm)
DNA	DeoxyriboNucleic Acid
DOC	Dissolved Organic Carbon
DT50	Degradation half-life or period required for 50 percent dissipation / degradation
DT90	Period required for 50 percent dissipation / degradation
E	Explosive (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
EASE	Estimation and Assessment of Substance Exposure Physico-chemical properties [Model]
EbC50	Effect Concentration measured as 50% reduction in biomass growth in algae tests

EC	European Communities
EC10	Effect Concentration measured as 10% effect
EC50	median Effect Concentration
ECB	European Chemicals Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECVAM	European Centre for the Validation of Alternative Methods
EDC	Endocrine Disrupting Chemical
EEC	European Economic Communities
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EN	European Norm
EPA	Environmental Protection Agency (USA)
ErC50	Effect Concentration measured as 50% reduction in growth rate in algae tests
ESD	Emission Scenario Document
EU	European Union
EUSES	European Union System for the Evaluation of Substances [software tool in support of the Technical Guidance Document on risk assessment]
F(+)	(Highly) flammable (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
FAO	Food and Agriculture Organisation of the United Nations
FELS	Fish Early Life Stage
GLP	Good Laboratory Practice
HEDSET	EC/OECD Harmonised Electronic Data Set (for data collection of existing substances)
HELCOM	Helsinki Commission -Baltic Marine Environment Protection Commission
HPLC	High Pressure Liquid Chromatography
HPVC	High Production Volume Chemical (> 1000 t/a)
IARC	International Agency for Research on Cancer
IC	Industrial Category
IC50	median Immobilisation Concentration or median Inhibitory Concentration
ILO	International Labour Organisation
IPCS	International Programme on Chemical Safety
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database (existing substances)
IUPAC	International Union for Pure and Applied Chemistry
JEFCA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
Koc	organic carbon normalised distribution coefficient
Kow	octanol/water partition coefficient

Kp	solids-water partition coefficient
L(E)C50	median Lethal (Effect) Concentration
LAEL	Lowest Adverse Effect Level
LC50	median Lethal Concentration
LD50	median Lethal Dose
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOED	Lowest Observed Effect Dose
LOEL	Lowest Observed Effect Level
MAC	Maximum Allowable Concentration
MATC	Maximum Acceptable Toxic Concentration
MC	Main Category
MITI	Ministry of International Trade and Industry, Japan
MOE	Margin of Exposure
MOS	Margin of Safety
MW	Molecular Weight
N	Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC
NAEL	No Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOEC	No Observed Effect Concentration
NTP	National Toxicology Program (USA)
O	Oxidizing (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limit
OJ	Official Journal
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic
P	Persistent
PBT	Persistent, Bioaccumulative and Toxic
PBPK	Physiologically Based PharmacoKinetic modelling
PBTK	Physiologically Based ToxicoKinetic modelling
PEC	Predicted Environmental Concentration
pH	logarithm (to the base 10) (of the hydrogen ion concentration $\{H^+\}$

pKa	logarithm (to the base 10) of the acid dissociation constant
pKb	logarithm (to the base 10) of the base dissociation constant
PNEC	Predicted No Effect Concentration
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
QSAR	(Quantitative) Structure-Activity Relationship
R phrases	Risk phrases according to Annex III of Directive 67/548/EEC
RAR	Risk Assessment Report
RC	Risk Characterisation
RfC	Reference Concentration
RfD	Reference Dose
RNA	RiboNucleic Acid
RPE	Respiratory Protective Equipment
RWC	Reasonable Worst Case
S phrases	Safety phrases according to Annex III of Directive 67/548/EEC
SAR	Structure-Activity Relationships
SBR	Standardised birth ratio
SCE	Sister Chromatic Exchange
SDS	Safety Data Sheet
SETAC	Society of Environmental Toxicology And Chemistry
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STP	Sewage Treatment Plant
T(+)	(Very) Toxic (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
TDI	Tolerable Daily Intake
TG	Test Guideline
TGD	Technical Guidance Document
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
UC	Use Category
UDS	Unscheduled DNA Synthesis
UN	United Nations
UNEP	United Nations Environment Programme
US EPA	Environmental Protection Agency, USA
UV	Ultraviolet Region of Spectrum
UVCB	Unknown or Variable composition, Complex reaction products of Biological material
vB	very Bioaccumulative

vP	very Persistent
vPvB	very Persistent and very Bioaccumulative
v/v	volume per volume ratio
w/w	weight per weight ratio
WHO	World Health Organization
WWTP	Waste Water Treatment Plant
Xn	Harmful (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
Xi	Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)

Appendix 1 Calculations with the EASE model

This log file was generated by the EASE system

Version: 2.0

The log-file-name is logprocess.log **Exposure of the plant operator**
The name is o-anisidine
The temperature of the process is 20
The physical-state is liquid
aerosol-formed is false
The exposure-type is dermal
The use-pattern is Non-dispersive use
The pattern-of-control is Segregation
The predicted dermal exposure to o-anisidine is very low

This log file was generated by the EASE system

Version: 2.0

The log-file-name is logproc2.log **Exposure during inspection, sampling and cleaning**
The name is o-anisidine
The temperature of the process is 20
The physical-state is liquid
aerosol-formed is false
The exposure-type is dermal
The use-pattern is Non-dispersive use
The pattern-of-control is LEV
The predicted dermal exposure to o-anisidine is very low

This log file was generated by the EASE system

Version: 2.0

The log-file-name is logproc3.log Exposure during the installing of gas compensation pipes
The name is o-anisidine
The temperature of the process is 20
The physical-state is liquid
aerosol-formed is false
The exposure-type is dermal
The use-pattern is Non-dispersive use
The pattern-of-control is Direct handling
The contact-level is Incidental
The predicted dermal exposure to o-anisidine is 0-0.1 mg/square cm/day
The predicted dermal exposure to o-anisidine is 0-0.1 mg/square cm/day
which is low

This log file was generated by the EASE system

Version: 2.0

The log-file-name is logprod.log **Exposure during production of o-anisidine**
The name is o-anisidine
The temperature of the process is 20
The physical-state is liquid
aerosol-formed is false
The exposure-type is dermal
The use-pattern is Closed system
significant-breaching is true
The use-pattern is Non-dispersive use
The pattern-of-control is LEV
The predicted dermal exposure to o-anisidine is very low

This log file was generated by the EASE system

Version: 2.0

The log-file-name is textile.log **Exposure during formulation and use of o-anisidine**
The name of the substance is pigment **based pigments for textile printing**
The temperature of the process is 20
The physical-state is liquid
The exposure-type is dermal
The use-pattern is Non-dispersive use
The pattern-of-control is Direct handling
The contact-level is Extensive

CONCLUSION: The predicted dermal exposure to pigment is 1-5 mg/square cm/day

Dermal exposure to a substance which is directly handled is determined by the use pattern (Non-dispersive use) and the contact level (Extensive), resulting in an exposure range of 1-5 mg/square cm/day

Appendix 2 Valid characterized end points used in the risk assessment

End point	Selected test result	Application	Section
Vapour pressure (20°C)	3.5 Pa (average from two valid test results)	Estimation of volatility from water	3.1.1
Water solubility (20°C)	15 g/l	Estimation of volatility from water	3.1.1
n-Octanol/water partition coefficient log K_{ow}	1.18 (measured)	Estimation of bioconcentration	3.1.1
Adsorption coefficient log K_{oc}	38 (calculated)	Estimation of soil and sediment sorption	3.1.1
Biodegradation	Pass levels within 28 d in a test on ´ready biodegradability´, failing 10 d time window	Elimination from water	3.1.1
Hydrolysis	not to be expected	Elimination from water	3.1.1
Photolysis/-oxidation in water	no data available	Assessment not possible	3.1.1
Photolysis/-oxidation in air	$t_{1/2} \approx 4$ h (calculated)	Elimination from air	3.1.1
Immobilization of daphnids	0.055 mg/l (NOEC)	Aquatic risk assessment	3.2.1, 3.3.1
Toxicity to aquatic microorganisms	800 mg/l (EC_{50})	Risk assessment sewage plant	3.2.1, 3.3.1
Toxicity to airborne organisms	no data available	Assessment not possible	3.3.2
Toxicity to terrestrial organisms	no data available	Assessment according to equilibrium partition method	3.3.3
Subacute toxicity (28 day oral according to OECD guideline 407)	NOEL: 16 mg/kg bw/d	Risk assessment human toxicity	4
Long-term carcinogenicity study (104 weeks)	NOEL not available	Risk assessment human toxicity	4

European Commission

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o-anisidine, Volume 15

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The report provides the comprehensive risk assessment of the substance o-anisidine. It has been prepared by Austria 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. 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 for o-anisidine concludes that there is at present concern for workers, consumers and humans exposed via the environment. The environmental risk assessment for o-anisidine concludes that there is at present no concern for atmosphere, aquatic ecosystem, terrestrial ecosystem and for microorganisms in the sewage treatment plant.

The conclusions of this report will lead to risk reduction measures to be proposed by the Commissions committee on risk reduction strategies set up in support of Council Regulation (EEC) No 793/93.

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