European Chemicals Bureau

Institute for Health and Consumer Protection

European Chemicals Bureau

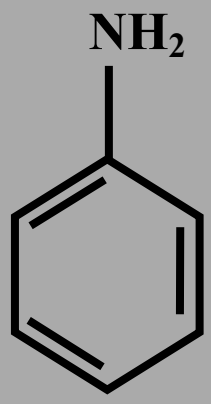
Existing Substances

European Union Risk Assessment Report

CAS No: 62-53-3

EINECS No: 200-539-3

aniline



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1st Priority List

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European Union Risk Assessment Report

ANILINE

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RISK ASSESSMENT

Luxembourg: Office for Official Publications of the European Communities, 2004

ANILINE

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RISK ASSESSMENT

Final Report, 2004

Germany

The risk assessment of aniline has been prepared by Germany on behalf of the European Union.

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Date of Last Literature Search:	**2001**
Review of report by MS Technical Experts finalised:	**2001**
Final report:	**2004**

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.

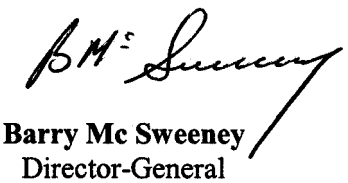
There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each 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
DG Joint Research Centre

Catherine Day
Director-General
DG Environment

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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 No: 62-53-3
EINECS No: 200-539-3
IUPAC name: aminobenzene
Synonym aniline

Environment

Aquatic compartment (incl. sediment)

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

This conclusion is reached because of the need for better information to adequately characterise the risks for the aquatic ecosystem as a consequence of exposure arising from rubber production sites.

The information and/or test requirements are:

- data about the formation of aniline from rubber chemicals, the releases into the wastewater and wastewater treatment processes which are representative for the European rubber industry.

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

This conclusion is reached because of:

- concerns for effects on the aquatic environmental spheres including sediment as a consequence of exposure arising from aniline production and further processing (4,4'-methylenedianiline and rubber chemicals) sites.

Atmosphere

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

This conclusion is reached because there is a need for better information to adequately characterise the risks to the atmosphere.

The information and/or test requirements are:

- data about releases into the atmosphere and the applied exhaust air purification techniques which are representative for the European rubber industry.

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

This conclusion is reached because of:

- concerns for effects on plants as a consequence of exposure via the air compartment arising from one aniline production site.

Terrestrial compartment

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

This conclusion is reached because there is a need for better information to adequately characterise the risks to agricultural soils from aniline as a degradation product of phenylurea and carbamate derivatives used as plant protection products.

The information and/or test requirements are:

- long term tests with plants, earthworms and micro-organisms.

However, since the risk to soil from the breakdown of plant protection agents is not covered by Regulation 793/93 it is proposed that this be considered within the frame of Council Directive 91/414/EEC.

Human health

Human health (toxicity)

Workers

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

This conclusion is reached because of:

- concerns for acute toxicity as a consequence of:
  - inhalation exposure and/or dermal contact in case of unsuitable gloves arising from production and further processing in the large-scale chemical industry;
  - inhalation exposure arising from thermal degradation of plastics in iron, steel and aluminium foundries;
  - dermal exposure arising from the use of dyes containing residual aniline;
- concerns for skin sensitisation as a consequence of dermal exposure arising from production and further processing in the large-scale chemical industry (in case of unsuitable gloves), and the use of dyes with residual aniline;
- concerns for systemic toxic effects as a consequence of
  - inhalation exposure and/or dermal contact in case of unsuitable gloves arising from production and further processing in the large-scale chemical industry;
  - inhalation exposure arising from vulcanisation of rubber chemicals, and from thermal degradation of plastics in iron, steel and aluminium foundries;
  - dermal exposure arising from the use of dyes containing residual aniline;
- concerns for mutagenicity and carcinogenicity in all workplace scenarios, as the substance is identified as a non-threshold carcinogen. However, for the following specific working scenarios risks are already low:

- release of aniline as a decomposition products in different industrial sectors (e.g. plastics processing, electrical engineering);
- use of products with residual aniline (e.g. adhesives, engineering, device and tool construction industries);

This should be taken into account when considering the adequacy of existing controls and the feasibility and practicability of further specific risk reduction measures.

- concerns for developmental toxicity as a consequence of dermal exposure in case of unsuitable gloves arising from production and further processing in the large-scale chemical industry.

Consumers

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

This concusion is reached because of:

- concerns for mutagenicity and carcinogenicity as a consequence of exposure arising from use of products containing the substance, as aniline is identified as a non-threshold carcinogen.

Humans exposed via the environment

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

This conclusion is reached because of:

- concerns for systemic toxic effects, developmental toxicity, mutagenicity and carcinogenicity as a consequence of exposure arising from point sources.
- concerns for mutagenicity and carcinogenicity as a consequence of possible exposures at a regional level, as aniline is identified as a non-threshold carcinogen. However, exposures are already very low and this should be taken into account when considering the adequacy of existing controls and the feasibility and practicability of further specific risk reduction measures.

<u>Human health (risks from physicochemical properties)</u>

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

This conclusion is reached because:

- The risk assessment shows that risks are not expected. Risk reduction measures already being applied are considered sufficient.

CONTENTS

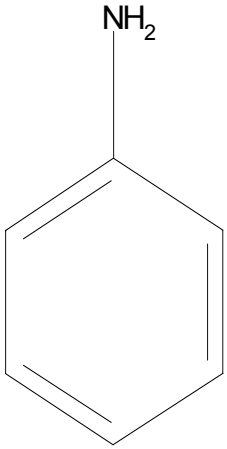
Euses Calculations can be viewed as part of the report at the website of the European Chemicals Bureau: http://ecb.jrc.it

TABLES

1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS No:	62-53-3
EINECS No:	200-539-3
IUPAC name:	aminobenzene
Synonyms:	aniline, phenylamine
Molecular weight:	74.08 $g \cdot mol^{-1}$
Molecular formula:	C_6H_7N
Structural formula:	



1.2 PURITY/IMPURITIES, ADDITIVES

Purity:	≥ 99.5% w/w	
Impurities:	water	0.1%
	nitrobenzene	< 20 ppm
	phenol	< 50 ppm
	low boiling fraction	50-250 ppm
	high boiling fraction	< 100 ppm
Additives:	none	

1.3 PHYSICO-CHEMICAL PROPERTIES

Table 1.1 Physico-chemical properties

Property	Value	Reference
Physical state	at 20°C and 1,013 hPa: colourless oily liquid with a characteristic odour and taste	
Melting point	- 6.2°C 1)	Sax and Lewis (1987)
Boiling point	184.4°C at 1,013 hPa 1)	Northcott (1978)
Relative density	1.022 at 20°C 1)	Budvari et al. (1989)
Vapour pressure	0.4 hPa at 20°C 1)	Bayer AG, (1993); Ullmann (1974)
Surface tension	70.5 mN/m at 20°C 1)	D'Ans Lax (1992)
Water solubility	35 g/l at 20°C 1)	Budvari et al. (1989)
Partition coefficient	log Pow 0.9 at 20°C (experimental) 2)	Susten et al. (1990)
Flash point	76°C (closed cup)	Chemsafe (1995)
Flammability	non flammable	Chemsafe (1995)
Ignition temperature	630°C (DIN 51794)	Chemsafe (1995)
Explosive properties	not explosive	no test conducted because of structural reasons
Oxidising properties	no oxidising properties	no test conducted because of structural reasons
Conversion factor	1 ppm = 3.87 mg/m^3	

1) There is no information about the applied method
2) Shaking-flask method

1.4 CLASSIFICATION

The classification and labelling of aniline has recently been discussed and MS agreement has been reached, as follows:

Classification	Carc. Cat. 3; R40 Muta. Cat. 3; R68 T; R23/24/25-48/23/24/25 Xi; R41 R43 N; R50
Labelling	T; N R: 23/24/25-40-41-43-48/23/24/25-68-50 S: (1/2-)26-27-36/37/39-45-46-61-63

Carc. Cat. 3; R40	Limited evidence of a carcinogenic effect
Muta. Cat. 3; R68	Possible risk of irreversible effects
T; R23/24/25-48/23/24/25	Toxic by inhalation, in contact with skin and if swallowed Danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed
Xi; R41	Risk of serious damage to eyes
R43	May cause sensitisation by skin contact
N; R50	Very toxic to aquatic organisms

S1/2	Keep locked up and out of the reach of children
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S27	Take off immediately all contaminated clothing
S36/37/39	Wear suitable protective clothing, gloves and eye/face protection
S45	In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)
S46	If swallowed, seek medical advice immediately and show this container or label
S61	Avoid release to the environment. Refer to special instructions/Safety data sheets
S63	In case of accident by inhalation: remove casualty to fresh air and keep at rest

Concentration limits

$C \geq 25\%$:	T, N; R23/24/25-40-41-43-48/23/24/25-68-50
$10\% \leq C < 25\%$:	T; R20/21/22-40-41-43-48/23/24/25-68
$1\% \leq C < 10\%$:	T; R20/21/22-40-43-48/23/24/25-68
$0{,}2\% \leq C < 1\%$:	Xn; R48/20/21/22

2 GENERAL INFORMATION ON EXPOSURE

For Western Europe, a total production capacity for aniline of 649,000 t/a in 1989 is reported, with an annual growth rate of 3.5%. The production volume was 500,000 t in 1990, 65,000 t were imported and 5,000 t exported in the same year (Chinn et al., 1991).

The following European companies are producers or importers of aniline:

- BASF AG, Antwerpen (BE)
- Bayer AG, Antwerpen (BEL)
- Bayer AG, Brunsbüttel (GER)
- Bayer AG, Uerdingen (GER)
- Enichem SpA, Brindisi (IT)
- Flexsys (BEL)
- Huntsman, Wilton (UK)
- Quimigal, Quimica de Portugal, Estarreja (POR)
- Uniroyal Chimica Srl, Latina Scalo (IT)

Two companies have recently stopped production and are therefore no longer cited above. However, in Section 3 both companies are still treated as producers because the available emission data are related to the former situation.

Aniline is exclusively used as an intermediate in the chemical industry (MC I, IC 3; UC 33). It can be a residual component of dyes and adhesives.

In **Table 2.1** below, market data and the use pattern according to different references are shown. BUA (1995) estimated the percentages for the sum of USA, Western Europe, Japan for 1988. Srour (1994) estimated the figures for Western Europe for 1993 and a prediction for 1998.

Table 2.1 Market data and use pattern of aniline for the sum of USA, Western Europe for 1988

	BUA (1995) 1988 use	**Srour (1994) 1993 use**	**Srour (1994) pred. for 1998**
Production vol.	-	530,000 t/a	605,000 t/a
Import	-	30,000 t/a	50,000 t/a
Export	-	3,000 t/a	-
European use	-	555,000 t/a	652,000 t/a
Processing to MDA	71%	410,000 t/a 74%	498,000 t/a 76%
Processing to rubber chem.	15%	88,000 t/a 16%	90,000 t/a 14%
Processing to dyes	5%	38,000 t/a 6.8%	42,000 t/a 6.4%
Processing to pesticides	3%	10,000 t/a 1.8%	10,000 t/a 1.5%
Processing to pharmaceuticals	1.2%	-	-
Processing to fibers	1%	-	-
Processing to others	3.7%	9,000 t/a 1.6%	12,000 t/a 1.8%

In addition, the information provided by industry for the present report was summarised (confidential Appendix G). Recent figures were supplied by the aniline producing/processing companies. From available production, export and import figures, the European use was calculated. The sum of the uses at known processing sites for which exposure scenarios were calculated in Section 3 covers 95% of the total European use. The actual production and use of aniline have increased more than predicted by Srour for 1998. The actual amount of aniline processed to MDA has increased to a large extent compared to the values estimated by Srour for 1998.

Releases of aniline occur into the atmosphere and into the hydrosphere during production and processing.

Furthermore, non-intentional environmental releases are considered in the exposure assessment:

- plant protection agents where aniline is formed as a degradation product,
- microbial reduction of nitrobenzene,
- rubber chemicals (degradation product),
- thermal degradation of polyurethanes,
- coal and oil industry,
- landfills.

For occupational exposure, the release of aniline by thermal degradation of plastics (e.g. in foundries and during rubber vulcanisation) as well as exposure caused by residual aniline in dyes and adhesives are considered.

The following occupational exposure limits apply in the EU (ILO, 1994):

- DK, S: 4 mg/m^3 (1 ml/m^3)
- FIN, B: 7.6 mg/m^3 (2 ml/m^3)
- D: 8 mg/m^3 (2 ml/m^3)
- UK, F: 10 mg/m^3 (2 ml/m^3)

In Germany, the short-time exposure limit amounts to 32 mg/m^3 (8 ml/m^3, 4·occupational exposure limit (MAK), 15 min, duration 1 h). Up to the end of 1996 it amounted to 40 mg/m^3 (10 ml/m^3, 5·MAK, during 30 minutes, 2 times per day).

3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 Environmental releases

3.1.1.1 Releases from production

The major starting product in the manufacture of aniline is nitrobenzene. The oldest method for the reduction of nitrobenzene uses iron and acetic acid. As a further product, high-grade synthetic iron oxides are produced which are used as pigments. A modern method is the catalytical reduction of nitrobenzene. After hydrogenation, the reaction mixture is separated to an organic phase containing aniline with dissolved water and an aqueous phase containing 4% aniline. The crude aniline is purified by distillation. Aniline is stripped from the aqueous phase and returned to the raw condensate.

Alternative production methods are ammonolysis of phenol or chlorobenzene. These methods are not applied in Germany (no further information available).

In the European Union, aniline is produced at 8 sites. From site-specific release amounts and production volumes, the release factors are calculated to:

Table 3.1 Release factors for production of aniline

	Hydrosphere	**Atmosphere**
For production only	99 ppm at 1 site which has no treatment plant 83 ppm at 1 site with a treatment plant, the factor includes WWTP purification	11 ppm
For production and processing	0.5 to 0.87 ppm at 2 sites with a treatment plant, the factors includes the releases from processing and WWTP purification	0.017 to 2.8 ppm

For 4 sites no relese factors were determined as more than two life-cycle steps take place at these sites.

3.1.1.2 Releases from processing

Processing to MDA

The total aniline production volume is processed by the chemical industry (MC I, IC 3; UC 33). The major subsequent product is 4,4'-methylenedianiline (MDA). MDA is synthesised by reaction of formaldehyde with aniline in the presence of hydrochloric acid. The condensation reaction may be carried out in a batch reactor or, alternatively, as a continuous process. The reaction product is a mixture of diamino and polyamino compounds. It is neutralised with an excess of caustic soda and allowed to settle into a two-layer mixture. The organic layer is separated, washed with hot water and distilled. The water recovered is recycled to provide the wash water for the washing stage. Unreacted aniline is recycled to the condensation reaction

stage. The aqueous layer produced after neutralisation is combined with the aqueous washings from the crude MDA washing stage. This mixture is then washed with aniline to remove dissolved MDA. The remaining aqueous layer is distilled to remove aniline and then discharged into the sewer (HMSO, 1995).

In the European Union, MDA is produced at 11 sites. From site-specific release amounts and production/processing volumes, the release factors are calculated to:

Table 3.2 Release factors for processing of aniline to MDA

	Hydrosphere	**Atmosphere**
For MDA production only	<0.013 to 78 ppm at 6 sites, all with treatment plants, the factors include WWTP purification	0.014 to 105 ppm
For MDA production, incl. aniline production and processing to other products	0.52 to 1 ppm at 2 site with treatment plant, the factors include releases from production and WWTP purification	0.02 to 2.9 ppm

For 3 sites no relese factors were determined as more than two life-cycle steps take place at these sites.

Processing to rubber chemicals

Aniline is processed to a serie of compounds being used in the rubber industry, e.g. mercaptobenzothiazole, diphenylguanidine, diphenylamine, aniline ketone condensates etc. 11 European sites are known to produce rubber chemicals. From site-specific release amounts and production/processing volumes, release factors are calculated to:

Table 3.3 Release factors for processing of aniline to rubber chemicals

	Hydrosphere	**Atmosphere**
For rubber chemicals only	0.44 to 75 ppm at 5 sites, all with treatment plants, the factors include WWTP purification 16,000 ppm (1.6%) at 1 site without treatment plant	24 to 380 ppm

For 2 sites release factors cannot be calculated, because site-specific release amounts were not submitted. Three other sites are known to produce rubber chemicals from aniline. The release factors were not calculated, because at these sites except the processing to rubber chemicals more than one other production or processing step takes place.

Processing to dyes

Table 3.4 Release factor for processing of aniline to dyes

	Hydrosphere	**Atmosphere**
For dyes only	500 ppm for 1 site, the factors include WWTP purification	110 ppm

Two other European sites are known to produce dyes from aniline. The release factors were not calculated, because at these sites except the processing to dyes more than one other production or processing step takes place.

Processing to plant protecting products

Table 3.5 Release factor for processing of aniline to plant protecting products

	Hydrosphere	Atmosphere
For ppps only	220 ppm for 1 site, the factors include WWTP purification	0 ppm

One other European site is known to produce plant protection products from aniline. The release factor was not calculated, because at this site except the processing to plant protection products more than one other production or processing step takes place.

Processing to pharmaceuticals

One site is known to produce pharmaceuticals from aniline. A release factor is not calculated, as the majority of the aniline is processed to another product, and the majority of the releases probably origin from those process.

Processing to other products

One other European site is known to produce other products (intermediates) from aniline. The release factor was not calculated, because at this site except the processing to this other products more than one other production or processing step takes place.

In Sections 3.1.3.2, and 3.1.4.2, the local PECs for the single sites are calculated. The summarised emissions during production and processing amount to 53 t into the atmosphere and 116 t into the hydrosphere.

3.1.1.3 Releases from microbial reduction of nitrobenzene

Aniline is metabolised from nitrobenzene under anaerobic conditions (Lu and Metcalf, 1975). However, because of the ready biodegradability of aniline, it is assumed that no significant environmental pollution will result from this source.

3.1.1.4 Releases from uses

Use of plant protecting agents

Aniline is processed to a series of plant protecting agents. It is known to be formed back by biotransformation from both phenylurea and phenylcarbamate derivatives. The major part is released in agricultural soils. When these agents are released into the hydrosphere, unknown amounts of aniline may be formed as well (cf. Section 3.1.3.2, Metabolisation of plant protecting agents).

Aniline forming plant protection products come under the Council Directive 91/414/EEC. Aniline is formed by metabolisation from these agents leading to an environmental exposure which adds to the emissions from production and processing. In the present report all known exposure pathways of aniline including those from the use of herbicides are assessed according to the principles of the Technical Guidance Document (TGD) (EC, 1996).

According to an inquiry among the EU member states, the following agents are presently applied:

Fenuron [3-phenyl-1,1-dimethylurea], CAS No. 101-42-8

Fenuron is applied as a non-selective herbicide. In soil, water and plants, the substance is degraded by a step-by-step N-demethylation (The Pesticide Manual, 1997).

In the UK, Fenuron is applied on arable crops, outdoor bulbs and flowers, grassland and fodder crops, and vegetables, with application rates between 0.09 and 1.49 kg/ha (total application 1.05 t/a). On paths and drives, 1.1 to 2.25 kg/ha are applied.

Fenuron is not used in Sweden, Finland, Belgium, Denmark, and Germany.

Propham [Isopropyl-N-phenylcarbamate], CAS No. 122-42-9

Propham is applied as a selective systemic herbicide and growth regulator. In soil and water, the substance is degraded to aniline. DT_{50} values of 15 days at 16°C and 5 days at 29°C are reported (The Pesticide Manual, 1997).

According to the Commission Decision 96/586/EC, plant protection products containing propham should be withdrawn by April 1997.

Siduron [N-(2-methylcyclohexyl)-N'-phenylurea], CAS No. 1982-49-6

The substance is applied as a selective herbicide. In soil and water, microbial degradation occurs with a DT_{50} of 120-150 days (The Pesticide Manual, 1997).

Siduron is not used in Sweden, Finland, Belgium, Denmark, UK, and Germany.

In France, siduron is homologated for grassland, with an application rate of 6 kg/ha (total application not known).

There are no figures about the environmental release amounts from these subsequent products available. The major part is set free in agricultural soils, where it reacts with humic substances (cf. Section 3.1.2.2 and Appendix D). A minor part, especially from the use of fenuron on sealed areas (paths and drives) may reach the hydrosphere via spray-drift and runoff.

In addition to the above cited substances there are other pesticides known to contain an aniline moiety, e.g. the N-substituted anilides carboxin [5,6-dihydro-2-methyl-N-phenyl-1,4-oxathiin-3-carboxamide, CAS-No. 5234-68-4], fenfuram [2-methyl-N-phenyl-3-furancarboxamide, CAS No. 24691-80-3] and propachlor [2-chloro-N-(1-methylethyl)-N-phenylacetamide, CAS No. 1918-16-7]. Carboxim and fenfuram are fungizides used for seed treatment, while propachlor is used as a herbicide (The Pesticide Manual, 1997). No further information is available whether aniline is remetabolised from these agents, or whether they are degraded via any other pathway. Furthermore, it is possible that further agents than the above mentioned can form aniline. Within the framework of this Risk Assessment report the conduction of a detailed

investigation and assessment of the releases of aniline due to metabolisation of pesticides is not possible.

Releases from rubber chemicals

Aniline is not used in the rubber industry, it is formed by reaction of other rubber chemicals which are its subsequent products. As precursor, a series of compounds comes into consideration: sulfenamide or guanidine accelerators (e.g. cyclohexyl-2-benzothiazolsulfen amide, diphenylguanidine), and N-phenyl-p-phenylenediamine derivatives (PPDs) which are used as anti-ageing agents. The contribution of each precursor is unknown, as there are no quantitative data about aniline formation rates available.

Releases at the rubber and tyre industry into the atmosphere

In a large-scale study about the composition of curing fumes, five carbon-black filled rubber compounds with typical sulphur-curing systems and one rubber compound with a peroxide-curing system, based on different polymers and other ingredients, were used. Samples were taken at a rotocure plant in the outlet channel before an installed scrubber. The loss of weight during curing was in all cases between 400 and 800 ppm. With the production volume of 25,000 t rubber/a, the author calculated the emissions of organic substances to 15 t/a. In the curing fume, totally 221 organic substances were identified. Aniline was detected in concentrations of 1,000 to 1,300 µg/m^3 in the sulphur-cured and 3,800 µg/m^3 in the peroxide-cured compound (Asplund, 1995).

With the available information, a precise release amount for aniline cannot be calculated. However, comparing the measured concentrations of aniline with those of the other substances, the aniline fraction can roughly be estimated to 10% of the total organic emissions. For an initial approach, the aniline releases are estimated to 60 ppm of the rubber production.

In the study of Asplund (1995), several methods for exhaust air cleaning are mentioned: thermal and catalytic oxidation, adsorption on active carbon, absorption by scrubbing, biofiltration and condensation. There is no information to what extent cleaning techniques are used in the European rubber industry, and how effective they are. Therefore, in the initial assessment no exhaust air purification is taken into account.

In the draft emission scenario document “additives in the rubber industry” (Baumann et al., 1999), a site with a daily production of 33 t tyres and 22 t rubber products is proposed for the local main source. With a total polymer production of 55 t/d and a release factor of 60 ppm, the daily releases are estimated to 3.3 kg/d.

In Europe, about 2.7 million t natural and synthetic rubber were consumed in 1997 (Baumann and Ismeier, 1998). With a release factor of 60 ppm, the annual release amount is 162 t. With a split of 10:90, 16 t/a are taken for the regional and 146 t/a for the continental model.

Releases at the rubber and tyre industry into the hydrosphere

It is expected that only a certain fraction of the aniline will be evaporated during rubber vulcanisation, another fraction may be released into the wastewater. Wastewater arises during vulcanisation (use of water vapour), cleaning of devices and working places, recycling of wasted rubber, and operation of scrubbing systems for exhaust air cleaning. Aniline may be discharged in aqueous solution or incorporated in or adsorbed onto rubber particles. The wastewaters are assumed to be supplied to industrial or municipal sewage treatment plants.

As no quantitative data about the aniline formation are available, an estimation of the environmental releases by rubber and tyre industry is uncertain. For a rough first exposure estimation, the following scenario is suggested:

- sulfenamide and guanidine compounds are used as vulcanisation accelerator. Vulcanisation agents are not released into the wastewater (Baumann et al., 1999),
- n-phenyl-p-phenylenediamine derivatives (PPDs) are used as anti-ageing agents. Measurements in 10 car and truck tyres resulted in contents between 3 to 16 g/kg rubber with an average of 8 g/kg (sum of the derivatives IPPD and 6PPD). Aniline was detected near the detection limit of 0.1 g/kg (Baumann and Ismeier, 1997). With the assumption that the aniline origins completely from PPDs, the conversion rate is 1.25%,
- in Germany, 4,000 t PPD derivatives were used in tyres and 590 t in rubber articles. With a conversion rate of 1.25%, and release factors of 1% for tyre and 2% for rubber production (Baumann et al., 1999), totally 650 kg aniline are released into the wastewater. Roughly assumed that the volumes in Europe are about fourfold, yearly 2.6 t aniline are released into the wastewater,
- if 70% of the wastewater are purified in a STP (elimination 87%) and 30% directly emitted, the total release into surface waters in Europe is 1 t/a,
- in the draft emission scenario document "additives in the rubber industry" (Baumann et al., 1999), a site with a daily production of 33 t tyres and 22 t rubber products is proposed for the local main source. Assuming an average PPD content of 8 g/kg, a conversion rate of 1.25%, and release factors of 1% for tyre and 2% for rubber production, the aniline release into wastewater is 77 g/d.
- the model input for rubber and tyre production is:

Table 3.6 Input data for calculation of PEC from rubber and tyre production

	Air	Wastewater	Hydrosphere
Local	3.3 kg/d	77 g/d	10 g/d
Regional	16 t/a	260 kg/a	100 kg/a
Continental	146 t/a	2,300 kg/a	900 kg/a

Releases during the use of tyres

In new and used tyres, aniline was detected in concentrations near the detection limit of 100 mg/kg rubber. Per one meter of interurban roads, in average yearly 90 g abraded rubber particles are released at each side containing 9 mg aniline. The total amount of rubber abrasion in Germany is about 60,000 t/a, with the measured concentration the total aniline content is calculated to 6 t/a (Baumann and Ismeier, 1997).

Beside tyre abrasion, aniline can be leached from the tyre surface by rain water. In a laboratory experiment, tyres were exposed to water which was analised after 2 months. In distilled water, 3.99-86.4 µg aniline/l were detected, while in artificial rain water (pH = 4), concentrations of 5.91-828 µg/l were found (Baumann and Ismeier, 1997). Thus further releases into the environment are expected during use and deposition of rubber goods.

Thermal degradation of polyurethanes

Aniline was detected in the working place atmosphere in foundries where it is formed by thermal degradation of MDI-based polyurethane bound foundry core materials (Renman et al., 1986). This issue is extensively described in Section 4. There are no data available about pollution of the outer atmosphere by these sources. However, it is assumed that this scenario is more relevant for the occupational risk assessment than for the environmnent.

Coal and oil industry

Bark et al. (1972) detected aniline in the effluents from coal carbonisation plants. The following levels were measured:

Table 3.7 Measured concentrations of aniline in effluents from coal carbonisation plants

Medium	Concentration (mg/l)
Tar-plant drainage water	1.2 / 2.3
Mixed effluent	3.1
Coke-oven limed spent liquor	5.4 / 8.1
Coke-oven unlimed spent liquor	16.5

At three shale oil manufacturing sites, aniline concentrations of 0.48 to 5.4 mg/l in the process retort wastewaters and of 0.57 to 14 mg/l in gas-condensate retort wastewaters were measured (Hawthorne and Sievers, 1984).

The substance was found in the wastewater of 2 coal conversion plants at a level of 21 resp. 12 mg/l (Singer et al., 1977; cited in Nielsen et al., 1993).

No more present information was found which could be used for quantification of the releases. Monitoring results from rivers in highly industrialised regions (listed in Section 3.1.3.1) are generally in a range between 0.1 to 1 µg/l, these levels are possibly caused by the coal or oil processing industry (a further candidate is rubber manufacture).

Landfills

Aniline was detected in the leachate plume from a mainly rural and municipal waste landfill in Canada. In one of two samples a level of 9.9 µg/l was detected; its presence was thought to be due to the small amounts of industrial waste deposited at the landfill site (Reinhard et al., 1984). The origin of the aniline may be rubber chemicals from rotting rubber articles. Because there is no more information available, the releases from this source cannot be quantified.

3.1.2 Environmental fate

3.1.2.1 Degradation

Elimination in wastewater treatment plants

Several experimental studies demonstrate that aniline is readily biodegradable in water under aerobic test conditions. In standardised tests the mineralisation amounts to 80-100% within 28 days. Due to the great number of available studies only a few tests are described here as representatives for the others.

Nyholm (1991) studied the mineralisation of aniline using 3 standard tests for ready biodegradability. With the closed bottle test (OECD 301 D) a mineralisation of 90% after 30 days was found; the modified OECD screening test (OECD 301 E) resulted in a mineralisation of 100% after 5 days and the modified Sturm test (OECD 301 B) in a mineralisation of 90% after 26 days.

The mineralisation of aniline in different tests on ready biodegradability was also examined by Gericke and Fischer (1979). The following mineralisation was found: 90% after 30 days (closed bottle test), 93% after 19 days (OECD screening test), 92% after 28 days (Sturm test) and 99% after 14 days (MITI I).

The partly great differences obtained in the different tests on ready biodegradability may be caused by the different stringency of the individual tests. This different stringency is mainly caused by origin and amount of the inoculum used and partly by different sum parameters used to quantify the mineralisation.

Under anaerobic conditions aniline was found to be not biodegradable. Battersby and Wilson (1989) found in a test using primary anaerobic digesting sludge from a WWTP treating domestic and industrial wastewater as inoculum a lag phase of more than 60 days. The net total gas production (CH_4 and CO_2) was only 6% of the theoretical value. Kuhn and Suflita (1989) tested the biodegradability of aniline in anaerobic aquifer slurries from two sites. Sulfate reducing conditions predominated at one site while methanogenesis prevailed in the other. Aniline was found to be recalcitrant under both redox conditons. No biotic transformation could be observed after 10 months under methanogenesis. Partial metabolism occurred in the sample from the sulfate reducing site after an adaptation period of several months. 39% of the aniline was biodegraded within 10 months.

According to the SIMPLETREAT model, an elimination rate in wastewater treatment plants (WWTP) of 87% (due to biodegradation) is used in the exposure assessment. 13% are emitted into the hydrosphere.

Biodegradation in surface water

Several investigations are available for the estimation of the biodegradability of aniline in surface waters. Only those studies are considered here that employed aniline concentrations of environmental relevance and that used aniline as sole carbon and energy source.

The primary degradation of aniline in river Rhine water was examined (Bayer, 1992). 10 µg/l aniline was added to the river water and incubated at 22°C in the dark. After 3 days a primary degradation of > 95% was achieved.

Subba-Rao et al. (1982) measured the mineralisation of ^{14}C-labelled aniline in lake water. Aniline concentrations of 5.7 ng/l to 500 µg/l were employed. The samples were incubated in the dark at 29°C for 21 days. The following results were obtained:

Table 3.8 Results from a mineralisation of aniline in lake water

Aniline concentration	Mineralisation
5.7 ng/l	98.5%*
54 ng/l	75.1%
505 ng/l	77.5%
5 µg/l	82.3%
50 µg/l	79.8%
500 µg/l	41.3%**

* Mineralisation was reached after 6 days
** Mineralisation was not complete at the end of incubation

From the slopes of the mineralisation curves rate constants of 0.138 d^{-1} for the concentration range between 5.7 ng/l to 5 µg/l and of 0.027 d^{-1} for the concentrations 50-500 µg/l were found. These rate constants are equivalent to half-lives of 5 days and 25.6 days, respectively.

Hwang et al. (1987) determined the rates of microbial degradation of ^{14}C-labelled aniline in estuarine water samples using aniline concentrations of 25 µg/l. The test flasks were suspended in an outdoor tank through which estuarine water was continuously circulated and water level in the flask was 3 cm below the surface. Investigations were conducted in summer (29°C) and in winter (14°C). Both mineralisation and transformation of aniline was measured. Half-lives for the mineralisation of aniline in samples exposed to sunlight at the upper layer of surface water were 33 days (k = 0.021-day^{-1}) in summer and 189 days (k = 0.0037 d^{-1}) in winter. In this experiment the rate constants are both due to biodegradation and photolysis. In experiments conducted in the dark that consider only biodegradation half-lives for mineralisation of 139 days (summer) and 770 days (winter) were found whereas in experiments that used poisoned water samples (only photolysis takes place) half-lives of 103 and 355 days were found for summer resp. winter. Half-lives for the transformation of aniline were much lower in all cases. They range from 27 hours (summer) resp. 71 hours (winter) for the unpoisened samples exposed to sunlight, over 36 hours (summer) and 125 hours (winter) for the poisoned water sample, to 173 hours (summer) for the dark experiment. It could be shown that microbial degradation rates were lower than photolysis rates throughout the year. However, if dullness and light absorption of surface waters is taken into account, biodegradation should be the major elimination pathway in the hydrosphere.

In several tests the mineralisation of ^{14}C-labelled aniline in natural seawater at low concentrations was examined: Nyholm (1991) found in a shake flask test employing an aniline concentration of 20 µg/l a half-life for mineralisation of 12 days. The sample was incubated at 15°C. With the same test design Nyholm et al. (1992) examined the mineralisation of aniline concentrations ranging from 2 to 40 µg/l in two different seawater samples. The following test results were obtained:

Table 3.9 Results from mineralisation of aniline in different seawater samples

Seawater sample	Aniline concentration	Rate constant [d^{-1}]	Half-life [d] for mineralisation
Sample from May 1985	21 µg/l	0.022	31
Sample from autumn 1987	2 µg/l	0.058	12
	10 µg/l	0.033	21
	20 µg/l	0.056	12
	40 µg/l	0.065	11

The above cited test results show that aniline in environmental relevant concentrations can be mineralised in both freshwater and seawater. The obtained half-lives for mineralisation range from 5 days (Subba-Rao et al., 1982) to 770 days (Hwang et al., 1987). These great differences can partly be explained by the test temperature, the site-specific characteristic of the microflora and the composition of the water sample.

The TGD proposes for readily biodegradable substances a half-life of 15 days for the biodegradation in surface waters. This value seems to be a compromise between the above reported half-lives. As microbial degradation processes are temperature dependent it is obvious that half-lives found in tests that were run at temperatures far above 20°C cannot be transferred unrestrictedly to the European standard environment that is defined in the TGD as having a temperature of 12°C. On the other hand the half-lives for mineralisation reported by Hwang et al. seem to be very high for a readily biodegradable substance. The half-lives found by Nyholm et al. in seawater at 15°C are in quite good agreement with the half-life proposed by the TGD.

Thus in the following exposure calculations an overall removal rate of $4.6 \cdot 10^{-2} \cdot d^{-1}$ (i.e. half-life 15 days) due to biodegradation in the hydrosphere is considered.

Abiotic degradation

A 10^{-5} M aniline solution was irradiated by May sunlight for 5 hours, and the reaction mixture was analyzed by liquid chromatography. The light conditions were: latitude 40°N, reaction in test tubes which corresponds water surface conditions. A degradation half-life of about one week was found in distilled water. The addition of commercial humic and fulvic acids as well as the use of river water (instead of distilled) lowered the degradation half-life to 4 to 8 hours. As reaction product, azobenzene was detected with a maximum yield of 0.2%, the major products (which are more polar than aniline) were not identified (Zepp et al., 1981).

Zepp and Schlotzhauer (1983) examined the influence of algae on the phototransformation rate of aniline. The degradation of aniline was determined both in distilled water and in the presence of 6 algae species (1-10 mg/l chlorophyll a). With algae, the reaction rate increased by a factor of 4 to 50 related to distilled water. Based on the portion adsorbed onto the algae, the reaction rate increased by a factor > 12,000. Even after the algae were heat-killed, the aniline degradation increased. The authors conclude that typical algae concentrations in natural freshwaters, which are several orders of magnitude lower than used in the study, have very little effect on the environmental aniline concentrations.

The influence of bicarbonate and carbonate radicals in the presence of H_2O_2 on the photodegradation of aniline was studied by Larson and Zepp (1988). A 1 µM aniline solution was irradiated with 313 nm light in a merry-go-round photoreactor. The degradation half-lives were 350 min for direct photolysis (0.092 M carbonate, pH 11.6), and 19 min (0.092 M

carbonate + 3 mM H_2O_2) resp. 20 min (0.092 M bicarbonate + 3 mM H_2O_2) for indirect photolysis. A near-surface half-life of 11 hours (latitude 40°N, summer) was computed based on the test results.

Hwang et al. (1987) determined the degradation of aniline in estuarine water (study cited above). The test flasks were suspended in an outdoor tank through which estuarine water was continuously circulated and water level in the flask was 3 cm below the surface. In experiments that used poisoned water samples (only photolysis takes place) mineralisation half-lives of 103 and 355 days were found for summer resp. winter. The respective half-lives for primary transformation were 36 hours and 125 hours.

The available studies show that aniline is photolytically degraded within about 4 to 11 h under spring or summer conditions in the top layer of surface waters. As no quantum yield was determined, a degradation rate cannot be estimated for a total water body (because of dullness and light disperion, photolysis occurs exclusively near the surface). Therefore a rate constant for the exposure modelling cannot be derived.

However, in the experiments of Hwang et al. (1987) photolysis was compared with biodegradation (see above). Under favoured light conditions, photolysis is an important elimination path near the water surface. It was found that, regarding the total water body, biodegradation should be the major elimination pathway in the hydrosphere.

Therefore, in the following exposure calculations only biodegradation ($t_{1/2}$ = 15 d) is considered.

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

Biodegradation in soil

Several tests are available that examine the biodegradation of aniline in soil.

Thompson and Corke (1969) found a rapid disappearance of aniline from soil at an initial concentration of 30 mg/kg. After 1 week incubation 18% of the aniline could be determined in the soil (no information about extraction method and analysis). After that time the elimination decreased and 7% of the aniline remained in soil after 10 weeks of incubation.

Medvedev and Davidov (1981) found 100% disappearance of aniline after 13 days of incubation at 19°C. Also the formed transformation product could not be detected after that time.

Süß et al. (1978) examined in a laboratory experiment with four soil types the mineralisation of ^{14}C-labelled aniline in a concentration of 1 mg/kg over 10 weeks. In the different soils between 16.2 and 26.3% of the aniline was mineralised to $^{14}CO_2$ after 10 weeks. The degradation maximum was already reached after 1 week. After 2 weeks 50% of the totally formed $^{14}CO_2$ was found. Then the weekly degradation rates remained constant at ca. 1% until the end of the test. After extraction with water between 57.3 and 67.4% of the ^{14}C activity could be detected in the four soils. Biodegradation in soil is impeded by the irreversible bonding of aniline to humic acids. Once those complexes are formed aniline is not accessible to degradation.

Kaufman and Blake (1973) used soil enrichment technique to isolate soil microorganisms capable of degrading the herbicide isopropyl carbanilate (prophame) in a muck and a silty clay loam. 5 g of the soil were added to an aqueous suspension (100 ml) of the herbicide (100 ppm). At 2-day intervals samples were taken and disappearance of the parent material, production of aniline and release of chloride ion were measured. It was found that 80% of the aniline formed

from propham was found to be degraded after 16 days in the muck soil. However, in the silty clay loam no degradation of aniline was observed.

From the available tests only the study by Süß et al. (1978) can be used to derive a biodegradation half-life for the reaction product of aniline with humic acids. In this study it was shown that at beginning of the test aniline is degraded relatively rapidly. At this time free aniline is available in the soil. During the test aniline becomes covalently bound to humic acids and the degradation rate decreases significantly. This property is known from several other aniline derivatives, further description is given in Appendix D.

From the degradation rate of about 1% per week found after 2 weeks a half-life of 350 days can be extrapolated approximately. For the risk assessment it has to be considered that a certain degree of aniline in soil is accessible to biodegradation before irreversible binding occurs. It is therefore assumed that 20% of the aniline in soil is rapidly mineralised and the other 80% are covalently bound to the organic fraction. For this bound aniline the above derived half-life of 350 days is used.

Sediments

No study on sediment degradation is available. Comparable to the fate in soils, aniline forms chemical bonds with the organic matter. For the reaction product, the same half-life as for soils (350 d) is used for the upper (aerobic) sediment layer, leading to a half-life of 3,500 days for the total sediment.

Atmosphere

In several tests on photochemical-oxidative degradation in the atmosphere the reaction constant with OH-radicals was determined (Atkinson, 1985; Witte et al., 1986). From this half-lives in the range of 3.2-3.5 h can be calculated ($C_{OH} = 5 \cdot 10^5$ molec/cm^3). In his critical review, Atkinson (1989) recommends the following Arrhenius expression:

$$k = 1.94 \cdot 10^{-11} \cdot e^{519/T} \text{ cm}^3 \cdot \text{molecule}^{-1} \cdot \text{s}^{-1}$$

With a temperature of 285 K and a OH radical concentration of $5 \cdot 10^5$ molecules $\cdot$ cm^{-3}, a half-life of 3.2 h is calculated.

Nitrobenzene was reported to be a reaction product of aniline and ozone or nitrogen monoxide (Atkinson et al., 1987; Nojima et al., 1975). Reaction constants with these species are not reported, but we expect that they are lower than with OH-radicals.

Summary

The following degradation rates are used in the further exposure assessment:

Table 3.10 Degradation rates

	k	$t_{1/2}$
$kdeg_{water}$	$4.6 \cdot 10^{-2} \cdot d^{-1}$	15 d
$kbio_{sed}$	$1.98 \cdot 10^{-4} \cdot d^{-1}$	3,500 d
$kbio_{soil}$	$1.98 \cdot 10^{-3} \cdot d^{-1}$	350 d
$kdeg_{air}$	$5.1\ d^{-1}$	3.2 h

3.1.2.2 Distribution

With a Henry's law constant in the range of 0.1-0.2 $Pa \cdot m^3 \cdot mol^{-1}$, aniline is expected to be low volatile from aqueous solution.

Experiments with aniline, various humus extracts and model substances revealed that the substance forms covalent bonds with the organic fraction in soils and sediments. Reaction partners of the amino moiety were found to be aldehyde or keto groups as well as double bonds of quinoid systems, which are typically for humic substances. The chemical attachment to humic substances occurs by at least two mechanisms, in a hydrolyzable and in a non-hydrolyzable manner (Parris, 1980). Therefore, the analytical determination of aniline in soils is not complete: with organic solvents only the physically adsorbed or in pore water dissolved fractions can be extracted, while by extraction with alkali the hydrolyzable fraction of the covalently bound aniline is removed additionally. The non-hydrolyzable fraction is inaccessible for analytical determination.

Because of the specifity of the reaction partners, chemisorption onto sewage sludge is not expected. The adsorption of aniline onto sludge should only be physisorption, which is described by the TGD models based on the log Kow of 0.9.

The binding properties to humic substances in aqueous solution were examined by Weber et al. (1996). Two different adsorption steps were identified: a rapid step which was attributed to electrostatic interactions, hydrophobic partitioning, and the formation of labile amine-carbonyl adducts, and a kinetically slower step which was attributed to irreversible covalent binding because of the inability to recover the parent compound by harsh extraction methods. With a fulvic acid concentration of 250 mg/l, 75% of the aniline (5 µM) disappeared over a time period of approximately 1 week, while with 25 mg/l only 10 to 20% of the substances were bound. The authors conclude that the dissolved organic matter is not a major sink of aromatic amines in most aquatic ecosystems. As a more likely scenario removal of the substances by sediment adsorption is supposed.

In a distribution experiment with radiolabelled aniline (6 concentrations, 0.0317-10 ppm), the radioactivity was measured in the supernatant water phase and the Freundlich adsorption constants were determined. Equilibrium was reached in nonsterile soils within 60 h, but was not attained in sterile soils by 120 h. With 2 nonsterile soils Koc values of 310 resp. 910 l/kg were calculated, while the values decreased to 130 resp. 410 l/kg when the same soils were autoclaved before the experiment. Aniline is degraded partially before adsorption, and the distribution

constants for the degradation products (azobenzene, azoxybenzene, phenazine) are much higher; therefore the constants determined in nonsterile soils seem to be overestimated. A minor effect is that the surface is slightly reduced leading to few lower constants (Pillai et al., 1982).

Similar Koc values (131 resp. 111 l/kg for two soils, 113 l/kg for one sediment) are reported from von Oepen (1990). Experimental conditions are not known.

Bishop et al. (1990) examined adsorption and desorption of ^{14}C-aniline on different sorbents. Using a soil with high organic content (20%) a Koc of 16 l/kg was determined. The low sorption constant is explained by the authors by saturation of the sorption sites. Desorption with 0.01 M $KClO_4$ and subsequent extraction with methanol yielded in a total removal rate of only 36% indicating that the major part of the test substance was irreversibly bound. On silica sand, kaolinite and an ironoxide containing soil (each with very low organic content) Kd values of 0.02 to 1.2 l/kg were found.

According to the TGD equation (log Koc = 0.62 log Kow + 0.85), a Koc value of 26 is calculated from the log Kow of 0.9. The underlying model presumes that sorption is only physically. As aniline undergoes chemisorption, this value is not appropriate for the exposure assessment.

The fate of aniline in the geosphere can be summarised as follows: The substance is reaching soils via deposition from the atmosphere and via degradation of plant protection agents which are its subsequent products. In soil, two competitive reactions occur: biodegradation and formation of covalent bounds onto the organic matter. The latter pathway leads to aniline-humic acid adducts which are immobile and only slowly degraded. Thus accumulation of the reaction product with humic substances occurs. The fate in sediments is considered to be similar than in soils. The formation of covalent bonds with humic matter is a general property of aniline derivatives. Appendix D gives an overview about this issue.

In the following model calculations, the empirically determined Koc-value of 410 l/kg is used. It should be kept in mind that the term "Koc" generally describes the distribution of a substance between the pore water and the organic matter when the substance is physically adsorbed; if chemisorption occurs the use of this term is not quite correct. The following distribution constants are calculated taking into account the organic carbon content of the individual compartments specified in the TGD:

Table 3.11 Distribution constants for aniline

Kp_{soil}	8.2 $l \cdot kg^{-1}$	$K_{soil\text{-}water}$	12.5 $m^3 \cdot m^{-3}$
Kp_{susp}	41 $l \cdot kg^{-1}$	$K_{susp\text{-}water}$	11 $m^3 \cdot m^{-3}$
Kp_{sed}	20.5 $l \cdot kg^{-1}$	$K_{sed\text{-}water}$	11 $m^3 \cdot m^{-3}$

With a concentration of 15 mg suspended matter per liter river water, 0.06% of the aniline is particle-bound. Based on the physico-chemical data it is predicted that the hydrosphere will be the target compartment of aniline released into the environment. The results of a Mackay-I calculation are presented in **Table 3.12**.

Table 3.12 Results of Mackay I calculation

Compartment	Percentage
Air	3.3
Water	87.1
Sediments	4.8
Soil	4.8

Figge et al. (1983) examined the distribution of chemicals in an environmental test system. A natural soil planted with equal part of grass, lettuce, radishes, and beans, was sprayed with 0.15 mmol ^{14}C-aniline and incubated for 30 days under June climate. The radioactivity was measured in the different compartments. For the activity the following distribution was observed:

Table 3.13 Distribution of aniline in an environmental test system

Compartment	Fraction of recovered radioactivity
Air	24% (from this 4.96% $^{14}CO_2$)
Soil (incl. seepage water)	49.44
Plants	26.56

It was not examined whether the radioactivity (which may be unreacted aniline or metabolites) was taken up via roots or leaves. Thus, no conclusion about the bioavailability for plants can be drawn.

3.1.2.3 Accumulation

There is only one reliable study available concerning the bioaccumulation of aniline in fish (Zok et al., 1991). *Brachydanio rerio* was exposed to ^{14}C-labelled aniline at a concentration of 0.2 µg/l under static conditions. The amount of radioactivity in the medium was kept constant by adding stock solution if required. After reaching a steady state of uptake and elimination, the remaining fish were transferred to a flow-through system containing clean water. A BCF of 2.6 ± 0.06 was determined. This result is in accordance with the measured log Kow of 0.9. The result shows no indication of a bioaccumulation potential due to the exposure of the organisms via water.

Bioaccumulation and uptake studies conducted with other aniline derivatives (mono- and dichloroaniline) are described in Appendix D. There are indications that the reaction product of chloroanilines is bioavailable for terrestrial and benthic organisms. Thus, there is some evidence (but no strong proof) that the reaction product of aniline with humic substancess is bioavailable and might accumulate via the benthic or terrestrial food chain.

The bioavailability of the reaction product of aniline with humic acids in sediment was examined in a test with the benthic oligochaete *Lumbriculus variegatus* that was performed in parallel to a sediment toxicity test (c.f. Section 3.2.1) (Egeler and Nésa, 2002). The sediment used in the test was an artificial sediment consisting of 5% peat, 20% kaolinite clay and 75% quartz sand. The

sediment had a mean organic matter content of 2 ± 0.5%. As food source for the test organisms urtica powder in a concentration of 0.5% of sediment dry weight was added. The test substance was added to the sediment as an aqueous solution. The test system was allowed to equilibrate for 48 hours before the worms were introduced. The oligochaetes were exposed to a nominal aniline concentration of 31.25 mg/kg dw for 28 days at 20 ± 2°C using a static system. Four replicate chambers, each containing 1-2 g wet weight of worms, were maintained. The worms were not fed throughout the test. Gentle aeration was used throughout the test, with the vessels being covered. During the study most of the worms in the replicates were observed lying on the sediment surface for one day. As possible reason the authors mention the relatively high stocking density in these replicates. Deviating from the study plan, the overlying water was renewed with uncontaminated water in order to improve water quality. The addition of uncontaminated water may lead to a further diffusion of test item from the sediment to the overlying water, as the formation of covalent bound to humic substances may not have been completed. A decrease of worm biomass was also observed in the replicates designated to determine body residues of aniline in the worms. The biomass in each single replicate was not sufficient for separate analysis per replicate. Therefore, the worms from the 4 replicates were pooled.

Analytical monitoring of the aniline concentration in the sediment shows a recovery of 22.27% after 28 days for this concentration level. The difference between nominal and measured aniline concentrations can be both attributed to biodegradation and formation of non-hydrolyzable binding to sediment components that is inaccessible for analytical determination. To consider the decrease of the aniline concentration by biodegradation, as a rough approach the revovery of 22.27% is used to correct the nominal aniline concentrations. As this recovery also covers formation of non-hydrolyzable binding of aniline to the sediment it represents a worst-case approach. The concentration of aniline in the pooled worm sample was 0.97 mg/kg worm tissue ww. The measured value was corrected for average recovery found in fortified worm tissue samples (73.3%). So a body residue of 1.32 mg/kg worm tissue ww was calculated. Body residue was normalised for the estimated dry weight and for the measured lipid content. The following table shows the obtained accumulation factors normalised for wet weight (AF_{ww}), dry weight (AF_{dw}) and lipid/TOC (AF_{lipid}) based on nominal and measured concentration.

Table 3.14 Accumulation factors of aniline in *Lumbriculus variegatus*

	AF_{ww}	AF_{dw}	AF_{lipid}
Nominal	0.06	0.21	0.08
Measured	0.28	0.95	0.36

In all cases the concentrations found in the worms were below the concentration in the sediment. However, the values for the accumulation factor were not statistically evaluated and they have not been evaluated for steady state conditions. Although the study shows some limitations and can therefore not be used unrestrictedly for a quantitative assessment of the bioaccumulation of aniline for sediment organisms, the result from this study can at least give an indication that no bioaccumulation of aniline in the test organisms from sediment occurs. Therefore, further tests on the bioaccumulation of aniline in sediment organisms are not of high priority for the risk assessment.

3.1.3 Aquatic compartment

3.1.3.1 Monitoring data

Aniline is part of a regular monitoring program in the Rhine and its tributaries. The following measured concentrations [µg/l] are reported (LWA, 1995; LUA NRW, 1999; RIWA, 1995, 1996).

Table 3.15 Aquatic monitoring data

Location	1994	1995	1996	1997	1998
Rhine - Bad Honnef	< 0.1 - 0.51 (∅ 0.10) *	< 1	< 1	< 1	< 1
Rhine - Düsseldorf	< 0.1 - 0.26 (∅ 0.07) *	-	-	-	-
Rhine - Kleve-Bimmen	< 0.1 - 0.50 (∅ 0.10) *	< 1	< 1	< 1	< 1
Rhine - Lobith	<0.01 - 0.99 (∅ 0.45)	<0.01 - 1.70 (∅ 0.85)	-	-	-
Sieg mouth	-	< 1	< 1	< 1	< 1
Wupper mouth	< 0.1 - 0.12 (∅ 0.06) *	< 1	< 1	< 1	< 1
Erft mouth	< 0.1 - 0.68 (∅ 0.10) *	< 1	< 1	< 1	< 1
Ruhr mouth	< 0.1 - 0.19 (∅ 0.06) *	< 1	< 1	< 1	< 1
Emscher mouth	< 0.1 - 1.60 (∅ 0.71) *	-	-	-	-
Lippe mouth	< 0.1 - 0.17 (∅ 0.06) *	< 1	< 1	< 1	< 1

* When values were below the detection limit, the half detection limit was used for the average value calculation

A comparison between mesasured and calculated aniline concentrations can be made on the basis of the 1994 data. The detected levels in the Rhine can clearly related to several production and processing sites which are covered by the present assessment. The origin of the aniline in the other rivers with positive detection is not known, other sources like rubber manufacturers, coal carbonisation plants or oil processing industry are possible.

Monitoring data from other European countries are not available.

3.1.3.2 Calculation of predicted environmental concentrations

Estimation of Clocal for production and processing / site-specific approach

The discharges of aniline during production and processing are assessed as point source emissions because the individual production/processing sites are identifiable. For the site-specific scenarios, all aniline producers as well as all known aniline processing sites are considered. In the tables, the available site-specific data and, when they are uncomplete, the used default values defined in the TGD are listed.

For the $C_{effluent}$, which is the basis for the assessment of treatment plants, the 90 percentile value or, when the compound was not detected, the detection limit is used.

For releases into rivers, the Clocal is calculated with the $C_{effluent}$ and a dilution factor resulting from wastewater and river low flow (10 percentile or 1/3 of the mean flow). For releases into estuaries, the default value of 1:10 for dilution while entering the sea is used, if further information is available which suggests that the dilution is higher (e.g. the pipe's end is far from coast, or a diffuser is installed), a factor of 100 is applied.

The PEClocal includes the PECregional of 0.13 µg/l (cf. Section 3.1.7).

Table 3.16 Local exposure at sites emitting into rivers

Site	Life stage	Site-specific data	Default values	Ceffl. [µg/l]	Clocal [µg/l]	PEClocal [µg/l]	Release [kg/a]
A	prod. + proc. to MDA, caout. chem, dyes	effluent conc. (∅ and 90%ile), sewage and river flow; prod. period	-	80	0.63	0.76	9,300
C	prod. + proc. to dyes, other intermediates and ppps	effluent conc. (∅ and 90%ile), sewage and river flow; prod. period	-	139	0.94	1.07	9,500
D	prod. + proc. to MDA	effluent conc. below dl, sewage and river flow; prod. period	-	< 10	< 0.38	< 0.51	< 14
E	prod. + proc. to MDA, caout. chem	effluent conc. (∅ and 90%ile), sewage and river flow; prod. period	-	100	0.51	0.64	240
F	prod. + proc. to MDA	effluent conc. below dl, sewage and river flow; prod. period	-	< 20	< 0.19	< 0.32	< 160
G	prod.	max. effluent concentration, sewage and channel flow; prod. period	-	20,000	280	280	< 7,900
H	prod.	effluent conc. (∅ and 90%ile), no WWTP	(dilution see remarks)	-	48	48	26,500
J	proc. to caout. chem.	effluent conc. below dl, sewage and river flow, proc. period	-	< 1	< 0.38	< 0.51	< 3.4
K	proc. to caout. chem.	mean and max effluent conc., sewage and canal flow, proc. period	-	2,000	3.4	3.5	120
M	proc. to caout. chemicals	processing volume, river flow	release factor 0.7%, sewage flow, WWTP elimin. 87%; proc. period	2,300	130	130	170
N	proc. to caout. chemicals	effluent conc. below dl, sewage flow, proc. period	river flow	< 50	< 0.0063	< 0.14	< 11

Table 3.16 continued overleaf

Table 3.16 continued Local exposure at sites emitting into rivers

Site	Life stage	Site-specific data	Default values	Ceffl. [µg/l]	Clocal [µg/l]	PEClocal [µg/l]	Release [kg/a]
O	proc.	proc. volume, river flow	release factor 0.7%, sewage flow, proc. period	1,500	0.068	0.20	180
P	proc.	proc. volume, river flow	release factor 0.7%, sewage flow, proc. period	500	0.022	0.15	36
Q	proc.	proc. volume, river flow	release factor 0.7%, sewage flow, proc. period	1,500	0.49	0.62	820
R	proc. to MDA	effl. conc. below dl, sewage and river flow; proc. period	-	< 100	< 0.37	< 0.50	< 2,770
V	proc. to dyes	emission amount per year, sewage and river flow	release factor 0.7% for Clocal calc., proc. period	150	0.21	0.34	1,000
W	proc. to ppps	emission amount per year, sewage and river flow	release factor 0.7% for Clocal calc., proc. period	49	0.033	0.16	220
Y	proc. to caout. chemicals	effluent conc. (∅ and 90%ile), proc. period, sewage and river flow	-	7.3	0.051	0.18	5.9
Z	proc. to caout. chemicals	effluent conc. (∅ and 90%ile), proc. period, sewage and river flow	-	120	0.27	0.40	114
ZA	proc. to caout. chemicals	processing volume, river flow	release factor 0.7%, sewage flow, WWTP elimin. 87%; proc. period	7,500	590	590	2,300

Remarks

Site F: the PEClocal calculation is based on figures from 1996. The figures reported recently by the company are much lower; however, as they cannot be followed by the rapporteur, it was decided to use the old figures. With these data the PEC/PNEC ratio is < 1 for this site.

Site G: the sewage is emitted into a channel, for which only an average flow is available. The flow is strongly influenced by tide effects. For the Clocal calculation, 1/3 of the mean channel flow is used.

Site H: the raw wastewater from the aniline plant is diluted 1:800 by the site drain flow and by the river entry channel. For the release into a river estuary, the default value of 1:10 is used. Plume model calculations are available which show that several km^2 of the estuary are polluted in concentrations above the PNEC of 1.5 µg/l.

Table 3.17 Local exposure at sites emitting into sea or estuaries

Site	Life stage	Site-specific data	Default values	Ceffl. [µg/l]	Clocal [µg/l]	PEClocal [µg/l]	Release [kg/a]
B	prod. + proc. to MDA + caout. chem.	effluent conc. below dl, sewage flow; proc. period	dilution 1:10	< 10	< 1	< 1.1	< 84
I	imp.; proc. to MDA	effluent conc., prod. period, sewage flow, pre-dilution 1:105; proc. period	dilution 1:10	< 50	< 0.048	< 0.18	< 160
L	proc. to caout. chem.	weekly emission (∅ and 90%ile), sewage flow; no treatment plant! proc. period	dilution 1:100	-	920	920	53,000
S	proc. to MDA	sewage conc. below dl, sewage flow; no treatment plant! proc. period	dilution 1:100	-	< 0.02	< 0.15	< 0.8
T	proc. to MDA and pharma	effluent conc. (∅ and 90%ile), proc. period, sewage flow	dilution 1:10	172	17	17	89
U	proc. to MDA	proc. volume, proc. period, sewage flow, yearly emission	dilution 1:50	710	14	14	1,200
X	proc. to MDA	sewage conc. below dl; proc. period; sewage flow	dilution 1:10	< 50	< 5	< 5.1	< 17

Remarks:

Site L: the raw sewage is emitted into an estuary, the pipe length is 1,500 m without a diffuser. Considering the available information a dilution factor of 100 seems to be appropriate for an initial step. The company states a flow of 162 m^3/s, where it is unclear how this figure is derived. Even with this flow, a Clocal of 32 µg/l would be calculated. Dilution factors of 1,000-100,000 stated by the company are not used, because any justification is missing.

Site S: the end of the sewage pipe is in a distance of 1,900 m from the coast in a depth of 25 m. The length of the diffuser section is 80 m. Considering the available information a dilution factor of 100 seems to be appropriate for an initial step.

Site T: the technical description for both MDA and pharmaceutical intermediate production reveals that the majority of the releases into the sewage origins from MDA production. The wastes from the other process are incinerated.

Site U: the used effluent concentration was calculated from the yearly release (sum of "normal operation" and incidents which obviously occur frequently) and the sewage flow. The wastewater is emitted into an industrial harbour which contains seawater and which has an open connection to the sea. A model calculation (which considers the tides) results in dilution factors of 1:10 after 7 m canal length, 1:100 after 500 m and 1:5,000 after 8,000 m. A model substance was detected in a concentration of 30 µg/l in the effluent and was not detected in the harbour with a detection limit of 1 µg/l. For the assessment the relevant exposure is at a distance of 100 m. We estimate roughly that the dilution for this distance is 50.

Site X: it is scheduled for 2001, that all industrial and domestic effluents in the region will be collected and released 3 km from the coast into the ocean. This will lead to an improvement of the dilution factor.

The total releases during aniline production and processing for all known production and processing sites are summarised to 116 t/a.

The specific release factors for each site are summarised in the confidential Appendix G.

Releases from rubber chemicals

In Section 3.1.1, the aniline releases into the wastewater are estimated to 77 g/d for the local main source. The concentrations are:

- sewage flow 2,000 m^3/d
- 87% are eliminated in the WWTP (SIMPLETREAT)
- $\Rightarrow C_{effluent}$ = 5.0 µg/l
- dilution 1:10 during release into the receiving water
- ⇒Clocal = 0.50 µg/l
- ⇒ PEClocal = 0.63 µg/l

Additionally, releases during the use of tyres have to be expected, as the substance is incorporated into the rubber matrix. Aniline was measured in the leaching water of a German highway. The concentrations in 8 samples were below the detection limit (2 resp. 8 µg/l). 2 weeks after a snowfall, the substance was detected in the snow in concentrations of 33.9, 4.01 and 19.9 µg/l, the samples were collected near a highway and an interurban road (Baumann and Ismeier, 1997).

Metabolisation of plant protecting agents

Phenylureas and phenylcarbamates may enter the hydrosphere via runoff from agricultural soil. From these aniline may be released by biotic or abiotic hydrolysis. There are no quantitative data about aniline exposure by this pathway. However, because of the ready biodegradability of aniline, this exposure pathway is probably of minor importance.

Releases from coal and oil industry

In the literature, wastewater concentrations between 0.48 and 21 mg/l are reported (cf. Section 3.1.1). No actual emission data from these branches are available.

Sediments

For the estimation of $PEClocal_{sediment}$, the aquatic PEClocal figures from Section 3.1.3.2 are used. With a Koc value of 410 l/kg and 10% organic matter in suspended particles, the following concentrations are calculated:

Table 3.18 Calculated sediment concentrations

Site	$PEClocal_{water}$ [µg/l]	$PEClocal_{sediment}$ [µg/kg ww]*
A	0.76	7.4
B	< 1.1	< 11
C	1.07	10.4
D	< 0.51	< 4.9
E	0.64	6.2
F	< 0.32	< 3.1
G	280	2,700
H	48	470
I	< 0.18	< 1.8
J	< 0.51	< 4.9
K	3.5	34
L	920	8,900
M	130	1,300
N	< 0.14	< 1.4
O	0.20	1.9
P	0.15	1.5
Q	0.62	6.0
R	< 0.50	< 4.9
S	< 0.15	< 1.5
T	17	170
U	14	140
V	0.34	3.3
W	0.16	1.6
X	< 5.1	< 49
Y	0.18	1.8
Z	0.40	3.9
ZA	590	5,700
rubber ind.	0.63	6.1

* ww = wet weight

It has to be kept in mind that in sediments aniline is always covalently bound onto the organic fraction. The sorption behaviour is described in Section 3.1.2.2. The PECs are the sum of the aniline fractions being dissolved in the porewater, physically and covalently (both hydrolysable and non-hydrolysable) bound.

3.1.4 Atmosphere

3.1.4.1 Monitoring data

In 1973, aniline was detected in aerosols in Germany, Ireland and Switzerland concentrations are not reported (Ketseridis et al., 1976). There are no monitoring data for the ambient air available reflecting the recent European exposure.

3.1.4.2 Calculation of predicted environmental concentrations

Estimation of PEClocal for production and processing / site-specific approach

In the TGD the following default release factors are defined:

- production: 10 ppm for main category Ib (stored on-site) resp. 100 ppm for main category Ic (stored off-site),
- processing: 0 for main category Ib (stored on-site) resp. 10 ppm for main category Ic (stored off-site).

For 16 sites, the emission factors are calculated from site-specific yearly release amounts into the air and the production/processing amount. These factors are in the range between 0.014 and 380 ppm and thus considerably higher than the TGD defaults. There are no striking differences between production and processing. For 4 sites no release factor was calculated because more than two life-cycle steps take place at these sites.

For the exposure calculation, site-specific data are used as far as they were submitted. For those sites (G, M, O, P, Q, ZA) where no release amount was submitted, a factor of 380 ppm was used for a worst-case approach.

The PECregional ($2.2 \cdot 10^{-4}$ µg/m^3, cf. Section 3.1.7) is far below the estimated Clocal values, thus the local concentrations can be taken as the PEClocal.

Table 3.19 Atmospheric concentrations

Site	Life-stage	Data basis	Release [kg/d]	PEClocal$_{air}$ [µg/m^3]	PEClocal$_{air,ann}$ [µg/m^3]
A	prod., proc. to MDA, caout. chem, dyes	yearly release	0.26	0.072	0.059
B	prod., proc. to MDA + caout. chem.	yearly release	0.92	0.26	0.21
C	prod., proc. to dyes	yearly release	2.2	0.61	0.50
D	prod., proc. to MDA	yearly release	0.26	0.072	0.059
E	prod., proc. to MDA + caout. chem	yearly release	< 0.087	< 0.024	< 0.020
F	prod., proc. to MDA	yearly release	0.011	0.0031	0.0025
G	prod.	default 380 ppm see remarks	120	3,900	1,200

Table 3.19 continued overleaf

Table 3.19 continued Atmospheric concentrations

Site	Life-stage	Data basis	Release [kg/d]	$PEClocal_{air}$ [µg/m³]	$PEClocal_{air,ann}$ [µg/m³]
H	prod.	yearly release	9.7	2.7	2.2
I	imp.; proc. to MDA	yearly release	3.0	0.83	0.75
J	proc. to caout. chem.	yearly release	0.15	0.042	0.038
K	imp., proc. to caout. chem.	yearly release	0.12	0.033	0.030
L	proc. to caout. chem.	yearly release	3.0	0.83	0.69
M	proc. to caout. chem.	default 380 ppm	1.9	0.53	0.055
N	proc. to caout. chem.	yearly release	0.76	0.21	0.19
O	proc.	default 380 ppm	1.3	0.36	0.059
P	proc.	default 380 ppm	0.42	0.12	0.012
Q	proc.	default 380 ppm	1.3	0.36	0.27
R	proc. to MDA	yearly release	2.2	0.61	0.50
S	proc. to MDA	yearly release	0.0024	0.00067	0.00067
T	proc. to MDA + pharma	yearly release	0.76	0.21	0.19
U	proc. to MDA	yearly release	5.5	1.5	1.3
V	proc. to dyes	yearly release	0.73	0.20	0.17
W	proc. to ppps	yearly release	incin.	-	-
X	proc. to MDA	yearly release	1.95	0.54	0.45
Y	proc. to rubber chem.	yearly release	5.9	1.6	1.3
Z	proc. to caout. chem.	yearly release	12	3.3	3.3
ZA	proc. to caout. chem.	default 380 ppm	6.3	1.8	0.72

Remarks:

Site G: The Company has no data for the release amount. The measured concentrations at 3 sampling sites are between 1.5 and 3.9 mg/m³ (maximum values) resp. 0.15 and 1.2 mg/m³ (average values). The upper values are used for the PEClocal. The yearly release is calculated on the basis of the default release factor (380 ppm).

The total releases into the air during production and processing are added to 53 t/a.

Releases from rubber chemicals

Aniline is formed from different rubber chemicals during vulcanisation. The substance was detected in the curing fume.

In Section 3.1.1, the releases into the air by the local main source are estimated to 3.3 kg/d. With a production period of 300 d/a, the environmental concentration is

$Clocal_{air} = 0.92$ µg/m³

$PEClocal_{air,ann} = 0.75$ µg/m³

3.1.5 Terrestrial compartment

Aniline reaches soils via deposition from the atmosphere or by degradation of plant protection agents. As elaborated in Section 3.1.2.2, aniline can be biodegraded or be bound onto the soil organics, where the reaction product accumulates. For the model calculation, it is assumed that 20% of the aniline is rapidly degraded, therefore significant concentrations of the unreacted aniline (both in the solid phase and in porewater) are not expected.

80% of the aniline is considered to react with humic substances. A significant exposure can only occur with this reaction product, which is degraded with an assumed half-life of 350 d. The covalently bound substance is considered to be immobile (kleach = 0). In the following, the soil exposure of the reaction product of aniline with humic substances is estimated. Because the molecular weight of humic substances is not homogenous, the concentrations are expressed as aniline equivalents.

Atmospheric deposition during production and processing / site-specific scenario

If the highest emission amount submitted by industry (12 kg/d, site Z) is used, the values are:

$DEP_{total,ann} = 4.8 \cdot 10^{-3}\ mg \cdot m^{-2} \cdot d^{-1}$
20% degraded
"effective" $DEP_{total,ann} = 3.8 \cdot 10^{-3}\ mg \cdot m^{-2} \cdot d^{-1}$

For the PEC calculation cf. Appendix I:

Table 3.20 Calculated PEC_{soil} from release from production and processing sites

	Soil [µg/kg dw]	Endpoint
$PEClocal_{soil}$	5.6	for terrestrial ecosystem
PEC_{agri_ind}	5.6	in agric. soil for indirect exposure
PEC_{grass_ind}	11	in grassland for indirect exposure

Releases from rubber chemicals

Aniline is emitted by the rubber industry, which leads to a soil exposure. With the data of the model site (cf. Section 3.1.1), the following values are calculated:

Emission 3.3 kg/d

$DEP_{total} = 1.32 \cdot 10^{-3}\ mg \cdot m^{-2} \cdot d^{-1}$
20% degraded
"effective" $DEP_{total} = 1.1 \cdot 10^{-3}\ mg \cdot m^{-2} \cdot d^{-1}$

Table 3.21 Calculated PEC_{soil} from release from rubber chemicals

	Soil [µg/kg dw]	Endpoint
$PEClocal_{soil}$	1.6	for terrestrial ecosystem
PEC_{agri_ind}	1.6	in agric. soil for indirect exposure
PEC_{grass_ind}	3.2	in grassland for indirect exposure

Substances incorporated in tyres can reach the environment via rubber abrasion. Aniline was measured in soil near roads. While in two samples from directly near a road the concentrations were below the detection limit of 0.1 mg/kg, in a third sample 0.42 mg/kg were detected. At a distance of 5 m no aniline was detected. The concentrations of other simultaneously measured substances occuring in higher concentrations dropped by a factor of about 100 within this distance (Baumann and Ismeier, 1997). Thus, no significant exposure of aniline in environmental soils near roads is expected.

Releases from plant protection agents

Aniline is formed as a metabolite during biodegradation of phenylurea and phenylcarbamate derivatives. Exposure scenarios are calculated below for the two compounds fenuron and siduron, because these substances are known to be applied within the EU. For the exposure model the highest application rates are considered. The aniline release is calculated assuming complete metabolisation. Other substances that may release aniline during metabolisation are not regarded here as they are no longer used within the EU (propham) or relevant information on application is missing (carboxin, fenfuram, propachlor) (cf. Section 3.1.1.4, Releases during use of plant protecting agents).

As there is no suitable exposure model for plant protecting agents available, the sewage sludge model proposed in the TGD is used. The initial concentration (after single application) is:

$$C_{soil1} = \frac{F_{bound}}{DEPTH_{soil} \cdot RHO_{soil}}$$

with F_{bound} = fraction bound to humic substances
$DEPTH_{soil}$ = mixing depth in soil (0.2 m)
RHO_{soil} = density of dry soil (1,500 $kg \cdot m^{-3}$)
C_{soil1} = initial concentration in soil in first year

Table 3.22 Local soil concentrations from use of plant protecting agents

Agent	Maximum application rate kg/ha]	Aniline content [kg/ha]	Bound fraction (80%) [kg/ha]	C_{soil1} [mg/kg dw]
Fenuron	2.25	1.28	1.02	0.34
Siduron	6.0	2.41	1.93	0.64

Furthermore, the accumulation during a 10-year period is estimated according to the TGD model (cf. Appendix B). The results are:

Table 3.23 Calculated PEC_{soil} from use of plant protecting agents

	Fenuron [µg/kg dw]	Siduron [µg/kg dw]	Endpoint
$PEClocal_{soil}$	640	1,200	for terrestrial ecosystem
PEC_{agri_ind}	560	0	in agric. soil for indirect exp.
PEC_{grass_ind}	0	2,000	in grassland for indirect exp.

Aniline has been measured in a monitoring program in Bavarian agricultural soils. The measurements were performed according to the Specht method, where the samples are subjected to an alkaline hydrolysis. The concentrations (not reported whether related to dry or wet weight) were above 50 µg/kg in 32 and above 30 µg/kg in 72 from 374 samples (Lepschy and Müller, 1991). It is unknown whether and how frequently the soils were treated with aniline herbicides. By the analytical method, an exact determination of the concentration is not possible as a part of the detected aniline may have its origin in the herbicides and the aniline-containing metabolites. On the other hand, aniline covalently bound to humic acids can only partially be detected. The recovery rate is not known.

As it is unclear whether the monitored soils were treated with aniline containing herbicides and their per-hectare-use figures are not known precisely, the PEC cannot be compared with the measured values.

3.1.6 Secondary poisoning

The BCF of 2.6 l/kg indicates that there is no bioaccumulation potential due to the exposure of the organisms via water. A biomagnification via food chain due to the route fish - fish-eating bird is not expected.

However, a possible bioaccumulation of the reaction product with humic acids could lead to a bioaccumulation for the route sediment or soil - sediment or soil dwelling worm - worm-eating mammal or bird. The available knowledge with other aniline derivatives is summarised in Appendix D. To assess this subject a bioaccumulation study was performed with the benthic oligochaete *Lumbriculus variegatus* (cf. Section 3.1.2.3). Bioaccumulation factors below 1 were obtained in this study this indicating that aniline will not bioaccumulate in sediment organisms. As the behaviour of aniline in soil is similar to that in sediment, it can be concluded that also for terrestrial organisms being exposed to aniline by similar pathways no significant bioaccumulation will occur. Therefore, further tests on the bioaccumulation of aniline in terrestrial organisms are not of high priority for the risk assessment.

3.1.7 Regional concentrations

For the estimation of the total releases into the environment, emissions during production and processing of aniline, and the emissions by the rubber industry are considered. Not considered are the releases from the agricultural use of the plant protection products and the releases from tyres into soils near roads, because in these cases the aniline is either rapidly biodegraded or reacts with soil organics under formation of a subsequent product which cannot be handled in one exposure model.

Releases from microbial nitrobenzene reduction, polyurethane degradation, coal and oil industry, and landfills are not quantifiable and therefore not considered.

Table 3.24 Total releases of aniline into the environment

Life-cycle step	Atmosphere reg. / cont.	Hydrosphere reg. / cont.
Production and processing	5.3 / 48 t/a	12 / 104 t/a
Rubber industry	16 / 146 t/a	100 / 900 kg/a
Total	**21 / 194 t/a**	**12 / 105 t/a**

The results of a EUSES calculation (cf. Appendix C) are:

Table 3.25 Regional and continental PECs

Endpoint	Continental concentration	Regional concentration
Water	0.017 µg/l	0.13 µg/l
Sediment	0.48 µg/kg dw	3.4 µg/kg dw
Atmosphere	$2.9 \cdot 10^{-5}$ µg/m³	$2.2 \cdot 10^{-4}$ µg/m³
Agric. soil	$1.8 \cdot 10^{-3}$ µg/kg dw	$14 \cdot 10^{-3}$ µg/kg dw
Agr. soil, porewater	$2.2 \cdot 10^{-4}$ µg/l	$1.7 \cdot 10^{-3}$ µg/l
Industr. soil	$4.7 \cdot 10^{-3}$ µg/kg dw	$36 \cdot 10^{-3}$ µg/kg dw
Nat. Soil	$4.7 \cdot 10^{-3}$ µg/kg dw	$36 \cdot 10^{-3}$ µg/kg dw

The results indicate that a regional exposure is only relevant for the hydrosphere. A high atmosphere pollution is only possible in the vicinity of a strong point source, and a relevant soil pollution due to atmospheric deposition can be excluded on the regional scale.

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

3.2.1 Aquatic compartment (incl. sediment)

3.2.1.1 Toxicity test results

For aniline many ecotoxicity tests are reported but most of them give only a rough estimation of the ecotoxic effect values as in most cases nominal concentrations are given. It has to be expected that the real concentrations are lower because of photolysis. This is a problem especially with the algae tests as it was shown that algae enhance the photo-transformation rate of aniline to a great extend.

3.2.1.1.1 Vertebrates

Short-term toxicity to vertebrates

In short-term tests with different fish species LC_{50} values for 48 to 96 hour exposure between 10.6 mg/l and 187 mg/l were found. The most relevant test results are reported below:

Oncorhynchus mykiss	48-hour LC_{50}	28.3 mg/l
	96-hour LC_{50}	10.6 mg/l

(flow-through system, measured concentration, Abram and Sims, 1982)

Oncorhynchus mykiss	96-hour LC_{50}	36.2 mg/l

(flow-through system, measured concentration, Hodson et al., 1984)

Lepomis macrochirus	96-hour LC_{50}	49 mg/l

(multispecies test, flow-through, measured concentration; Holcombe et al., 1987)

Brachydanio rerio	96-hour LC_{50}	32-33 mg/l

(static, nominal concentration; Wellens, 1982)

Brachydanio rerio	96-hour LC_{50}	57.5 mg/l

(semistatic, measured concentration, Zok et al., 1991)

Pimephales promelas	96-hour LC_{50}	68.6 mg/l
	7-day LC_{50}	60.2 mg/l
	7-day NOEC	15.7 mg/l

(flow-through, measured concentration, larvae < 24-hours old were used, NOEC related to survival and growth, Marchini et al., 1992)

Long-term toxicity to vertebrates

Birge et al. (1979) examined the long-term toxicity of aniline in an embryo-larval test with the largemouth bass *Micropterus salmoides* as test organism with hard, respectively soft water. In a flow-through system (temperature: 19-24°C; dissolved oxygen: 7.7-9 mg/l; water hardness: 50, respectively 200 mg/l $CaCO_3$; pH: 7.3-8.1) eggs were exposed to the test substance 1-2 hours after spawning. Exposure was maintained through 4 resp. 8 days after hatching giving exposure

periods of 6.5-7.5 resp. 10.5-11.5 d. Aniline concentration was measured daily. Test parameters were egg hatchability and survival 4 and 8 days posthatching. Log probit analysis was used by the authors to determine the LC_1 and LC_{50} 4 and 8 days after hatching. LC_{50} values of 10.4 resp. 5.2 mg/l were obtained for soft water and of 8.4 and 4.4 mg/l for hard water The LC_1 values calculated for 4 days posthatching were 5 µg/l and 55 µg/l in hard and soft water, respectively. The LC_1 values are not used as NOECs in the further assessment as an effect of 1% compared to the control seems not significant. However, from the available test results a NOEC of 45 µg/l for hard water and of 51 µg/l for soft water can roughly be estimated.

The effect values found by Birge and Black for several substances are usually very low compared to effect values found by other authors. No explanation for these large discrepancies could be found. A careful examination of the entire information provided by Birge et al. and Black et al. gave no plausible reason for the inconsistency of the data. Nevertheless it was decided by the EU member states not to use these data for a derivation of a $PNEC_{aqua}$ if other valid fish early life stage tests are available.

A 28-day early-life-stage test with *Brachydanio rerio* was conducted by van Leeuwen et al. (1990). Fertilised eggs were exposed to aniline in a semistatic manner about 1 day after spawning. Test solutions were renewed three times a week. The actual aniline concentration was analysed before and after renewal of the test solution. It was found that the measured aniline concentrations were far below the nominal concentrations. A 28-day NOEC of 1.8 mg/l for survival, hatching and growth and a 28-day NOLC (no observed lethal concentration) of 5.6 mg/l was found. Both values are based on nominal concentrations and are therefore not highly reliable. As no further information is given on the measured aniline concentration it is not possible to take into account the decreasing of the substance for the interpretation of the results. Therefore the NOEC from this study cannot be used for the effects assessment.

Russom and Broderius (1991) conducted early-life stage studies with *Pimephales promelas* with a variety of chemical substances, among them aniline. Embryos < 24 h old were exposed in a flow-through system to aniline for 32 days. The test substance concentration was measured at least twice a week. Test endpoints included percentage of normal larvae at hatch, percent survival and growth effects. A "chronic value", i.e the geometric mean of the NOEC and the LOEC, of 0.557 mg/l was found for the most sensitive endpoint, wet weight and total length. From this a NOEC of 0.39 mg/l can be derived according to the TGD.

3.2.1.1.2 Invertebrates

Short-term toxicity to invertebrates

The most relevant studies with invertebrates are presented below:

Daphnia pulex	48-hour EC_{50}	0.1 mg/l
Daphnia cucullata	48-hour EC_{50}	0.68 mg/l

(effect: immobilisation, nominal concentration; Canton and Adema, 1978)

Daphnia magna	48-hour EC_{50}	0.17 mg/l

(effect: immobilisation, nominal concentration; Gersich and Mayes, 1986)

Daphnia magna	24-hour -EC_{50}	0.9 mg/l
	48-hour EC_{50}	0.3 mg/l

(effect: immobilisation, nominal concentration; Kühn et al., 1988)

Daphnia magna 48-hour EC_{50} 0.16 mg/l
(effect: immobilisation, measured concentration; semi-static, Danish Environmental Protection Agency 1996)

Daphnia magna 48-hour EC_{50} 0.25 mg/l
(effect: immobilisation, flow-through, measured concentration; multispecies test, Holcombe et al., 1987)

*Gammarus fasciatu*s 96-hour LC_{50} 2.3 mg/l
(measured concentration, flow-through, Boeri 1989)

Long-term tests with invertebrates

Hutton (1989) examined the effects of long-term exposure of *Daphnia magna* to aniline. The daphnids were exposed in a flow-through system at about 20°C. As dilution water hardened unfiltered fish tank water was used. During the test the daphnids were fed with trout chow and yeast daily. Five test concentrations; a DMF control and a water control were used. Nominal test concentrations were between 11 µg/l and 105 µg/l. However, weekly HPLC analysis of the test samples showed that the measured test concentration in all samples was about 50% of the nominal concentration. A 21-day NOEC related to reproduction of 16 µg/l (based on measured concentrations) was derived.

The decreasing aniline concentration that was found in this flow-through study is attributed by the author to the presence of daphnid food. This statement is confirmed by Lieder (1989). He found a complete disappearance of aniline (concentration: 1 mg/l) caused by degradation in well water in the presence of daphnid food (trout chow and yeast, 30 mg/l) after 9,696-hours, whereas in the absence of this food after 9,696-hours a loss of aniline of 19% was found. Both solutions were exposed to 16-hour light – 8-hour dark photoperiods.

In a semi-static test (three renewals per week) Kühn et al. (1988) studied the long-term toxicity of aniline to Daphnia magna. The study was conducted at 25°C. During the test the daphnids were fed on fish food and activated sludge. Eight test concentrations ranging nominally from 0.1 µg/l to 316 µg/l were employed. A 21-day NOEC for reproduction of 10 µg/l based on nominal concentration was found. As it was not possible to analyse the real aniline concentration in the samples (detection limit of the used method was 0.1 mg/l) an additional test vessel without daphnids and food but with a nominal aniline concentration of 316 µg/l was employed. After 2 days the aniline concentration in this sample was only 40 to 60% of the nominal concentration. Therefore, a NOEC value of 4 µg/l is extrapolated from this recovery rate. This extrapolation does not take into account the possibly enhanced degradation of aniline in the presence of daphnid food.

Gersich and Milazzo (1988) examined the effect of aniline to Daphnia magna in a long-term study. The daphnids were exposed under semi-static conditions at 20°C for 21 days. During the tests the daphnids were fed on green algae. Aniline concentrations ranging nominally from 10.6 µg/l to 170 µg/l were employed. The stability of aniline over the renewal period was examined by analyzing particular test solutions at 0-hour and 48-hour. A mean deviation of about 82% was found. A NOEC of 24 µg/l for reproduction based on mean analysed concentrations was reported.

In two other tests conducted over 14 days at 24°C NOECs of 20.8 resp. 10.2 µg/l were found for reproduction (Gersich and Milazzo, 1990).

3.2.1.1.3 Plants

It is reported that algae catalyze the phototransformation of aniline (Zepp and Schlotzhauer, 1983). In another test aniline disappeared completely after 96 hours in the presence of daphnid food (Lieder, 1989). Therefore the following nominal concentrations overestimate the real effective concentrations for algae substantially.

Selenastrum capricornutum	96-hour EC_{50}	19 mg/l
	96-hour NOEC	2 mg/l

(effect: biomass; nominal concentrations; Calamari et al., 1980)

Scenedesmus subspicatus	48-hour E_bC_{10}	22 mg/l
	48-hour E_bC_{50}	68 mg/l
	48-hour E_rC_{10}	48 mg/l
	48-hour E_rC_{50}	> 750 mg/l

(effect:biomass and growth rate; nominal concentrations; Kühn and Pattard, 1990)

Microcystis aeruginosa	8-day TGK	0.16 mg/l

(effect: biomass; nominal concentrations; Bringmann, 1975)

The TGK or "toxic threshold concentration" was determined at 3% effect compared to the controls and can therefore be considered as NOEC.

3.2.1.1.4 Microorganisms

Pseudomonas putida	16-hour TGK	130 mg/l

(effect: biomass; nominal concentrations; Bringmann and Kühn, 1976, 1977 and 1979)

The TGK or "toxic threshold concentration" was determined at 3% effect compared to the controls.

Nitrosomonas spec.	2-hour EC_{76}	2.5 mg/l
	2-hour EC_{50}	< 1 mg/l

(effect: inhibition of nitrification; nominal concentrations; Hockenbury and Grady, 1977)

activated sludge	10 min-EC_{50}	2,500 mg/l

(effect: inhibition of respiration; nominal concentration; Mihara et al., 1991)

Tetrahymena pyriformis	48-hour-EC_{50}	53 mg/l

(effect: biomass; nominal concentration; Schultz et al., 1989)

Uronema parduczi	20-hour TGK	91 mg/l

(effect: biomass; nominal concentrations; Bringmann and Kühn, 1980b)

Chilomonas paramaecium	48-hour TGK	250 mg/l

(effect: biomass; nominal concentrations; Bringmann and Kühn, 1980a)

Entosiphon sulcatum	72-hour TGK	24 mg/l

(effect: biomass; nominal concentrations; Bringmann, 1978)

Activated sludge, industrial	2-hour EC_{50}	7 mg/l
	2-hour NOEC	2 mg/l

(effect: inhibition of nitrification, nominal concentration, Bayer AG, 2000c)

The TGK or "toxic threshold concentrations" were determined at 5% effect compared to the controls and can therefore be considered as a NOEC.

3.2.1.2 Determination of Predicted No Effect (PNEC)

3.2.1.2.1 Determination of $PNEC_{aqua}$

Among the tested species *Daphnia* was most sensitive in both short-term and long-term tests. Therefore, the results from the *Daphnia* reproduction tests are used for the derivation of the $PNEC_{aqua}$.

Three 21-day NOECs in the narrow range of 4 µg/l to 24 µg/l are available for *Daphnia*. None of these tests was conducted according to international guidelines but careful examination of the test reports allows the conclusion that they can all be regarded as valid (with restriction) and that it cannot be justified to prefer one of the tests. It could be stated that the NOEC of 4 µg/l derived from the study of Kühn is not as reliable as the other 2 daphnia tests, because this value was extrapolated from a nominal value of 10 µg/l, based on the recovery rate that was determined at a much higher concentration. However, the decrease in test substance concentration of 40–60% is confirmed by the flow-though study of Hutton. Also in this test it was found that the measured concentrations were about 50% of the nominal values. In addition, the recovery rate of 40% does not take into account the possibly enhanced degradation of aniline in the presence of daphnid food. Therefore, the real NOEC may be lower than 4 µg/l.

The three available NOECs are lying close together and thus are supporting each other. As NOECs are dependent on the range and the spacing of the substance concentration used in the tests it is obvious that variation in the test concentrations in different tests is an important and relevant reason for different NOEC values obtained. Therefore, to reduce this uncertainty it seems adequate that the mean value of these three NOECs is calculated and used as basic value for the effects assessment. It could be stated that the NOEC of 4 µg/l should be used for the derivation of the PNEC because it is possible that effects occur at concentrations below 4 µg/l. However, as three *Daphnia* long-term tests are available that are regarded of equal value, the calculation of the arithmetic mean seems to be most appropriate.

Calculating the arithmetic mean of the three NOECs results in a value of 15 µg/l.

For the derivation of the $PNEC_{aqua}$ an assessment factor of 10 seems appropriate, as reliable long-term tests are available for daphnids and fish. An effective NOEC on algae cannot be determined due to the rapid phototransformation of aniline in the presence of algae. However, as the nominal effect values from the algae tests are about 2-3 orders of magnitude higher than the NOECs from the *Daphnia* long-term tests, it can be expected with high probability that the effective algae NOEC would not be below 15 µg/l.

Therefore: $PNEC_{aqua} = 15\ \mu g/l / 10 = 1.5\ \mu g/l$.

3.2.1.2.2 Determination of $PNEC_{microorganisms}$

From the several tests available, four are selected to demonstrate the derivation of this PNEC. According to the endpoints and sensitivities of the test systems the following assessment factors have to be applied:

Tetrahymena pyriformis	EC_{50}= 53 mg/l	F = 10	=> PNEC = 5.3 mg/l
activated sludge (respiration inhibition)	EC_{50} = 2,500 mg/l	F = 100	=> PNEC = 25 mg/l
Nitrosomonas spec.	EC_{50} = < 1 mg/l	F = 10	=> PNEC < 0.1 mg/l
		EC_{76} = 2.5 mg/l	
activated sludge, industrial	EC_{50} = 7 mg/l	F = 10	=> PNEC = 0.7 mg
(nitrification inhibition)	NOEC = 2 mg/l	F = 1	=> PNEC = 2 mg

The most sensitive endpoint was nitrification inhibition, both in the test with *Nitrosomonas* and with industrial activated sludge. As nitrification is an important step in both municipal and industrial sewage treatment plants a $PNEC_{microorganism}$ based on this endpoint has to be used for the risk characterisation for all kinds on sewage treatment plants.

For the risk assessment of industrial sewage treatment plants the $PNEC_{microorganism}$ based on the nitrification inhibition test with industrial activated sludge is used as this test is more realistic for this kind of treatment plants than the test with *Nitrosomonas spec*. Therefore, the $PNEC_{microorganism}$ is 2 mg/l.

3.2.1.3 Toxicity test results for sediments

A prolonged sediment toxicity test using spiked sediment has been carried out with the midge *Chironomus riparius* (Egeler et al., 2002). The test protocol was based on the OECD draft test Guideline 218 with some deviations. The sediment used in the test was an artificial sediment consisting of 5% peat, 20% kaolinite clay and 75% quartz sand. The sediment had a mean organic matter content of 2 ± 0.5%. As food source for the test organisms urtica powder in a concentration of 0.5% of sediment dry weight was added. The test substance was added to the sediment as an aqueous solution. The test system was allowed to equilibrate for 48 hours before the midge larvae were introduced.

In the test, groups of midge larvae were exposed to a series of 6 test concentrations in the range of 31.25 mg/kg dw to 1,000 mg/kg dw and control sediment for 28 days at 20 ± 2°C using a static system. The larvae used in the test were first-instar larvae (6-24 h old). Four replicate chambers, each containing 20 larvae, were maintained in each treatment group. For the control group, 6 replicates were used. The larvae were not fed throughout the test. Gentle aeration was used throughout the test, with the vessels being covered.

The endpoints determined in the study were emergence ratio, developmental rate and total number of adults emerged.

Analysis of the concentration of the test substance in sediment, pore water and overlying water was carried out for the control and three test concentrations at days –2, 0, and 28 of the test. The results of the analysis are shown in **Table 3.26**.

Table 3.26 Recoveries of aniline in sediment, pore water and overlying water

			Recovery of test item			Sum of recoveries	Mean recovery	Overall mean recovery
Conc. Level code	**Nominal conc. (mg/kg dw)**	**Test period (days)**	**Sediment %**	**Pore water %**	**Overlying water %**	**%**	**%**	**%**
Co (control)	0 0 0	-2 0 28	n.a. n.a. n.a.	n.a. n.a. n.a.	n.a. n.a. n.a.	n.a. n.a. n.a.		
C1	31.5 31.5 31.5	-2 0 28	22.8 15.7 11.7	2.2 0.8 0.2	n.a. < 7.4 < 7.4	25.1 16.5 11.8	14.2	
C4	252.1 252.1 252.1	-2 0 28	48.6 26.8 5.8	13.2 6.0 0.2	n.a. 27.8 < 0.9	61.8 60.6 6.9	33.7	
C6	1,008.4 1,008.4 1,008.4	-2 0 28	53.5 28.8 4.4	21.4 8.6 0.2	n.a. 42.1 0.09	74.9 79.5 4.7	42.1	30.0

The difference between nominal and measured aniline concentrations can be both attributed to biodegradation and formation of non-hydrolysable binding to sediment components that is inaccessible for analytical determination. The competing mechanisms biodegradation and chemisorption in soil and sediment are described in Section 3.1.2.1.

To consider the decrease of the aniline concentration by biodegradation, as a rough approach the overall mean recovery of 30% is used to correct the nominal aniline concentrations. As this recovery also covers formation of non-hydrolyzable binding of aniline to the sediment it represents a worst-case approach.

Ammonium concentration was measured twice during the test period. The measurements were performed with overlying water removed from replicate of control and one of each concentration level (**Table 3.27**).

Table 3.27 Ammonium content in mg/l NH_4^+

Sediment concentration of aniline [mg/kg dw]	Day 0 of test	Day 28 of test
Control	0.8	0.0
31.25	3.0	0.0
62.5	1.6	0.0
125	1.2	0.0
250	3.0	0.0
500	2.0	8.0
1,000	8.0	8.0

As ammonia in its unionised form is toxic to most aquatic organisms, it cannot be excluded that the increased ammonium concentration has an influence on the biological results and that the observed results are not directly related to the toxicity of aniline. However, since the increased

ammonium concentration was observed only in treated vessels, it may have occurred as a secondary effect of the presence of aniline in the sediment-water system.

The result of the study shows a significant, dose-dependent effect of aniline to the development and emergence of the test species *Chironomus riparius*. The results of the study are described in **Tables 3.28** and **3.29.**

Table 3.28 Result from the study with *Ch. riparius* for the endpoint emergence ratio

	mg/kg Sediment (dw)	
	Nominal	**Corrected for mean recovery of 30%**
NOEC	125	37.5
LOEC	250	75
EC_{10}	113.2	34
EC_{50}	220.2	66.1
EC_{90}	327.3	98.2

Table 3.29 Result from the study with *Ch. riparius* for the endpoint developmental rate

	mg/kg Sediment (dw)	
	Nominal	**Corrected for mean recovery of 30%**
NOEC	250	75
LOEC	500	150
EC_{10}	245.3	73.6
EC_{50}	348.5	104.6
EC_{90}	451.7	135.5

The overall EC_{10} from this study is therefore 113 mg/kg dry weight (nominal). The actual measured concentrations appear to be lower than the nominal concentrations with a mean recovery of 30% containing both biodegradation and formation of non-hydrolyzable bindings. The EC_{10} corrected by this mean recovery is 34 mg/kg dry weight.

A prolonged sediment toxicity test using spiked sediment has been carried out with the oligochaete *Lumbriculus variegatus* (Egeler and Nésa, 2002). The sediment and test system used was the same as in the *Chironomus riparius* test (see above).

In the test, groups of oligochaetes were exposed to a series of 6 test concentrations in the range of 31.25 mg/kg dw to 1,000 mg/kg dw and control sediment for 28 days at 20 ± 2°C using a static system. The worms used in the test were "synchronised" to be in a similar physiological state. For the "synchronisation" the worms were artificially fragmented, and the posterior ends left to regenerate for about 2 to 4 weeks before start of the test. Four replicate chambers, each containing 10 worms, were maintained in each treatment group. For the control group, 6 replicates were used. The worms were not fed throughout the test. Gentle aeration was used throughout the test, with the vessels being covered.

The endpoints determined in the study were survival, reproduction and biomass.

Analysis of the concentration of the test substance in sediment, pore water and overlying water was carried out for the control and three test concentrations at days –2, 0, and 28 of the test. The resultsof the analysis are shown in **Table 3.30**.

Table 3.30 Recoveries of aniline in sediment, pore water and overlying water

			Recovery of test item			Sum of recoveries	Mean recovery	Overall mean recovery
Conc. Level code	**Nominal conc. (mg/kg dw)**	**Test period (days)**	**Sediment %**	**Pore water %**	**Overlying water %**	**%**	**%**	**%**
Co (control)	0 0 0	-2 0 28	n.a. n.a. n.a.	n.a. n.a. n.a.	n.a. n.a. n.a.	n.a. n.a. n.a.		
C1	31.25 31.25 31.25	-2 0 28	81.6 68.09 22.27	12.48 4.08 0.26	- < 7.5 < 6.18	94.08 72.17 22.89	47.5	
C4	250 250 250	-2 0 28	45.09 29.06 4.53	13.53 5.57 0.39	- 15.5 < 0.74	58.62 50.12 4.2	27.5	
C6	1,000 1,000 1,000	-2 0 28	53.25 30.96 3.05	20.81 7.42 0.53	- 30.97 0.37	74.06 69.35 3.96	36.7	37.2

The difference between nominal and measured aniline concentrations can be both attributed to biodegradation and formation of non-hydrolyzable binding to sediment components that is inaccessible for analytical determination. The competing mechanisms biodegradation and chemisorption in soil and sediment are described in Section 3.1.2.1.

To consider the decrease of the aniline concentration by biodegradation, as a rough approach the overall mean revovery of 37.2% is used to correct the nominal aniline concentrations. As this recovery also covers formation of non-hydrolyzable binding of aniline to the sediment it represents a worst-case approach.

Ammonium concentration was measured twice during the test period. The measurements were performed with overlying water removed from replicate of control and one of each concentration level. Directly prio to addition of the test organisms, the measured ammonium concentration appeared to be positively correlated with the aniline concentration. At the end of the test the measurements of control vessels showed 0 mg/l ammonium (indicating sufficient nitrifying capacity of the sediment in the absence of aniline) while in all treatment vessels 8 mg/l ammonium were measured (**Table 3.31**).

Table 3.31 Ammonium content in mg/l NH_4^+

Sediment concentration of aniline [mg/kg dw]	Day 0 of test	Day 28 of test
Control	3.0	0.0
31.25	3.0	8.0
62.5	n.m.	8.0
125	n.m.	8.0
250	n.m.	8.0
500	5.0	8.0
1,000	8.0	8.0

As ammonia in its unionised form is toxic to most aquatic organisms, it cannot be excluded that the increased ammonium concentration has an influence on the biological results and that the observed results are not directly related to the toxicity of aniline. The ammonium concentration of 8 mg/l corresponds to approximately 0.9 mg/l of unionised NH_3 at an average measured pH of 8.4 at the end of the test. A LC_{50} for unionised NH_3 of 1.2 mg/l is reported for *Lumbriculus variegatus* (Schubauer-Berigan et al., 1995). However, since the increased ammonium concentration was observed only in treated vessels, and it is known that aniline inhibits nitrification, the ammonia associated effects have most likely occurred as secondary effect of the presence of aniline in the sediment-water system.

The results of the study show a clear dose-response relationship for the endpoint survival. For the endpoints biomass and reproduction there were clear effects from the lowest concentration level on. Therefore, no dose-response relationship could be calculated and no NOEC/LOEC estimation could be made. It can only be stated that the NOEC for reproduction and growth is < 31.25 mg/kg dw (nominal) or < 11.6 mg/kg dw (measured). The result of the study for the endpoint survival is described in **Table 3.32**.

Table 3.32 Result from the study with *Lumbriculus variegatus* for the endpoint survival

	mg/kg Sediment (dw)	
	Nominal	Corrected for mean recovery of 37.2%
NOEC	125	46.5
LOEC	250	93.1
EC_{10}	34.5	15.3
EC_{50}	191.5	72.8
EC_{90}	1,064.9	346.8

3.2.1.4 Determination of $PNEC_{sediment}$

As from the study with *Lumbriculus* no NOEC could be determined for the sublethal endpoints reproduction and growth and as it is unclear whether the ammonium concentration up to 8 mg/l in all test vessels contributes to the toxicity (also in nature), it can be proposed not to use the *Lumbriculus* study for the $PNEC_{sediment}$ derivation. Following this approach the lowest EC_{10} of

34 mg/kg dw from the *Chironomus* study has to be used as basic value. With an assessment factor of 100 a $PNEC_{sediment}$ of 340 µg/kg dw can be determined.

However, from the available data for *Lumbriculus* it can be concluded that this species is more sensitive to aniline in sediment than *Chironomus*. Although no NOEC could be determined for the sublethal endpoints, the EC_{10} determined for the endpoint survival (15.3 mg/kg dw) is about a factor of 2 lower than the EC_{10} from the *Chironomus* study for the endpoint emergence. Therefore, another approach could be to use the EC_{10} for survival of *Lumbriculus* as basic value. To consider, that the sublethal endpoints reproduction and growth are more sensitive, an assessment factor of 100 could be applied to the EC_{10} of 15.3 mg/kg dw resulting in a $PNEC_{sediment}$ of 153 µg/kg dw.

Another possibility would be the performance of a new study with *Lumbriculus variegatus* from which a NOEC for reproduction and biomass can be derived. However, as there is a valid study for *Chironomus riparius* available, the performance of further sediment tests with aniline is not regarded to be of high priority.

For the risk assessment of the sediment compartment, the PNEC of 153 µg/kg dw, that is equivalent to a PNEC of 58.8 µg/kg ww is used. It has to be considered that in both tests the sediment was pre-incubated with aniline for 2 days only. Therefore, it cannot be excluded that at the beginning of the test there was still free aniline in the sediment that may be more bioavailable to the test organisms than aniline covalently bound to humic acids.

3.2.2 Atmosphere

Cheeseman et al. (1980) examined the effect of aniline on pine seedlings exposed by fumigation. The aim of the study was to correlate symptoms found in the field with symptoms evoked by controlled fumigation with different chemicals. Several experiments were conducted. All given concentrations are nominal.

First, the effects of aniline on active plants (plants that were growing in a heated greenhouse with extended photoperiod) were examined. 5-10 trees, at least 10 month old, were exposed for 3 hours at a temperature of 25-30°C. It was found that aniline at concentrations of 0.4 ppm (1.5 mg/m^3) produced as much damage as higher concentrations (up to 10 ppm) and that some trees were injured at 0.07 ppm (0.27 mg/m^3). No further information, e.g. on the number of trees affected at 0.07 ppm or on the time needed after exposure to observe first effects is given.

A further experiment studied the effect of aniline on dormant and dormant/active trees. Dormant trees were growing outside and were so going to the normal winter dormancy cycle. After fumigation, dormant trees were either returned to the outside or were placed in the greenhouse to become active (dormant/active trees). The trees were fumigated with aniline concentrations of 1, 3 and 10 ppm for 3 hours. Dormant/active trees developed severe necrosis with minor abscission after 7 days and severe abscission after 14 days. Dormant trees showed minor banding after 14 days and severe necrosis after 21 to 35 days. No further information is given.

At last the effect of aniline on known resistant and sensitive genotypes was studied. For the first exposure 4 groups of plants comprising 1 resistant and 2 sensitive active trees and 2 resistant and 2 sensitive dormant/active trees were tested at 0, 0.025, 0.07 and 0.25 ppm, respectively for 3 hours. After 14 days both active sensitive trees at 0.25 ppm (0.97 mg/m^3) and 1 active sensitive tree at 0.07 ppm (0.27 mg/m^3) showed necrosis. 14 days after the first test all trees, except the 2 sensitive trees showing symptoms at 0.25 ppm were exposed a second time to 0.4 ppm

(1.5 mg/m^3). Exposure time varied between 1 and 3 hours. 5 days after the second exposure, developing necrosis was visible on 8 susceptible trees. 11 days after exposure two of the trees were severely chlorotic. After 14 days 10 sensitive trees showed moderate to severe necrosis and/or abscission. Resistant trees and controls showed no symptoms.

The authors give the additional information that a preliminary screening of three other tree species, seven horticultural species and twenty weed species showed no response to aniline at 1 ppm for 1-3 hours. Neither the species names are given nor the studied endpoints.

These studies cannot be used to derive a reliable $PNEC_{plant}$ because relevant information is missing. However, it can be concluded from the study that aniline may cause effects on plants even at very short exposure periods of a few hours. In addition, the studied endpoints chlorosis/necrosis and needle abscission are possibly less sensitive than e.g. growth inhibition.

To allow the derivation of a $PNEC_{plant}$ for a risk assessment of the atmosphere, a plant fumigation test was performed with aniline (BASF, 2002). Three species of higher plants (*Avena sativa*, *Brassica pekinensis* and *Abies grandis*) were exposed in laboratory exposure chambers for 14 days to 3 aniline concentrations (0.1 mg/m^3, 0.3 mg/m^3 and 1 mg/m^3) and a control. Aniline concentrations were measured by HPLC. Daily means were calculated based on tow measured samples per concentration and exposure. Mean measured concentrations were 0.158 mg/m^3, 0.334 mg/m^3 and 1.22 mg/m^3. Seeds (*Avena* and *Brassica*) and germinated plants (*Abies,* 1 year old plants) were acclimatised in the exposure chambers for 8 days before exposure to aniline. Endpoints were plant length, wet and dry weight as well as macroscopic changes for *Avena sativa* and *Brassica pekinensis*. For *Abies grandis* only macroscopic and microscopic changes were recorded. The EC_{50} for all tested parameters for *Avena sativa* and *Brassica rapa* was > 1 mg/m^3. The NOEC values for all tested endpoints were 1 mg/m^3 for *Avena sativa* and 0.3 mg/m^3 for *Brassica pekinensis*. Macroscopic and stereo microscopic observations in *Abies grandis* showed no changes in the habit of the plants. For the derivation of the $PNEC_{plant}$ the lowest NOEC of 0.3 mg/m^3 found for *Brassica pekinensis* is used as basic value. An assessment factor of 50 is proposed as the exposure period for the three tested plant species was only 14 days. There are indications from the study of Cheeseman et al. (1980) that certain tree species may react sensitive to aniline. As it is not appropriate to examine adequately the effect of aniline on trees in a 14-day laboratory test this is considered by the height of the assessment factor.

Therefore $PNEC_{plant} = 0.3 \text{ mg/m}^3 / 50 = 6 \text{ µg/m}^3$.

3.2.3 Terrestrial compartment

Hulzebos et al. (1993) determined the toxicity of aniline to *Lactuca sativa* in natural soil and in nutrient solution. Both tests were conducted by two institutes (TNO and RIVM). A 14-day EC_{50} for growth inhibition of 33 mg/kg soil (dw) was found by RIVM and of 56 mg/kg soil (dw) by TNO. The content of organic matter was 1.4% for the soil used by TNO and 1.8% for the soil used by RIVM. For the test with nutrient solution different exposure times were used by the two laboratories. TNO derived a 16-day EC_{50} of 7.9 mg/l and RIVM found for 21-day exposure an EC_{50} of 17 mg/l. All effect values are related to nominal concentrations. The authors showed that the concentration of aniline had dropped to $\leq$ 30% after 14 days.

The lowest EC_{50} value found for soil exposure is used for the determination of the $PNEC_{soil}$. An assessment factor of 1,000 has to be applied. This leads to a PNEC of 33 µg/kg (dry weight).

Considering the fate of aniline in soils (partial degradation, rapid formation of covalent bonds with soil organics, cf. Sections 3.1.2.1 and 3.1.2.2) the practicability of this test for the risk assessment appears to be questionable. In the test system, the organisms were initially (during a few hours or days) exposed to a high dosis of "free" aniline, while later (when the equilibrium was reached) they were exposed to both free aniline in the porewater and bound aniline in the solid phase. The test system doesn't differentiate between both exposure mechanisms. Under natural conditions, soil organisms will mainly be exposed to the bound substance, as aniline is set free relatively slowly from the agents and is always in equilibrium with the bound form.

Applying the equilibrium partitioning approach (TGD, eq. 56), a $PNEC_{soil}$ of 11 µg/kg ww is calculated from a $PNEC_{aqua}$ of 1.5 µg/l. As discussed above, this approach is not appropriate for the aniline assessment as only the exposure via porewater is considered by this model.

An investigation with other aniline derivatives indicates that the reaction product of anilines with humic acids is bioavailable. Similar to aniline, 4-chloroaniline and 3,4-dichloroaniline form covalent bounds to the humic fraction of soils and sediments. In a plant-uptake test, radiolabelled chloroanilines were pre-incubated into soils until the covalent bonds had been formed. Then different plants were sowed and the radioacticity was measured. It was shown that radioactivity was taken up by the plants indicating that the complexes of the humic substances with aniline derivatives are bioavailable (Fuchsbichler, 1978 a; b). This topic is elaborated more extensive in Appendix D.

With the available information, only a rough approach for an initial assessment is possible:

$PNEC_{soil}$ = 33 mg/kg / 1,000 = 33 µg/kg (dry weight)
= 24 µg/kg (wet weight)

Effect values for terrestrial organisms from three trophic levels are available for the substance 3,4-dichloroaniline, that shows a similar behaviour like aniline in soil and sediment (formation of covalent bonds to humic acids). Although the effect values found for 3,4-dichloroaniline cannot be used directly for an assessment of aniline, the data can be used to indicate whether further terrestrial tests with aniline are necessary for risk assessment purposes.
The following effect values are availabe for 3,4-dichloroaniline (for a detailed description of the tests cf. Appendix D):

Table 3.33 Short-term tests with 3,4-DCA with freshly contaminated soil

Lactuca sativa	7d/14d EC_{50}	10 mg/kg dw	growth
Eisenia fetida	7d/14d LC_{50}	130 mg/kg dw	mortality

Table 3.34 Long-term tests with 3,4-DCA with freshly contaminated and pre-incubated soil

Avena sativa	51-day EC_{50} 51-day NOEC	202–242 mg/kg dw 125 mg/kg dw	14 d pre-incubation	shoot lenght, shoot dry weight, number of flowers, dry weight of flowers, total dry weight
Brassica rapa	35-day EC_{50} 35-day NOEC	25–264 mg/kg dw 125 mg/kg dw	14 d pre-incubation	shoot lenght, shoot dry weight, number of seed pods, dry weight of seed pods, total dry weight
Eisenia fetida	56-day NOEC	100 mg/kg dw	freshly contaminated	mortality, body weight, offsprings
Eisenia fetida	56-day NOEC	> 320 mg/kg dw	5 weeks pre-incubation	mortality, body weight
Eisenia fetida	56-day NOEC	100 mg/kg dw	5 weeks pre-incubation	offspring
Microorganisms	28-day NOEC	32 mg/kg dw	freshly contaminated	nitrification inhibition
Microorganisms	28-day NOEC	100 mg/kg dw	5 weeks pre-incubation	nitrification inhibition

The effect values found for 3,4-dichloroaniline clearly shows that pre-incubation of the soil with the test substance significantly reduce the toxicity. This reduction in toxicity can even be observed in long-term tests performed with pre-incubated soil compared to short-term tests performed with freshly contaminated soil. A comparison of short- and long-term tests with plants shows a factor of about 20 between the EC_{50} values. For *Eisenia fetida* the NOEC from the reproduction test with pre-incubated soils is a factor of about 2.5 higher than the LC_{50} from the short-term test with freshly contaminated soil. Microorganisms show in long-term tests a factor of about 3 between the NOEC found in freshly contaminated soil and the NOEC obtained in pre-incubated soil.

As 3,4-dichloroaniline and aniline show a similar behaviour in soils, it can be assumed with high probability, that also the toxicity of aniline in soils pre-incubated with the test substance for a certain time is reduced compared to the toxicity in freshly contaminared soils.

However, the degree of the reduction in toxicity cannot be estimated based on the available information. It is also not possible to state that aniline in soil will generally be least toxic than 3,4-dichloroaniline, as the tests performed with sediment organisms (cf. Section 3.2.1.3) do not support such a statement (*Chironomus* spec: aniline: 28-day EC_{10} = 34 mg/kg dw, 3,4-DCA: 28-day EC_{10} = 104 mg/kg dw; *Lumbriculus variegatus*: aniline: 28-day NOEC (reproduction) < 11.6 mg/kg dw; 3,4-DCA: 28-day NOEC (reproduction): 5 mg/kg dw). A statement concerning the relative toxicity of aniline in comparison to 3,4-DCA is not possible.

Therefore, for an assessment of the effects of aniline in soil further tests are necessary. To improve the effect data, tests on terrestrial organisms should be performed. The binding properties can be considered by using soils pre-incubated with aniline before the test is started.

3.2.4 Secondary poisoning

A biomagnification via food chain is not expected via the route water - fish. The result of a recently performed bioaccumulation study with the benthic oligochaete *Lumbriculus variegatus* indicates that also for sediment and soil dwelling organisms bioaccumulation of aniline is low. On the basis of mammalian toxicity data, aniline is classified as toxic and harmful. According to the TGD it is assumed that the available test data with laboratory animals can give an indication

on the possible risk of the chemicals to top-predators in the environment. The NOAELs found in these studies have to be converted into a food concentration by using the ratio between body weight and daily food intake as conversion factor. In the TGD conversion factors for several laboratory test species (rats, mice, etc.) are given.

In a repeated dose toxicity study with rats conducted over 104 weeks a LOAEL of 7 mg/kg bw/d was found (CITI, 1982). As no NOAEL is available, the PNECoral is derived from the LOAEL This LOAEL have to be converted into a food concentration by using a conversion factor of 10. With this a LOEC of 70 mg/kg food can be derived. According to the TGD, for the calculation of the PNECoral an assessment factor of 30 has to be applied to this value based on a LOAEL found in a chronic study. Therefore, a PNECoral of 2.3 mg/kg food is calculated.

Regarding this PNECoral, it has to be kept in mind, that it is derived from a LOAEL. In addition, for carcinogenic substances like aniline it is not possible to set a threshold value below which no adverse effects will occur.

3.3 RISK CHARACTERISATION

3.3.1 Aquatic compartment (incl. sediment)

The result of the aquatic effects assessment are a $PNEC_{microorganism}$ of 2,000 µg/l and a $PNEC_{aqua}$ of 1.5 µg/l. In the following tables, the risk characterisation rations for all known aniline production and processing sites together with the databasis laying down for the PEC calculations are presented.

3.3.1.1 Production and processing

Production

The exposure scenarios for the sites G and H are based on site-specific emission data. The PEC/PNEC ratio for the aquatic compartment is above 1, thus a risk to the aquatic environment is expected: **conclusion (iii)**.

Processing to MDA

The exposure scenarios for the sites T, U and X are based on site-specific emission data. The PEC/PNEC ratio for the aquatic compartment is above 1, thus a risk to the aquatic environment is expected: **conclusion (iii)**.

At site X it is scheduled for 2001, that all industrial and domestic effluents in the region will be collected and released 3 km from the coast into the ocean. This will lead to a higher dilution and a reduction of the PEC_{aqua}.

Table 3.35 Risk characterisation for sites emitting into rivers

Site	Life stage	Site-specific data	Default values	Ceffl. [µg/l]	Ceffl. / $PNEC_{micro.}$	PEClocal [µg/l]	PEClocal / $PNEC_{aqua}$
A	prod. + proc. to MDA, caout. chem, dyes	effluent conc. (∅ and 90% percentile), sewage and river flow; prod. period	-	80	0.04	0.76	0.51
C	prod. + proc. to dyes, other intermediates and ppps	effluent conc. (∅ and 90%ile), sewage and river flow; prod. period	-	139	0.07	1.07	0.71
D	prod. + proc. to MDA	effluent conc. below dl, sewage and river flow; prod. period	-	< 10	< 0.005	< 0.51	< 0.34
E	prod. + proc. to MDA, caout. chem	effluent conc. (∅ and 90%ile), sewage and river flow; prod. period	-	100	0.05	0.64	0.43
F	prod. + proc. to MDA	effluent conc. below dl, sewage and river flow; prod. period	-	< 20	< 0.01	< 0.32	< 0.21
G	prod.	max. effluent concentration, sewage and channel flow; prod. period	-	20,000	10	280	190
H	prod.	effluent conc. (∅ and 90%ile), no WWTP	(dilution see Section 3.1.3.2)	No WWTP	-	48	32
J	proc. to caout. chem.	effluent conc. below dl, sewage and river flow, proc. period	-	< 1	< 0.0005	< 0.51	< 0.34
K	proc. to caout. chem.	mean and max effluent conc., sewage and canal flow, proc. period	-	2,000	1	3.5	2.3
M	proc. to caout. chemicals	processing volume, river flow	release factor 0.7%, sewage flow, WWTP elimin. 87%; proc. period	2,300	1.15	130	87
N	proc. to caout. chemicals	effluent conc. below dl, sewage flow, proc. period	river flow	< 50	< 0.025	< 0.14	< 0.09
O	proc.	proc. volume, river flow	release factor 0.7%, sewage flow, proc. period	1,500	0.75	0.20	0.13

Table 3.35 continued overleaf

Table 3.35 continued Risk characterisation for sites emitting into rivers

Site	Life stage	Site-specific data	Default values	Ceffl. [µg/l]	Ceffl. / $PNEC_{micro.}$	PEClocal [µg/l]	PEClocal / $PNEC_{aqua}$
P	proc.	proc. volume, river flow	release factor 0.7%, sewage flow, proc. period	500	0.25	0.15	0.1
Q	proc.	proc. volume, river flow	release factor 0.7%, sewage flow, proc. period	1,500	0.75	0.62	0.41
R	proc. to MDA	effl. conc. below dl, sewage and river flow; proc. period	-	< 100	< 0.05	< 0.50	< 0.33
V	proc. to dyes	emission amount per year, sewage and river flow	release factor 0.7% for Clocal calc., proc. period	150	0.075	0.34	0.23
W	proc. to ppps	emission amount per year, sewage and river flow	release factor 0.7% for Clocal calc., proc. period	49	0.02	0.16	0.11
Y	proc. to caout. chemicals	effluent conc. (∅ and 90%ile), proc. period, sewage and river flow	-	7.3	0.004	0.18	0.12
Z	proc. to caout. chemicals	effluent conc. (∅ and 90%ile), proc. period, sewage and river flow	-	120	0.06	0.40	0.27
ZA	proc. to caout. chemicals	processing volume, river flow	release factor 0.7%, sewage flow, WWTP elimin. 87%; proc. period	7,500	3.75	590	390

Table 3.36 Risk characterisation for sites emitting into sea or estuaries

Site	Life stage	Site-specific data	Default values	Ceffl. [µg/l]	Ceffl. / $PNEC_{micro}$	PEClocal [µg/l]	PEClocal / $PNEC_{aqua}$
B	prod. + proc. to MDA + caout. chem.	effluent conc. below dl, sewage flow; proc. period	dilution 1:10	< 10	< 0.005	< 1.1	< 0.73
I	imp.; proc. to MDA	effluent conc., prod. period, sewage flow, pre-dilution 1:105; proc. period	dilution 1:10	< 50	< 0.025	< 0.18	< 0.12
L	proc. to caout. chem.	weekly emission (∅ and 90% percentile), sewage flow; no treatment plant! proc. period	dilution 1:100	No WWTP	-	920	610
S	proc. to MDA	sewage conc. below dl, sewage flow; no treatment plant! proc. period	dilution 1:100	No WWTP	-	< 0.15	< 0.1
T	proc. to MDA and pharma	effluent conc. (∅ and 90% percentile), proc. period, sewage flow	dilution 1:10	172	0.086	17	11
U	proc. to MDA	proc. volume, proc. period, sewage flow, yearly emission	dilution 1:50	710	0.35	14	9.3
X	proc. to MDA	sewage conc. below dl; proc. period; sewage flow	dilution 1:10	< 50	< 0.025	< 5.1	< 3.4

Processing to rubber chemicals

The exposure scenarios for the sites K and L are based on site-specific emission data. The PEC/PNEC ratio for the aquatic compartment is above 1, thus a risk to the aquatic environment is expected: **conclusion (iii)**.

The exposure scenarios for the sites M and ZA are largely based on default parameters. The PEC/PNEC ratio for the aquatic compartment is above 1, thus a risk to the aquatic environment is expected: **conclusion (iii)**. Industry was asked repeatedly for the missing data, without effect.

Processing to dyes

The exposure scenarios for the sites A, C and V are based on site-specific emission data. The PEC/PNEC ratio for the aquatic compartment is below 1, thus a risk to the aquatic environment is not expected: **conclusion (ii)**.

Processing to plant protection products

The exposure scenarios for the site C is based on site-specific emission data. The PEC/PNEC ratio for the aquatic compartment is below 1, thus a risk to the aquatic environment is not expected: **conclusion (ii)**.

The exposure scenario for the sites W is largely based on default parameters. The PEC/PNEC ratio for the aquatic compartment is below 1, thus a risk to the aquatic environment is not expected: **conclusion (ii)**.

Processing to pharmaceuticals

One site (T) is known to produce pharmaceutical intermediates. The PEC/PNEC ratio for this site is above 1. The technical description of the processes reveals that the releases via wastewater origin primarily from MDA production: **conclusion (ii)**.

3.3.1.2 Rubber chemicals

In a first rough approach, a PEC_{local} of 0.63 µg/l was estimated for emissions of rubber manufacturers. So far, the database for the exposure estimation is extremely poor (cf. Section 3.1.1.4), and the resulting PECs cannot be considered as safe.

Data about the formation of aniline from different precursors, the releases into the wastewater and wastewater treatment which are representative for the European rubber industry are needed: **conclusion (i)**.

3.3.1.3 Coal and oil industry

The present information is not sufficient to carry out a risk characterisation for this emission source. The releases resulting from coal and oil industry are not covered by the life-cycle of aniline produced or imported into the EU. Data improvement sufficient for risk assessment purposes is judged disproportionate within the scope of this programme. Therefore, no formal conclusion is drawn for this emission scenario.

3.3.1.4 Plant protecting agents

Releases of phenylureas and -carbamates and their metabolisation to aniline in the hydrosphere are probably of minor importance: **conclusion (ii)**.

3.3.1.5 Sediments

A $PNEC_{sediment}$ of 58.8 µg/kg ww (153 µg/kg dw) was determined for aniline. The risk characterisation ratios for all known aniline production and processing sites are presented in **Table 3.37**.

Table 3.37 Risk characterisation for sediment for production and processing sites

Site	$PEClocal_{water}$ [µg/l]	$PEClocal_{sediment}$ [µg/kg ww]*	PEC/PNEC
A	0.76	7.4	0.12
B	< 1.1	< 11	< 0.19
C	1.07	10.4	0.18
D	< 0.51	< 4.9	< 0.08
E	0.64	6.2	0.1
F	< 0.32	< 3.1	< 0.05
G	280	2,700	45.9
H	48	470	8
I	< 0.18	< 1.8	< 0.03
J	< 0.51	< 4.9	< 0.08
K	3.5	34	0.58
L	920	8,900	151
M	130	1,300	22.1
N	< 0.14	< 1.4	< 0.02
O	0.20	1.9	0.03
P	0.15	1.5	0.03
Q	0.62	6.0	0.1
R	< 0.50	< 4.9	< 0.08
S	< 0.15	< 1.5	< 0.03

Table 3.37 continued overleaf

Table 3.37 continued Risk characterisation for sediment for production and processing sites

Site	PEClocalwater [µg/l]	PEClocalsediment [µg/kg ww]*	PEC/PNEC
T	17	170	2.9
U	14	140	2.4
V	0.34	3.3	0.06
W	0.16	1.6	0.03
X	< 5.1	< 49	< 0.83
Y	0.18	1.8	0.03
Z	0.40	3.9	0.07
ZA	590	5,700	97

Production

The exposure scenarios for the sites G and H are based on site-specific emission data. The PEC/PNEC ratio for the sediment compartment is above 1, thus a risk to the benthic environment is expected: **conclusion (iii)**.

However, as the PEC/PNEC ratio for surface water is higher for these sites than the PEC/PNEC ratio for sediment, any risk reduction measure that has to be applied for surface water will cover also the sediment compartment. Therefore, no further risk reduction measures are necessary for the sediment compartment.

Processing to MDA

The exposure scenarios for the sites T and U are based on site-specific emission data. The PEC/PNEC ratio for the sediment compartment is above 1, thus a risk to the benthic environment is expected: **conclusion (iii)**.

However, as the PEC/PNEC ratio for surface water is higher for these sites than the PEC/PNEC ratio for sediment, any risk reduction measure that has to be applied for surface water will cover also the sediment compartment. Therefore, no further risk reduction measures are necessary for the sediment compartment.

Processing to rubber chemicals

The exposure scenario for the site L is based on site-specific emission data. The PEC/PNEC ratio for the sediment compartment is above 1, thus a risk to the benthic environment is expected: **conclusion (iii)**.

The exposure scenarios for the sites M and ZA are largely based on default parameters. The PEC/PNEC ratio for the sediment compartment is above 1, thus a risk to the benthic environment is expected: **conclusion (iii)**. Industry was asked repeatedly for the missing data, without effect.

However, as the PEC/PNEC ratio for surface water is higher for these sites than the PEC/PNEC ratio for sediment, any risk reduction measure that has to be applied for surface water will cover also the sediment compartment. Therefore, no further risk reduction measures are necessary for the sediment compartment.

Processing to dyes

The exposure scenarios for the sites A, C and V are based on site-specific emission data. The PEC/PNEC ratio for the sediment compartment is below 1, thus a risk to the benthic environment is not expected: **conclusion (ii)**.

Processing to plant protection products

The exposure scenarios for the site C are based on site-specific emission data. The PEC/PNEC ratio for the sediment compartment is below 1, thus a risk to the benthic environment is not expected: **conclusion (ii)**.

The exposure scenario for the sites W is largely based on default parameters. The PEC/PNEC ratio for the sediment compartment is below 1, thus a risk to the benthic environment has not to be expected.

Processing to pharmaceuticals

One site (T) is known to produce pharmaceutical intermediates. The PEC/PNEC ratio for this site is above 1. The technical description of the processes reveals that the releases via wastewater origin primarily from MDA production: **conclusion (ii)**.

3.3.2 Atmosphere

3.3.2.1 Production and processing

The result of the effects assessment is a $PNEC_{plant}$ of 6 µg/m^3. In **Table 3.38**, the risk characterisation ratios for all known aniline production and processing sites together with the databasis laying down for the PEC calculations are presented.

Table 3.38 Risk characterisation for atmosphere for production and processing sites

Site	Life-stage	Data basis	Release [kg/d]	$PEClocal_{air,ann}$ [µg/m^3]	$PEC/PNEC_{plant}$
A	prod., proc. to MDA, caout. chem, dyes	yearly release	0.26	0.059	0.01
B	prod., proc. to MDA + caout. chem.	yearly release	0.92	0.21	0.03
C	prod., proc. to dyes	yearly release	2.2	0.50	0.08
D	prod., proc. to MDA	yearly release	0.26	0.059	0.01
E	prod., proc. to MDA + caout. chem	yearly release	< 0.087	< 0.020	< 0.003
F	prod., proc. to MDA	yearly release	0.011	0.0025	0.0004

Table 3.38 continued overleaf

Table 3.38 continued Risk characterisation for atmosphere for production and processing sites

Site	Life-stage	Data basis	Release [kg/d]	PEClocal$_{air,ann}$ [µg/m^3]	PEC/PNECplant
G	prod.	default 380 ppm see remarks	120	1,200	200
H	prod.	yearly release	9.7	2.2	0.37
I	imp.; proc. to MDA	yearly release	3.0	0.75	0.125
J	proc. to caout. chem.	yearly release	0.15	0.038	0.006
K	imp., proc. to caout. chem.	yearly release	0.12	0.030	0.005
L	proc. to caout. chem.	yearly release	3.0	0.69	0.12
M	proc. to caout. chem.	default 380 ppm	1.9	0.055	0.009
N	proc. to caout. chem.	yearly release	0.76	0.19	0.03
O	proc.	default 380 ppm	1.3	0.059	0.01
P	proc.	default 380 ppm	0.42	0.012	0.002
Q	proc.	default 380 ppm	1.3	0.27	0.04
R	proc. to MDA	yearly release	2.2	0.50	0.08
S	proc. to MDA	yearly release	0.0024	0.00067	0.0001
T	proc. to MDA + pharma	yearly release	0.76	0.19	0.03
U	proc. to MDA	yearly release	5.5	1.3	0.22
V	proc. to dyes	yearly release	0.73	0.17	0.03
W	proc. to ppps	yearly release	incin.	-	-
X	proc. to MDA	yearly release	1.95	0.45	0.07
Y	proc. to rubber chem.	yearly release	5.9	1.3	0.22
Z	proc. to caout. chem.	yearly release	12	3.3	0.55
ZA	proc. to caout. chem.	default 380 ppm	6.3	0.72	0.12

Remarks

Site G: The company has no data for the release amount. The measured concentrations at 3 sampling sites are between 1.5 and 3.9 mg/m^3 (maximum values) resp. 0.15 and 1.2 mg/m^3 (average values). The upper values are used for the PEClocal.

Production

The exposure scenarios for the site G is based on a limited number of measured concentrations. The PEC/PNEC ratio for the atmosphere is above 1, thus a risk to plants exposed via the vapor phase is expected: **conclusion (iii)**.

Processing to MDA

The PEC/PNEC ratios are below 1, thus a risk to the atmosphere is not expected: **conclusion (ii)**.

Processing to rubber chemicals

The PEC/PNEC ratios are below 1, thus a risk to the atmosphere is not expected: **conclusion (ii)**.

Processing to dyes

The PEC/PNEC ratios are below 1, thus a risk to the atmosphere is not expected: **conclusion (ii)**.

Processing to plant protection products

The PEC/PNEC ratios are below 1, thus a risk to the atmosphere is not expected: **conclusion (ii)**.

Processing to pharmaceuticals

The PEC/PNEC ratios are below 1, thus a risk to the atmosphere is not expected: **conclusion (ii)**.

3.3.2.2 Rubber industry

For rubber industry, only an initial exposure estimation with an unsafe data basis was possible. More representative information on aniline releases are necessary, especially whether exhaust air purification techniques are commonly applied, together with their effectiveness: **conclusion (i)**.

3.3.2.3 Thermal degradation of polyurethanes

Aniline was detected in the working place atmosphere in foundries where it is formed by thermal degradation of MDI-based polyurethane bound foundry core materials. This issue is extensively described in Section 4. There are no data about pollution of the outer atmosphere by these sources. Compared with aniline production and processing plants with releases above 1 t/a, this source is expected to be of minor importance.

The present information is not sufficient to carry out a risk characterisation for the environment for this emission source. As these releases are expected to be of minor importance, data improvement is not of high priority. No formal conclusion is drawn for this scenario.

3.3.3 Terrestrial compartment

3.3.3.1 Production and processing

Aniline emitted into the atmosphere is deposited into the soil near the source. With a $PNEC_{soil}$ (related to dry weight) of 33 µg/kg, the following ratio is calculated:

highest submitted emission (site Z): $PEClocal_{soil}$ = 5.6 µg/kg, PEC/PNEC = 0.17

Conclusion (ii).

3.3.3.2 Rubber chemicals

A first rough exposure assessment for the rubber industry resulted in a $PEClocal_{soil}$ of 1.6 µg/kg, from this a $PEC_{soil}/PNEC_{soil}$ ratio of 0.05 is calculated: **conclusion (ii)**.

3.3.3.3 Plant protecting agents

With a PNEC of 33 µg/kg dw, the PEC/PNEC ratios are:

Table 3.39 PEC/PNEC ratios for use of plant protecting agents

Agent	PEC_{soil} [µg/kg dw]	PEC_{soil} / $PNEC_{soil}$
Fenuron	640	19.4
Siduron	1,200	36.4

The result of the effects assessment was that it is not possible to derive a PNEC which considers the realistic exposure situation. The risk characterisation is only a rough initial approach. For an improved effects assessment, tests with terrestrial organisms with pre-incubated aniline should be performed. Long-term tests with plants, earthworms and microorganisms are proposed to enable a proper effects assessment. It is proposed that this problem should be considered for the assessment of plant protection agents within the frame of Council Directive 91/414/EEC. **Conclusion (i)**.

3.3.4 Secondary poisoning

Because of the low accumulation of aniline in fish via water, the exposure route water - fish -fish eating bird or mammal is likely to be not relevant.

However, the reaction product of aniline with humic acids accumulates in sediments and soils and is probably bioavailable. A bioaccumulation via the route sediment or soil - sediment or soil dwelling worm – worm-eating mammal or bird cannot be excluded. However, the result of a recently performed bioaccumulation study with the benthic oligochaete *Lumbriculus variegatus* indicates that also for sediment and soil dwelling organisms bioaccumulation of aniline is low: **conclusion (ii)**.

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 General discussion

Aniline is produced from nitrobenzene by means of catalytic hydrogenation under pressure or by reduction with iron. The raw product is purified by distillation and is delivered via pipeline, tanker and, to a small extent, in drums.

Aniline is used as a chemical intermediate. A EU use pattern is given in BUA, 1995:

- MDA (4,4`-methylenedianiline, 71%, starting material for polyurethane plastics),
- initial dye products (5%),
- rubber chemicals (15%, stabilisers, activators etc.),
- initial products and intermediates for fibre (1%) and pesticide production (3%),
- pharmaceuticals (1.2%),
- other products (3.7%).

It is not known whether aniline is used as a component in consumer products. Analyses of a dye used for dying shoes in Spain have revealed concentrations of 1-2% of aniline. Cases of intoxications were suspected to be due to use of dyed shoes. However, there is no more information available to the rapporteur.

4.1.1.2 Occupational exposure

Since aniline is exclusively used as a chemical intermediate, exposure during production and further processing are the main exposure scenarios. Additionally, exposures are to be expected if formulations with residual aniline contents are handled, e.g. dyes and adhesives, or if aniline occurs during further processing as a result of decomposition, e.g. in foundries and during rubber vulcanisation. Aniline may arise in coal carbonisation plants.

The following occupational exposure limits apply in the EU (ILO, 1994):

- DK, S: 4 mg/m^3 (1 ml/m^3)
- FIN, B: 7.6 mg/m^3 (2 ml/m^3)
- D: 8 mg/m^3 (2 ml/m^3)
- UK, F: 10 mg/m^3 (2 ml/m^3)

In Germany, the short-term exposure limit amounts to 32 mg/m^3 (8 ml/m^3, 4·occupational exposure limit (MAK), 15 min, duration 1 h). Up to the end of 1996 it amounted to 40 mg/m^3 (10 ml/m^3, 5·MAK, during 30 minutes, 2 times per day).

4.1.1.2.1 Production and further processing in the chemical industry (Scenario 1-4)

Production

In Western Europe aniline is synthesised by the reduction of nitrobenzene. The reduction mainly occurs catalytically with hydrogen, under pressure. According to information provided by one German manufacturer, production is "in part continuous". However, there is no information which production step is non-continuous. In addition, one manufacturer uses the discontinuous Bechamp process which is based on the reduction of nitrobenzene with iron. The yield amounts to approx. 99% for both methods. The raw aniline which is obtained is purified by distillation and filled into tankers via pipelines (gas displacement equipment). According to information provided by one manufacturer in Germany, less than 1% is filled into drums. Converted to the quantity of aniline produced in Germany, this percentage amounts to approx. 2,000 t.

Aniline is synthesised in closed systems. One manufacturer states that reduction according to the Bechamp process is performed in a plant at which natural ventilation is available but not exhaust ventilation. Reduction using hydrogen takes place in an open-air plant.

Possibilities for exposure exist during sampling as well as during cleaning, maintenance and repair work. Exposure by inhalation can additionally occur during filling and the performance of checks on the fill levels (using a gauge stick). According to information provided by one manufacturer in Germany, the workers wear safety goggles and gloves. If packing drums and opening containers or pipelines, protective gloves, safety goggles and/or respirators are used. One manufacturer describes the collective of exposed persons as comprising 140 workers who are permanently based in the areas concerned and 25 workers who are there occasionally. Another manufacturer states that approx. 50 workers sometimes handle aniline. No further information on duration and frequency is available. Although differing levels of exposure are to be expected in dependence on the particular activities, it is, however, not possible to undertake a differentiated individual assessment of all of the activities on the basis of the available data. In the case of workers who, due to the continuous process, handle aniline on a permanent basis, the assumptions made about the duration and the frequency are that they are daily and continue throughout the entire shift.

The Health and Safety Executive (HSE, UK) has recently published a report about occupational exposure to aniline in UK (HSE, 1997). It is described, that in the UK aniline is produced within enclosed plants and that exposure to aniline is only likely to occur when this containment is breached for sampling, during maintenance and during tanker loading and unloading. In addition, fugitive emissions from pipe flanges, pump glands and vents are considered as possible contributions to airborne aniline.

Further processing

Aniline is processed further in closed systems, either continuously or batchwise, to form a number of different products. In the case of two manufacturers, further processing takes place in 16 and 20 plants, respectively. One manufacturer states that approx. 800 workers sometimes handle aniline.

One company states that during the further processing of aniline to MDA and other organic products, inhalation exposures occur in the areas of sampling, cleaning, the preparation of the product, transfer and maintenance or repair work. The collective of exposed persons is described

as comprising 170 workers who work at the installations on a permanent basis and a further 80 workers who occasionally work there. Safety goggles and gloves are worn as personal protective measures. In another company, 100 workers are employed at the installations at which aniline is converted to MDA.

Although differing levels of exposure and durations are to be expected in dependence on the particular activities, it is, however, not possible to undertake a differentiated individual assessment of all of the activities on the basis of the available data. In the case of workers who, due to the continuous process, handle aniline on a permanent basis, the assumptions made about the duration and the frequency are that they are daily and continue throughout the entire shift.

In the HSE report the further processing of aniline to fine chemicals and rubber chemicals is described to occur within enclosed plants. Exposure to aniline is only likely to occur when this containment is breached for sampling, during maintenance and during tanker unloading. In addition, fugitive emissions from pipe flanges, pump glands and vents are considered as possible contributions to airborne aniline. In the UK, a relative small amount (quantity is not given) of unmodified aniline is used to produce azo-dyes. The processing takes place in closed reactors and partially lidded mixing vessels. In the report it is stated, that exposure to aniline is mainly given during the period when aniline is added and during sampling (HSE, 1997).

Inhalation exposure

Workplace measurements

Workplace measurements from 3 production sites were submitted by industry (cf. **Table 4.1**, source: producer).

The measurement method used in the performance of workplace measurements was the NIOSH method 2002 (adsorption to silica gel, elution with ethanol, GC-FID). Due to the measurement method which was applied and the measurement strategy employed by the German manufacturers and users (TRGS 402, 1986), the present measurement values are regarded as valid although workplaces, activities, durations of exposure and collectives of exposed persons have not been described by all of the companies in a sufficiently differentiated manner.

Additional to the data provided by industry the HSE (UK) has published a report about occupational exposure to aniline in the UK (HSE, 1997). Therefore workplace measurement results regarding the production and further processing of aniline are also given in **Table 4.1** (source: HSE, 1997).

Table 4.1 Aniline exposures at workplaces during production and further processing (Industry and by HSE, 1997)

Work area / activities	Year of the measurements	Number of measurements	Measurement range [mg/m³]	Geometric mean [mg/m³]	95% value [mg/m³]	Duration and frequency	Source
8-hour time-weighted average							
Production							
Aniline production, reduction using H_2	1990-1994	27	< 0.8	-	-	-	producer
Aniline production, reduction using H_2, - All workplaces	1990-1996	238	< 1	< 0.04	0.6	-	producer
- Production	1990-1996	53	0-0.9	0.08	0.54	-	producer
- Pilot plants	1990-1996	9	0-0.56	0.07	0.084	-	producer
- Filling area/store	1990-1996	3	0.01-0.4	-	-	-	producer
- Workshop	1990-1996	3	0.02-2.7	-	-	-	producer
Aniline production reduction using H_2	1991-1996	152	≤ 2.8	0.45 (a.m.[1)])	-	-	HSE (1997)
Maintenance	1991- 996	29	≤ 1.8	0.4 (a.m.[1)])	-	-	HSE (1997)
Aniline production, reduction using Fe	1990-1994	9 6	< 0.8 0.95 - 1.5	-	-	-	producer
Further processing							
Further processing to MDA	1990-1994	20 15	< 0.8 < 0.08	-	-	-	producer
Further processing to NaMBT [(3)]	1993-1994	5	< 0.08	-	-	-	producer
Further processing to organic products	1990-1994	82	< 0.8	-	-	-	producer
Further processing to phenylhydrazine	-	4 [(2)] 2 [(2)]	< 0.8 < 1	-	-	-	producer
Further processing to acetoacetic anilide	-	13 [(2)]	< 0.8	-	-	-	producer
Further processing of aniline	1988-1994	-	0.01-0.2	-	-	-	producer
Further processing of aniline to dyes, plant-protection and initial pharmaceutical products	1990-1996	141	0-0.8	0.04	0.64	-	producer
Further processing, initial dye products	-	14 [(2)]	< 0.8	-	-	-	producer

Table 4.1 continued overleaf

Table 4.1 continued Aniline exposures at workplaces during production and further processing (Industry and by HSE, 1997)

Work area / activities	Year of the measurements	Number of measurements	Measurement range [mg/m³]	Geometric mean [mg/m³]	95% value [mg/m³]	Duration and frequency	Source
Further processing of fine chemicals	1993	26	0.16-3.6	1 (a.m. 1))	94% < 2	-	HSE (1997)
Further processing of aniline to rubber chemicals Laboratory	1992- 995 1992-1996	176 1 277 2 4	< 2 2.4 < 2 2.4, 4.7 < 0.64	0.04-0.12 0.04-0.12	-		HSE (1997)
Further processing of aniline to dyes	-	-	< 2 0.32 < 0.8	-	-		HSE (1997)
Short-term value							
Production							
Further processing to MDA, connection and closing containers	1990-1994	3	< 0.8; 0.8; 3.2	-	-	30 min	producer
Further processing to other organic products, activities unknown	1990-1994	24	< 0.8	-	-	10-60 min	producer
Further processing							
Further processing of aniline to rubber chemicals, different activities including sampling	1992-1995	62	< 0.5 - 6	-	-	1-30 min	HSE (1997)
Further processing of aniline to dyes, charging activities	-	-	< 0.2	-	-	20-25 min	HSE (1997)
Further processing to other organic products involving the filling of drums, work at the filter press, sampling, container-closing work, sieve cleaning	1990-1994	9 1	0.8 - 8 12	-	-	10-60 min 10 min	HSE (1997)

1) a.m.: arithmetic mean
2) Same results for person-related and stationary sampling
3) NaMBT: Sodiummercaptobenzthiazole

With regard to aniline production, measurements are only available from three manufacturers. Two manufacturers provide exposure levels < 0.8 mg/m^3 (0.2 ml/m^3) for the reduction of nitrobenzene using hydrogen. The third manufacturer provides a value of 0.9 mg/m^3 (0.23 ml/m^3) for the maximum exposure and a 95% value of 0.6 mg/m^3 (0.15 ml/m^3) is given regarding activities in the entire plant including the areas production, distillation, filling, sampling, tank farm and quality control in the laboratory. Measurements up to 1.5 mg/m^3

(0.375 ml/m^3) are provided for the Bechamp process (reduction with Fe). One manufacturer provides further information on the areas pilot plants, store and workshops. In the pilot plants, involving the steps filling activities to synthesis work, as well as during the checking of supply stores, the 95% value amounts to 0.084 mg/m^3 (0.02 ml/m^3), the highest value in the storage areas being 0.4 mg/m^3. In workshops, e.g. the rubber workshop, pump repair, vulcanisation and rubber technology, the highest exposure amounts to 2.7 mg/m^3 (cf. **Table 4.1**). According to information provided by 6 companies, the exposures during further processing within the large-scale chemical industry are mainly located below 0.8 mg/m^3 (0.2 ml/m^3). One manufacturer gives the 95% value as 0.64 mg/m^3 (0.16 ml/m^3). This data item includes activities during distillative preparation, sampling, filling, laboratory and storage. During particular activities, e.g. the filling of drums, work at the filter press, sieve cleaning etc., short-term exposure levels (10 min) of up to 12 mg/m^3 (3 ml/m^3) may be reached.

The HSE (1997) reports on exposure during the production of aniline by reduction with H_2. The highest exposure level amounts to 2.8 mg/m^3 (0.7 ml/m^3). From exposure data concerning the further manufacturing of aniline to fine and **Table 4.1**, source: HSE).

Former data provided by the HSE (UK) relating to the chemical industry (databases, not given in **Table 4.1**) reveal a 95% value of 2.1 mg/m^3 (0.525 ml/m^3).

Additional information on exposure to aniline during the production of polymers using 2,2,4-trimethyl-1,2-dihydrochinoline is cited in BUA (1995). Exposure levels were measured in two companies (n = 15, 0.1–3.1 mg/m³, mean: 0.6 mg/m³, n = 27; 0.024–1.015 mg/m³, mean: 0.13). Since the original Italian paper (Pozzoli et al., 1982) is not available, there is a lack of information, e.g. it is not known, if these measurement results are shift averages. It is not clear, if the data regard to the production of 2,4-trimethyl-1,2-dihydrochinoline due to the fact that aniline is a starting material in the production of chinolines. On the other side, chinolines can be used to give polymers ion-exchanging properties. It might be possible, that 2,2,4-trimethyl-1,2-dihydrochinoline is decomposed during the production of such polymers because of thermal processing. Since no detailed information is available, it cannot be judged which processes are relevant for exposure. Taking into account, that the data are rather old (year of publication 1982), it is very questionable to take these data as a basis for a separate exposure scenario. It is to be assumed, that the production of chinolines as well as the production of special polymers with ion-exchanging properties are performed within large-scale or specialised chemical companies with high levels of protection. Therefore, this exposure situation is included in the scenario "further processing of aniline".

EASE estimation

EASE for windows, Version 2, 1995, for the production and further processing of aniline in closed systems within the large-scale chemical industry:

Input parameters: T = 20 C, closed system, significant breaching, LEV present
Level of exposure: 2-12 mg/m^3 (0.5-3 ml/m^3).

Conclusion of inhalation exposure

For the purpose of the assessment of the risks resulting from exposure by inhalation during the production of aniline, here: the reduction of nitrobenzene by means of hydrogen, an exposure level is derived by expert judgement as a reasonable worst case on the basis of measurement results provided by the UK. The highest value of these data amounts to 2.8 mg/m^3 (0.7 ml/m^3, n = 152, see **Table 4.1**). Since a 95th percentile cannot be calculated, a reasonable worst case is

estimated by expert judgement to 2.5 mg/m^3 (0.625 ml/m^3). This value has to be considered as the 8-hours time-weighted average for daily exposure (Scenario 1, cf. **Table 4.5**).

In the case of reduction using elemental Fe, a lower exposure of 1.5 mg/m^3 (0.38 ml/m^3; 8-hour time-weighed average, highest measurement result) should be taken for assessing the risks of daily inhalation exposure (Scenario 2, cf. **Table 4.5**).

Results of workplace measurements were submitted by the producers for three sites. Additional information is reported by the HSE. On the basis of all presented data it is not possible to decide whether the data are also representative for the remaining sites. Therefore the estimation according to the EASE model of 2-12 mg/m^3 (0.5-3 ml/m^3) is used additionally in the description of the exposure by inhalation for the companies which did not submit any data in default of workplace measurements (Scenario 3, cf. **Table 4.5**).

For the purpose of assessing the risks during further processing in the chemical industry, 2 mg/m^3 (0.5 ml/m^3) should be considered as an 8-hours-time-weighted average for daily exposure (expert judgement of the worst case derived from measurements described in the UK report). This exposure scenario should be regarded to include the production of polymers using 2,2,4-trimethyl-1,2-dihydrochinoline (Scenario 4, cf. **Table 4.5**).

In the case of sampling, filling activities and similar work, short-term (10 min) exposures of up to 12 mg/m^3 (3 ml/m^3) are possible.

Dermal exposure

Dermal exposure in the large-scale chemical industry is estimated considering that aniline is manufactured and further processed primarily in closed systems and that the use of gloves is highly accepted within the chemical industry. The extent of protection of the personal protective equipment (here gloves) depends inter alia on the suitability of the recommended material with regard to the permeation properties of aniline.

On condition that suitable gloves are worn, dermal exposure is assessed as low. One manufacturer performed tests of several glove types according to DIN EN 374. The measurement results revealed that the material butyl rubber, fluorocarbon rubber and layers of LLDPE are stable against penetration of aniline for at least 8 hours (Bayer AG, 2000a).

However, the knowledge about the used glove materials is incomplete since only four producers have submitted appropriate information. Additionally, there is still a lack of information with regard to the suitability of all recommended materials. Therefore, it cannot be excluded that, besides suitable protective gloves, also unsuitable gloves providing only limited protection are worn. For this scenario, dermal exposure is assessed as a worst-case estimation applying the EASE model (EASE for windows, Version 2, 1995), estimating a dermal exposure level for immediate dermal contact without gloves. It is not possible to consider the limited protection provided by unsuitable glove material.

For production and further processing in the large-scale chemical industry, worst-case estimation for the immediate handling of aniline assuming that gloves are not used (the limited protection of unsuitable gloves cannot be considered).

Input parameters: T = 20°C, non-dispersive use, direct handling, intermittent
Level of exposure: 0.1-1 mg/cm^2/day

The same scenario is taken to predict exposure during occasionally performed cleaning and maintenance (e.g. during shut down of a plant).

Conclusion of dermal exposure

In case of using suitable gloves, dermal exposure is assessed as low (Scenario 1a–4a, cf. **Table 4.6**).

Supposing that unsuitable gloves are worn, a worst-case estimation of the daily dermal exposure according to the EASE model on the basis of dermal contact without using protective gloves is made. The estimation results in a dermal exposure level of 0.1-1 mg/cm^2 per day. Considering an exposed area of 420 cm^2 (corresponding to the surface of the palms of two hands) the exposure level amounts to 42-420 mg/person/day (Scenario 1b–4b, cf. **Table 4.6**).

In the case of occasional (not daily) cleaning and maintenance of the plant (e.g. during "shut down" of the plant), larger skin areas than during usual daily work may be exposed (hands and part of the forearms) leading to exposure levels of 130–1,300 mg/person/day.

4.1.1.2.2 Release of aniline as a decomposition product (Scenario 5–7)

Aniline is practically not used outside the chemical industry. However, possibilities of exposure exist during the further processing of products from which aniline is released, e. g. as a thermal decomposition product of polyurethane plastics (e.g. in foundries). From the literature (BUA, 1995) and the German Workers Compensation Funds data about aniline exposure in different branches are available. The EASE model (EASE for windows, Version 2, 1995) cannot be used for purposes of estimation exposure since aniline is released as a result of thermal decomposition.

Inhalation exposure is described for rubber vulcanisation, foundries and different branches. After that, dermal exposure is assessed for these three scenarios.

Scenario 5: Rubber vulcanisation

Inhalation exposure

During rubber vulcanisation aniline can be released from vulcanisation accelerators (sulphonamide or guanidine accelerators). These accelerators are added to the rubber in quantities of 0.1-2% during the mixing process which is performed in lidded mixing devices (kneaders). The vulcanisation is an intermittent process performed in presses or autoclaves at 160°C-220°C. The vulcanisation presses are rarely provided with local exhaust ventilation. It is more frequent for the tables on which the vulcanised parts cool down to be equipped with local exhaust ventilation. Extruded parts and thin bands are vulcanised in continuous processes. As a rule, such installations are encapsulated.

Workplace measurements

Table 4.2 Aniline exposures during vulcanisation

Work area / activities	Year of the measurements	Number of measurements	Measurement range [mg/m³]	Geometric mean [mg/m³]	95% value [mg/m³]	Country reference
Vulcanisation of rubber	1981	1	1	-	-	DK Berg et al. (1982)
Production of remoulded tyres	-	10	0.0003–0.0098	-	-	I Menichini et al. (1989)
Vulcanisation of tyres 1)	1992	2 2 1 1	< 0.75 2) < 0.85 2) 0.75 < 0.37 2)		-	D Bayer (2000b)

1) Duration of the measurements 2.5 h. It is assumed, that exposure relevant activities could be performed during the whole shift.
2) Below detection limit

Data provided by industry were obtained during the vulcanisation of tyres (cf. **Table 4.2**). Three of four measurement results are below the detection limits of 0.75 mg/m³ (0.18 ml/m^3), 0.85 mg/m^3 (0.21 ml/m^3) or 0.37 mg/m^3 (0.09 ml/m^3). One stationary collected sample yields in an exposure level of 0.75 mg/m^3 (0.18 ml/m^3). Two exposure levels (0.85 and 0.75 mg/m^3) were measured at an old plant which was shut down later and at a new plant not operating under optimal conditions, respectively.

Aniline exposure at the workplace amounting to 1 mg/m^3 (0.25 ml/m^3) or < 0.01 mg/m^3 (0.00.25 ml/m^3) are provided in the literature (Berg et al., 1982; Menichini et al., 1989).

Based on the presented data, 0.8 mg/m^3 (2 ml/m^3) is estimated as representing the reasonable worst-case situation.

Conclusion of inhalation exposure

For the purpose of assessing the risks as a result of exposure by inhalation in the rubber industry and during rubber processing, 0.8 mg/m^3 (0.2 ml/m^3) is to be considered as the 8-hours-time-weighted average representing a reasonable worst-case situation for daily exposure (Scenario 5, cf. **Table 4.5**).

Scenario 6: Foundries

Inhalation exposure

In foundries, aniline can be released by pyrolysis during the use of polyurethane foam binders in casting moulds.

Workplace measurements

Table 4.3 Aniline exposures in foundries

Work area / activities	Year of the measurements	Number of measurements	Measurement range [mg/m³]	Geometric mean [mg/m³]	95% value [mg/m³]	Country reference
Iron and steel foundries	-	40	0.004–0.098	-	-	S Renman et al. (1986)
Aluminium foundry	-	4	0.16–1.8	-	-	S Renman et al. (1986)
Aluminium foundry	1988	1	0.8	-	-	D BIA (1989)
Aluminium foundry	1992-1995					S Westberg et al. (2001)
in total		33	< 0.1–6.4	0.65	-	
Aluminium sand foundries:						
Moulding		4	1.3–2.6	2.0	-	
Pouring		4	2.0– .6	2.2	-	
Shake-out		5	0.20–6.4	3.9	-	
Aluminium static die casting						
Static die casting		10	0.1–1.3	0.31	-	
Core knock out		2	1.1	< 0.27-< 0.31	-	

Measurements in German foundries revealed aniline exposures of 0.8 mg/m^3 (0.2 ml/m^3, BIA, 1989). Four measurements performed in aluminium foundries produced values of 0.16-1.8 mg/m^3 (0.04-0.45 ml/m^3).

A recent study on exposure in Swedish aluminium foundries and aluminium remelting plants covers different types of aluminium foundries sand, die and static die casting–, diffferent sizes, and melting, molding, core making, casting, and shake out techniques as well as various binders and additives (Westberg et al., 2001). The corresponding exposure levels given in **Table 4.3** were obtained at different workplaces. The authors state that aniline is formed during the thermal degradation of cold-box binders. The highest 8-hour TWA of 6.4 mg/m^3 (1.6 ml/m³) was found at shakeout operators. Earlier studies in Swedish iron and steel foundries reveal lower exposure levels below 0.1 mg/m^3 (0.025 ml/m^3, cf. **Table 4.3**).

The extent of aniline exposure in foundries depends on numerous factors, such as the casting temperature, the ratio of the quantities of metal and binder, the casting process and the level of protection realised in the foundry.

Conclusion of inhalation

An estimation of the exposure in iron, steel and aluminium foundries can only be undertaken on the basis of literature data and one additional measurement result from BIA (1989). Model estimations using the EASE model are not possible, because this model is not applicable for the assessment of exposure caused by decomposition processes. Taking into account that the number

of measurement results is limited, the highest level of 6.4 mg/m^3 (1.6 ml/m^3, 8-hour TWA) should be taken for assessing the risks of daily inhalation exposure.

Scenario 7: Different branches

Inhalation exposure

Generally, the possibility of aniline to be released from products containing polyurethane during thermal processes exists, e.g. during grinding of thermoplastic polyurethane materials which are used in injection moulding machines, baking of polyurethane lacquers, welding of materials which are coated with polyurethane (BIA, 1995). In part, these activities can be performed in many different branches.

Workplace measurements

Measurement results are only available from the German MEGA database (BIA 1994; 1997; 1999; cf. **Table 4.4**). These measurement results date from different periods and include, in part, exposure in old plants in the German Democratic Republic. Therefore these data are judged to be obsolete.

In order to base the exposure assessment on up-to-date exposure data, the evaluation from BIA, 1999 and 1997 are taken. Measurements were performed in different branches. The 95% value of 0.1 mg/m^3 (0.025 ml/m^3) is in agreement with the highest measurement result given in BIA (1997). Additional information on the workplaces and the exposure relevant activities are not available.

Table 4.4 Aniline exposures at workplaces belonging to different industries

Work area / activities	Year of the measurements	Number of measurements	Measurement range [mg/m^3]	Geometric mean [mg/m^3]	95% value [mg/m^3]	Country reference
8-hour shift average						
Chemical industry, production and processing of plastics, plastic foams and rubber	1981-1993	15	< 0.01- < 0.6	-	0.14 (90% value)	D BIA (1994)
Branches outside the chemical industry, e.g. electrical engineering, iron foundries	1981-1993	39	0.05 - < 1	0.05	-	D BIA (1994)
Cable production, welding, use of granules of luminous material, soldering, dismantling, gluing of wood, use of lacquers, plastic processing, foundries, screwing using cooling lubricants, sewerage sanitation	-	40	< 0.1			D BIA (1997)
Different branches e.g. production of plastics, use of adhesives, disposal	1990-1995	28	-	-	90%: 0.06 95%: 0.1	D BIA (1999)

Finland has provided workplace exposures from the years 1986–1992. Exposure during surface treatment was 0.004 mg/m^3 (arithmetric mean) with 2 workers exposed and during foam insulation 0.003 mg/m^3 (arithmetric mean) with 7 workers exposed. A higher exposure level of 0.48 mg/m^3 (arithmetric mean) was given for a not specified job (2 workers exposed, no information relating to job/activity). Further information is not available. Because it is not known, if these values represent 8-hour shift averages, these values are not taken as a basis for exposure assessment.

Based on the presented data, 0.1 mg/m^3 (0.025 ml/m^3, highest measurement results of a measurement collective and 95th percentile of another collective) is regarded to be an appropriate measure for a reasonable worst case.

Conclusion of inhalation exposure

For other branches (e.g. plastics processing, electrical engineering) in which aniline may be released as a decomposition product, 0.1 mg/m^3 (0.025 ml/m^3, 95% value of one measurement collective, highest measurement result of another measurement collective, 8-hour TWA) should be taken for assessing the risks of daily inhalation exposure (Scenario 7, cf. **Table 4.5**).

Dermal exposure (Scenario 5–7)

Because aniline is released during thermal processes, normally no immediate skin contact occurs. According to a rough estimation of dermal exposure caused by touching aniline contaminated surfaces (indirect exposure), the exposure level in foundries and in plastics and rubber processing is regarded as being low (here: < 1 mg/person/day, rough estimation, based on the air concentration of aniline. This estimation is valid for Scenarios 5–7, cf**. Table 4.6**).

4.1.1.2.3 Use of products with residual aniline

Apart from the release of aniline as a result of thermal decomposition, the substance can also be released from products possessing residual aniline contents, e.g. dyes and adhesives.

Scenario 8: Use of dyes

Dyes are used in closed or open systems. Whereas only a few dyes are produced directly from aniline (e. g. nigrosines), it is an important starting product for the synthesis of precursors for the production of dyes. Only one measurement result from Finland (0.1 mg/m^3, 0.025 ml/m^3) is available in context of the handling of dyes. The level of the residual aniline contents in products is unclear. Only one company has provided confidental information about dyes with residual content of aniline being purchased mainly as solutions. Additionally UK (HSE, 1997) describes the use of aniline for the production of azo-dyes. Azo-dyes are applied in the textile industry but also for dyeing of oil or paper. Within the framework of the risk assessment of aniline Spain provided the information that dyes containing aniline are produced in Spain and are used as consumer products for the purpose of dyeing shoes. The content of aniline has recently been reduced from 9% to 2%. Since it cannot be excluded that similar dyes are used at the workplace, too, the following exposure assessment is made for powdery dyes or liquid dying fromulations containing 2% aniline.

Based on general experience within the framework of the notification of new substances, it is known, that dyes are often placed on the market in a low-dust form or as aqueous formulations.

Generally, powdery dyes can be used to prepare liquid or dispersive dyeing formulations, so that exposure may occur during preparatory activities like dosing and filling. In order to assess the exposure for a worst-case scenario, it is assumed, that the content of residual aniline in dyes amounts to 2% and that the duration and frequency of exposure is over the entire shift and daily. Since no further measurement data are available, an estimation of the inhalation and dermal exposure is undertaken applying the EASE model.

Companies belonging to the textile industry, where many dyes are applied, often provide protective gloves to the worker. Within the framework of research projects (Gmehling et al., 1991; Voullaire, 1995) and company inspections (with the Landesamt für Arbeitsschutz in Nordrhein-Westfalen, 1996-1997), it appeared that workers often do not use the provided gloves. In the companies, workers with coloured hands were observed in the area of filling and dosing of dyes. Often, cleaning of hands was not performed immediately after exposure because the works had to be done under pressure of time.

Inhalation exposure

Workplace measurements

Workplace measurements are not available.

Within a research project of the Federal Institute for Occupational Safety and Health, a model was developed for the prediction of inhalation exposure during activities like applying dyes or adhesives at ambient temperatures without the formation of aerosols (Weidlich, Gmehling, 1986). In this approach specific standard scenarios were developed. One standard scenario is: continuous release of the substance, evaporation surface: 200 cm^2, evaporation time: 100 min, room size: 100 m^3 and air ventilation rate: 1 h^{-1}. This standard scenario is applicable for preparations of dyes and small-scale applications.

For comparison with model estimates measurements were taken at 4 workplaces: gluing of shoes (6 substances), preparation of a glue (3 substances) cleaning of glue drums (10 substances) and cleaning of circuits boards (3 substances). Single measurement results were taken, or, if appropriate, shift averages were calculated based on measurements with shortened measurement duration.

A comparison of model estimates and measurement results revealed good correspondence. It turned out, that the exposure levels were, as a rule, more than 100 times below the saturation concentrations of the substances under consideration. This relation was also checked by model estimates for other substances/workplaces.

EASE estimation

EASE for windows, Version 2, 1995

For liquid dyes, the estimation is performed on the basis of a dye for which aniline contents of 2% are assumed. The partial vapour pressure of aniline in the dye is estimated to be approx. < 0.2 Pa (calculation based on Raoult's Law regardless of the intermolecular interactions). Because of the resulting low partial vapour pressure (< 1 Pa), the exposure level estimated by the EASE model is independent of the pattern of use and the pattern of control.

Input parameters: T = 20°C, non dispersive use/wide dispersive use,
direct handling, dilution ventilation present/absent

Level of exposure: 0-0.4 mg/m^3 (0-0.1 ml/m³)

Use of powdery dyes in a low-dust form with an aniline content of 2% (filling, dosing) with LEV present

Input parameters: T = 20°C, low-dust technique, LEV present
Level of exposure: 0-1 mg/m^3

Considering a content of 2% in the dye, the exposure level amounts to
0-0.02 mg/m^3

Use of powdery dyes in a low-dust form with an aniline content of 0.5% (filling, dosing), without LEV

Input parameters: T = 20°C, low-dust technique, LEV absent
Level of exposure: 0-5 mg/m^3

Considering a content of 2% in the dye, the exposure level amounts to
0-0.1 mg/m^3

Conclusion of inhalation exposure

In the case of exposure to vapours (here during the use of liquid dyeing solutions), exposure levels do not exceed the saturation concentration of a substance. According to a rough estimation, the saturation concentration (unit ml/m^3) can be regarded to be ten times higher than the partial vapour pressure of the substance (unit Pa). On the basis of the partial vapour pressure of 0.2 Pa a saturation concentration of aniline approx. of 2 ml/m^3 will result. Based on the general finding that exposure levels are considerably below the saturation concentration of substances (Weidlich and Gmehling, 1986), the EASE estimate is regarded to overestimate inhalation exposure. A more realistic exposure level is a factor 100 beyond the saturation concentration of 2 ml/m^3. The resulting exposure level of ≤ 0.08 mg/m^3 (≤ 0.02 ml/m^3) should be taken for the risk assessment for the use of dyeing solutions (expert judgement, Scenario 8a, cf. **Table 4.5**).

As a worst-case estimation for the handling (filling, dosing; shift length, daily) of dyes in low-dust forms with 2% residual aniline, the estimation of exposure in application of the EASE model results in 0-0.02 mg/m^3 when LEV is present and 0-0.1 mg/m^3 without LEV (Scenario 8b and 8c, cf. **Table 4.5**).

Dermal exposure

EASE for windows, Version 2, 1995

Based on the above described observation regarding the use of dyes, for exposure estimation in application of the EASE model, the contact level “extensive” is chosen.

Use of dyes with residual aniline contents in the textile industry, immediate contact with dyes

Input parameters T = 20°C, non-dispersive use, direct handling, extensive
Level of exposure: 1-5 mg/cm^2/day

Considering a content of 2% aniline, the exposure level amounts to
0.02-0.1 mg/cm^2/day

Conclusion of dermal exposure

Immediate dermal contact during the unprotected handling of powdery dyes or liquid dye formulations, e.g. in the textile industry, which contain residual aniline may lead to dermal exposure. For the purpose of estimation, daily extensive skin contact during dyeing works is assumed. Taking into consideration a concentration of 2%, the estimation using the EASE model results in an exposure of 0.02-0.1 mg/cm^2 per day. Considering an exposed area of 840 cm^2, the exposure amounts to 17-84 mg/person/day (Scenario 8a, cf. **Table 4.6**).

The EASE model was developed on the basis of liquids and possibly overestimates exposure to low-dust powders, because liquids wet the skin faster than powders and probably lead to more intensive dermal contacts. Therefore it seems to be reasonable to choose the lower value of the estimated exposure range of 17 mg/person/day for further risk characterisation for dermal contacts with low-dust dyes (Scenario 8b, cf. **Table 4.6**). However, it has to be kept in mind, that this exposure estimation is based on assumptions and expert judgement. Since the knowledge is limited, the uncertainty of this exposure assessment might be higher than for other scenarios described in this exposure assessment.

Scenario 9: Use of adhesives

2-Package polymerisation adhesives, which are used to glue glas and metal, contain as an activator a butyraldehyde-aniline condensation product and additionally small amounts of free aniline (0.3%). In the field of engineering, device and tool construction industries, adhesives are used to bond metals during assembly. Automatic or semi-automatic bonding machines are employed within continuous production processes (production lines). Inhalation and dermal exposures are possible during charging and bonding work (semi-automatic machines), during cleaning, maintenance and repair work. It cannot be excluded that gloves are not worn.

In the skilled trade sector, 2-package polymerisation adhesives containing aniline are used for repairing metal workpieces and for glueing metal and glass. It may be assumed that exhaust ventilation systems are absent, and that suitable personal protective equipment is not worn.

Inhalation exposure

Workplace measurements

Workplace measurements are not available.

Within a research project of the Federal Institute for Occupational Safety and Health, a model was developed for the prediction of inhalation exposure during activities like applying dyes or adhesives at ambient temperatures without the formation of aerosols (Weidlich and Gmehling, 1986). In this approach specific standard scenarios were developed. One standard scenario is: continuous release of the substance, evaporation surface: 200 cm^2, evaporation time: 100 min, room size: 100 m^3 and air ventilation rate: 1 h^{-1}. This standard scenario is applicable for preparations of dyes and small-scale applications.

For comparison with model estimates measurements were taken at 4 workplaces: gluing of shoes (6 substances), preparation of a glue (3 substances) cleaning of glue drums (10 substances) and cleaning of circuits boards (3 substances). Single measurement results were taken, or, if appropriate, shift averages were calculated based on measurements with shortened measurement duration.

A comparison of model estimates and measurement results revealed good correspondence. It turned out, that the exposure levels were, as a rule, more than 100 times below the saturation concentrations of the substances under consideration. This was also checked by model estimates for other substances/workplaces.

EASE estimation

EASE for windows, Version 2, 1995

Use, outside the chemical industry, of products manufactured from aniline, e. g. adhesives.

The estimation is performed on the basis of an adhesive for which aniline contents of 0.3% aniline are assumed. The partial vapour pressure of aniline in the adhesive is estimated to be approx. < 0.2 Pa (calculation based on Raoult's Law regardless of the intermolecular interactions). Because of the resulting low partial vapour pressure (< 1 Pa), the exposure level estimated by the EASE model is independent of the pattern of use and the pattern of control.

Input parameters:	T = 20°C, non dispersive use/wide dispersive use, direct handling, dilution ventilation present/absent
Level of exposure:	0-0.4 mg/m³ (0-0.1 ml/m³)

Conclusion of inhalation exposure

In the case of exposure to vapours, exposure levels do not exceed the saturation concentration of a substance. According to a rough estimation, the saturation concentration (unit ml/m^3) can be regarded to be ten times higher than the partial vapour pressure of the substance (unit Pa). On the basis of the partial vapour pressure of 0.2 Pa a saturation concentration of aniline approx. of 2 ml/m^3 will result. Based on the general finding that exposure levels are considerably below the saturation concentration of substances (Weidlich, Gmehling, 1986), the EASE estimates are regarded to overestimate inhalation exposure. A more realistic exposure level might be a factor 100 beyond the saturation concentration of 2 ml/m^3. The resulting exposure level of ≤ 0.08 mg/m^3 (≤ 0.02 ml/m^3) should be taken for the risk assessment for the use of adhesives (expert judgement, Scenario 9, cf. **Table 4.5**).

Dermal exposure

EASE for windows, Version 2, 1995

For the use of adhesives with residual aniline contents in the industrial area

Input parameters:	T = 20°C, non dispersive use, direct handling, intermittent
Level of exposure:	0.1-1 mg/cm^2 / day

Considering a content of 0.3% aniline in the adhesive, the exposure level amounts to
0.0003-0.003 mg/cm^2 / day

For the use of adhesives in the skilled trade area, it is to be assumed that the exposure levels are the same or even lower than in the industrial area.

Conclusion of dermal exposure

Generally workers avoid immediate skin contact with adhesives that can be removed only with difficulties. For the adhesive under consideration, a reactive 2-package adhesive, dermal

exposure with the aniline-containing component has to be assumed. At present, it is unknown in which adhesives residual aniline is found. For immediate skin contact with slowly hardening adhesives, it has to be assumed, that there is the opportunity to penetrate skin. These adhesives are removed later with the aid of skin cleaning agents which are also employed after contact with paints. The corresponding exposure level is assessed applying the EASE model.

Dermal exposure must be assumed when adhesives are used in the further processing industry. The content of aniline (0.3%) is taken into account to estimate an exposure level of 0.0003-0.003 mg/cm^2/day in application of the EASE model. Considering an exposed area of 210 cm^2 (corresponding to the surface of half of the palms), the exposure of 0.06-0.6 mg/person/day is assumed for the assessment of the risks of regular dermal exposure (Scenario 9, cf. **Table 4.6**).

4.1.1.2.4 Summary of occupational exposure

Aniline is used as a chemical intermediate which is mainly (71%) used to produce MDA, a starting product for polyurethane plastics. Minor amounts are used to produce initial dye products (5%) and rubber chemicals (15%).

For occupational exposure there are three sources of exposure:

- handling aniline during manufacturing and further processing,
- release of aniline as a decomposition product during thermal degradation of plastics,
- use of products with residual aniline (dyes, adhesives).

Relevant inhalation and dermal exposure levels are given in **Tables 4.5** and **4.6**, respectively.

For the large-scale chemical industry, it is assumed that the production and further processing of aniline is mainly performed in closed systems. Exposure occurs if the closed systems are breached for certain activities e.g. filling, (cf. **Tables 4.5** and **4.6**). For companies which did not submit any data in default of workplace measurements, inhalation exposure is assessed in application of the EASE model (**Table 4.5**).

The assessment of dermal exposure in the large-scale chemical industry is divided into two subscenarios: dermal exposure assessed for works which are performed daily, e.g. filling, transfer, cleaning and maintenance on the one side and on the other side for occasional cleaning and maintenance works e.g. during the shut down of a plant, being more intensive than the daily activities. In this subscenario, a higher skin area is considered.

Inhalation exposure to aniline may be caused by the release of aniline during thermal decomposition in the areas of foundries, during rubber vulcanisation and during thermal processes in other branches. Because aniline is released during thermal processes, dermal exposure is restricted to touching contaminated surfaces and is assessed as low (here: < 1 mg/p/day).

Based on the available information, dyes and adhesives may contain residual aniline. The corresponding exposure levels are assessed in application of the EASE model (see **Tables 4.5** and **4.6**).

Table 4.5 Summary of inhalation exposure data of aniline relevant for the occupational risk assessment

Area of exposure	Form of exposure	Activity	Duration [h/day]	Frequency [day/year]	Shift average [mg/m³]	Method	Short-term exposure [mg/m³]	Method
Production and further processing in the large-scale chemical industry								
Production, reduction of nitrobenzene 1) by means of H_2	vapour	filling transfer cleaning maintenance repair work	shift length	daily	2.5	expert judg.[1)]	-	-
2) by means of Fe		see above	shift length	daily	1.5	highest measured result	-	-
3) Production by means of H_2 or by means of Fe	see above	see above	shift length	daily	2-12	EASE	-	-
4) Further processing to various products [2)]	vapour	filling transfer , cleaning, repair-work, maintenance	shift length	daily	2.0	expert judg. [3)]	12	highest measurement, n=10
Release of aniline as a decomposition product								
5) Vulcanisation of rubber plastics and rubber processing	vapour	release during thermal processes	shift length	daily	0.8	expert judg.[1)]	-	-
6) Iron, steel and aluminium foundries	vapour	release during casting	shift length	daily	6.4	data from literature	-	-
7) Different branches (e.g. plastics processing, electrical engineering)	vapour	release during thermal processes	shift length	daily	0.1	95th percentile	-	-
Use of products with residual aniline								
8) Use of dyes with residual aniline (2%), used e.g. in the textile industry								
8a) Liquid dyeing solutions	vapour	dyeing work	shift length	daily	0-0.08	expert judg.[4)]	-	-
8b) Powdery dyes	dust	filling, dosing	shift length	daily	0-0.02	EASE (with LEV)	-	-

Table 4.5 continued overleaf

Table 4.5 continued Summary of inhalation exposure data of aniline relevant for the occupational risk assessment

Area of exposure	Form of exposure	Activity	Duration [h/day]	Frequency [day/year]	Shift average [mg/m³]	Method	Short-term exposure [mg/m³]	Method
8c) Powdery dyes	dust	filling, dosing	shift length	daily	0-0.1	EASE (without LEV)	-	-
9) Use of adhesives (0.3%) engineering, device and tool construction industries	vapour	charging bonding cleaning , repair work, maintenance	shift length	daily	0-0.08	expert judg.[4)]	-	-

1) Reasonable worst case derived from available data
2) The scenario includes exposure to aniline during the production of polymers on the basis 2,2,4-trimethyl-1,2-dihydrochinoline
3) Worst case, derived from given data
4) Estimated by comparison with the saturation vapour pressure

Table 4.6 Summary of dermal exposure data of aniline relevant for the occupational risk assessment

Area of exposure	Form of exposure	Activity	Frequency [day/year]	Contact level (accord. to EASE model)	Level of exposure [mg/cm²/day]	Exposed area [cm²]	Shift average [mg/p/day]	Method, (use of gloves)
Production and further processing in the large-scale chemical industry								
1a–4a) [1)] Production and further processing [2)]	liquid	filling, transfer cleaning, repair, maintenance [(2)]	daily	--	low	-	low	exp. judg.(suitable gloves)
1b–4b) [1)] Production and further processing [2)]	liquid	see above	daily	intermittent	0.1–1	420 (palms of hands)	42-420	EASE [3)] (unsuitable gloves)
Release of aniline as a decomposition product								
5) Vulcanisation of rubber plastics and rubber processing	re-condensation	release during thermal processes	daily	-	low	-	low	exp. judg.[4)]
6) Iron, steel and aluminium foundires	re-condensation	release during casting	daily	-	low	-	low	exp. judg. [4)]
7) Different branches, (e.g. plastics processing, electrical engineering)	re-condensation	release during thermal processes	daily	-	low	-	low	exp. judg. [4)]

Table 4.6 continued overleaf

Table 4.6 continued Summary of dermal exposure data of aniline relevant for the occupational risk assessment

Area of exposure	Form of exposure	Activity	Frequency [day/year]	Contact level (accord. to EASE model)	Level of exposure [mg/cm²/day]	Exposed area [cm²]	Shift average [mg/p/day]	Method, (use of gloves)
Use of products with residual aniline								
8) Use of dyes with residual aniline (2%), used e.g. in the textile industry								
8a) Liquid dyeing solutions	liquid	dyeing work	daily	extensive	0.02-0.1	840 (hands)	17-84	EASE (without gloves)
8b,c) Powdery dyes	powder	filling, dosing	daily	extensive	0.02-0.1	840 (hands)	17	EASE [5] (without gloves)
9) Use of adhesives (0.3%), engineering, device and tool construction industries	liquid	charging bonding cleaning maintenance repair work	daily	intermittent	0.0003-0.003	210 (fingers)	0.06-0.6	EASE (without gloves)

1) Separation of cleaning and maintenance performed daily (included in the scenario production) and cleaning and maintenance performed only occasional, e.g. during shut down of a plant, for the latter case: EASE: 0.1–1 mg/cm²/day, exposed skin area: 1,300 cm², shift average: 130–1,300 mg/p/day

2) The scenario 4a,b (further processing) includes exposure to aniline during the production of polymers on the basis 2,2,4-trimethyl-1,2-dihydrochinoline

3) Worst case estimation for the unprotected worker without gloves

4) Rough estimation; aniline is released during heating, secondary contact with contaminated surfaces (< 1 mg/p/d)

5) Expert judgement; the upper value of the EASE calculation is assumed to lead to an overestimation (see text)

4.1.1.3 Consumer exposure

Aniline may occur in rubber articles in small amounts in the rubber matrix. As a result of migration and leaching, consumer exposure to aniline in low concentrations is conceivable but not quantifiable.

It is not known whether aniline is used as a component in consumer products.

Comment

There is information from Spain that aniline (concentration < 9%) is a component in a formulation for dyeing shoes (National Network of Vigilance, Control and Santion of Chemical Products 1999; 2000). At present, the Spanish authorities are proving the data. Because severe health hazards have been attributed to exposure with aniline from shoe dyeing, a worst-case assessment has been made to estimate a possible risk from those uses. It should be mentioned that no measured data are available, neither from measurements in humans nor migration rates from using shoe dyes. It is also assumed that the shoes are dyed outside. The recent information that is available is that the amount of aniline in dyes for shoes is 1-2%.

Two different kinds of exposure have to be considered: a) consumers dying shoes themselves and b) consumers wearing dyed shoes.

Scenario a) can be assumed to be a single event of short duration. Hence, exposure will be minimal. Scenario b) considers the exposure from wearing dyed shoes. The variables used for this worst-case estimate have to be understood as defaults.

Area of surface of dyed shoe	Area	3,190 430	cm² cm² (children)	= surface of lower legs + feet (TGD) = only feet (AUH Report [1)]
Thickness of layer of dye	T_{Lay}	0.01	cm	default (estimated)
Amount of aniline in the layer	Perc	2	%	measured
Amount of aniline on shoe (density: 1)	A_{shoe}	0.638 0.086	g (adults) g (children)	$= A_{rea} \cdot T_{Lay} \cdot P_{erc}$
Portion of aniline migrating through the leather to inside of shoe per day	Mig	0.001	%	default (estimated)
Amount of aniline migrating per day through the leather	A_{mig}	$6.38 \cdot 10^{-6}$ $0.86 \cdot 10^{-6}$	g/d (adult)g/d children)	$= A_{rea} \cdot T_{Lay} \cdot P_{erc} \cdot M_{ig}$
Maximum absorption rate through human skin	R_{abs}	3.0	mg/cm²/h	(compare Section 4.1.2.1)
Amount of aniline on skin	A_{skin}	$2.0 \cdot 10^{-6}$	mg/cm²	$= A_{mig}/A_{rea}$

Because the maximum absorption rate of 3 mg/cm²/h, the very small amount of $2.0 \cdot 10^{-6}$ mg/cm² of aniline on skin would be completely absorbed ($A_{mig} = A_{abs}$).

Amount absorbed	A_{abs}	$6.38 \cdot 10^{-6}$ $0.86 \cdot 10^{-6}$	g/d g/d	$= A_{mig}$
Body weight (female)	BW	60	kg	TGD
Body weight (child)		20	kg	
Internal exposure		$1.0 \cdot 10^{-7}$ $4.3 \cdot 10^{-8}$	g/kg/d (adult) g/kg/d (children)	$= A_{abs}BW$
Internal exposure per year		$3.65 \cdot 10^{-5}$ $1.57 \cdot 10^{-5}$	g/kg/year (adult) g/kg/year (children)	$= A_{abs}\,BW \cdot 365$

[1)] Arbeitsgemeinschaft der Leitenden Medizinalbeamtinnen und-beamten der Länder, Bericht des Ausschusses für Umwelthygiene (1995). Standards zur Expositionsabschätzung. Hrsg.: Behörde für Arbeit, Gesundheit und Soziales, Hamburg

It has to be mentioned that one of the most important factors influencing the extent of exposure the migration through the leather of shoes is not known. This lack of knowledge could only be filled out by measurements of aniline migrating through shoes.

It is not known whether this kind of dyes is used for other purposes nor its usage in other European countries other than Spain.

4.1.1.4 Humans exposed via the environment

Industrial emission sources

As a worst-case scenario, the maximum intake due to exposure in the vicinity of the greatest point source which emits into a river (site G; cf. **Table 3.35**) is calculated. This is compared to an average intake due to exposure via the regional background concentration. The calculation according to the TGD model is given in Appendix C:

Table 4.7 Indirect exposure by industrial emission sources

	Local	Regional
PEC_{water} [µg/l]	280	0.13
$PEC_{air,ann}$ [µg/m³]	3,900	$2.2 \cdot 10^{-4}$
$DOSE_{drw}$ [$mg_{chem} \cdot kg_{bw}^{-1} \cdot d^{-1}$]	$8 \cdot 10^{-3}$	$3.7 \cdot 10^{-6}$
$DOSE_{fish}$ [$mg_{chem} \cdot kg_{bw}^{-1} \cdot d^{-1}$]	$1.2 \cdot 10^{-3}$	$5.5 \cdot 10^{-7}$

Table 4.7 continued overleaf

Table 4.7 continued Indirect exposure by industrial emission sources

	Local	Regional
$DOSE_{stem}$ [$mg_{chem} \cdot kg_{bw}^{-1} \cdot d^{-1}$]	0.47	$8.9 \cdot 10^{-8}$
$DOSE_{root}$ [$mg_{chem} \cdot kg_{bw}^{-1} \cdot d^{-1}$]	0	$9.4 \cdot 10^{-9}$
$DOSE_{meat}$ [$mg_{chem} \cdot kg_{bw}^{-1} \cdot d^{-1}$]	$6.9 \cdot 10^{-6}$	$2.6 \cdot 10^{-11}$
$DOSE_{milk}$ [$mg_{chem} \cdot kg_{bw}^{-1} \cdot d^{-1}$]	$1.3 \cdot 10^{-4}$	$4.8 \cdot 10^{-10}$
$DOSE_{air}$ [$mg_{chem} \cdot kg_{bw}^{-1} \cdot d^{-1}$]	0.26	$4.8 \cdot 10^{-8}$
$DOSE_{tot}$ [$mg_{chem} \cdot kg_{bw}^{-1} \cdot d^{-1}$]	0.74	$4.4 \cdot 10^{-6}$

The main contribution to the intake in the case of the local exposure are the $DOSE_{stem}$ and the $DOSE_{air}$ with fractions of 64% and 35%, respectively, of the total daily dose. This is caused by the high releases into the air at the main source.

On the regional scale, a relevant exposure is expected only for the hydrosphere. In this case, the fraction of the $DOSE_{drw}$ is 84% and of $DOSE_{fish}$ is 13%.

However, some investigations concerning the exposure via drinking water have to be considered additionally:

Aniline was measured in a drinking-water-work producing from bank filtrate. While the average river water concentration was about 2.3 µg/l, in the bank filtrate 1.4 µg/l were detected. The elimination rate during bank filtration was estimated to be 20% with an average storage time of 20 days; the proportion of river water within the raw drinking water was about 80%. The raw water was subsequently treated with ozone and three-stage filtration over activated carbon, after this aniline was not detected in the purified water with a detection limit of 0.1 µg/l (Kussmaul, 1978).

In the Netherlands, in drinking water produced from Rhine water (1988-1991) no aniline was detected with a detection limit of 0.1 µg/l (RIWA, 1994).

These investigations reveal that aniline is removed in the waterworks. Thus, the total daily intake calculated above is reduced to $0.7 \cdot 10^{-6}$ $mg_{chem} \cdot kg_{bw}^{-1} \cdot d^{-1}$ for the regional scenario.

Intake from plant protecting agents

In agricultural soils, aniline is released from plant protecting agents. The substance is partially degraded and partially bound on soil organics. Figge et al. (1983) observed that aniline and its metabolites is taken up by plants (cf. Section 3.1.2.2).

There are no data available about the uptake of the bound aniline by plants. Therefore, the indirect exposure caused by the plant protecting agents cannot be modelled. However, there are some measured data available:

Table 4.8 Measured aniline concentration in food

Food	Concentration	Reference
Tea	qualitative	Vitzthum et al. (1975)
Fruit, vegetables	0.6 - 30.9 mg/kg	Neurath et al. (1977)
Garlic	qualitative	Yu and Wu (1989)

Assuming a mean concentration of 15 mg/kg in plants and a consumption of 0.5 kg/d, a daily dose of 0.11 $mg_{chem} \cdot kg_{bw}^{-1} \cdot d^{-1}$ is calculated.

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

4.1.2.1 Toxicokinetics, metabolism and distribution

4.1.2.1.1 Oral exposure

Studies in animals

After single oral dosages of ^{14}C aniline in rabbits (160 to 500 mg/kg bw) during 3-8 days 60-90% of the radioactivity is eliminated from the body in the urine, 0.7-1.5% in the faeces, less than 0.2% in the expired air and 3-7% remains in the body (Parke, 1960).

Following oral administration of ^{14}C aniline (50 mg/kg) to rats, sheep and pigs, more than half the dose was excreted by each animal in 24 h. In the rat, 96% of the administered dose was recovered in the urine within 24 h, whereas in the sheep the recovery was in the range of 80%. In pigs 56% of the dose was eliminated in urine during the first 24 h. Fecal excretion of aniline in all three species was 2% of the dose (Kao et al., 1978).

Bus et al. (1978) observed the peak plasma radioactivity at 0.5, 1.0 and 2.0 h after a single oral administration of 10, 30, and 100 mg/kg ^{14}C aniline HCl in rats. By 24 h after dosing, the radioactivity in plasma decreased to less than 2% of the peak concentration for all doses. ^{14}C radioactivity was found in all examined tissues, with the highest peak levels observed in kidney, followed by liver, plasma, lung, heart, spleen and brain for all doses. By 48 h after dosing, less than 0.1% of the administered radioactivity of any of the three doses remained in any of the tissues examined. Recovery of administered radioactivity excreted in the urine 48 h after dosing was 96, 91 and 77% for the 10, 30, and 100 mg/kg doses, respectively.

Male Fischer-344-rats were given 100 mg/kg/d ^{14}C aniline HCl orally for 1 or 10 days and killed 24 h after the last dose (Bus and Sun, 1979). In rats treated for 1 day, radioactivity (in µg-equiv. aniline HCl/ml or g wet weight) was 25.2 in erythrocytes and 0.4-4.0 in plasma, spleen, kidney, liver, lung, heart, brain and fat. After 10 days treatment, spleenic radioactivity concentration was 12.3 times greater than in 1 day treated rats, while other tissues concentrations were increased only 1.8-3.8 times. Covalent binding of radioactivity was minimal in spleen and liver after 1 day treatment. After 10 days treatment covalent binding in spleen was significantly greater than in liver (265 vs. 46 ng-equiv./mg protein).

Rats and mice were predosed with unlabeled aniline (50 and 100 mg/kg, respectively) by gavage for 7 consecutive days (McCarthy et al., 1985). On the eighth day, rats were dosed by gavage with ^{14}C aniline at either 50 or 250 mg/kg; mice were dosed p.o. with ^{14}C aniline at either 100 or 500 mg/kg. In 24 h, rats and mice excreted 89 and 72%, respectively, of the dose in the urine. Out of 11 tissues examined, the highest levels of binding of ^{14}C radioactivity to DNA were in the kidney, large intestine, and spleen of high-dose rats.

Male F-344 rats were gavaged daily with 100 mg/kg ^{14}C aniline HCl for 1 or 10 days (Sun and Bus, 1980). Covalently bound radioactivity (in pmol/ml or g wet weight) 2 h and 6 days after rats were given one dose was 160 and 4 in red blood cells (RBCs), 5 and 1 in spleen, and 15 and 1.5 in liver. In rats dosed for 10 days, covalently bound radioactivity was 360 and 170, 85 and 44, and 28 and 5 in RBC, spleen and liver, respectively. Thus, repeated treatment with aniline HCl leads to the accumulation of covalently bound radioactivity in RBC and spleen.

After *in vivo* administration of one or three doses (1 mmol/kg) ^{14}C aniline to rats, maximal binding to blood components occurred in RBCs. Plasma contained only 40% and 16% radioactivity as compared to RBCs after 1 and 3 doses, respectively. Liver did not show any appreciable increase in the radioactivity at three doses (Khan et al., 1995).

Human experience

No information is available.

4.1.2.1.2 Inhalation exposure

Studies in animals

Rats were exposed to 100 ppm aniline for either 8 or 12 h for 1 day (Kim and Carlson, 1986). The Met-Hb level reached a steady state by 8 h. The half-life of Met-Hb following the exposure was estimated to be 75 min for both the 8- and the 12-h exposure groups. The aniline concentration in blood or fat did not increase when the exposure period was extended to 12 h.

Comparison between oral and inhalation routes in animals

Four adult Beagle dogs were exposed nose-only to an aniline vapour concentration of 174 mg/m^3 air for four hours. The calculated exposure total dose was 14.6 mg/kg. This exposure dose was tolerated without specific clinical signs by 3 dogs. One dog experienced signs of stress. Met-Hb levels were maximal at the end of the 4-hour exposure period and were in the range of 5%.

Administration of equal doses of aniline by gavage (15 mg/kg; vehicle: saline; four adult Beagle dogs) resulted in Met-Hb levels that were maximal approximately 3 hours after administration and were in the range of 25-30%. All dogs displayed a cyanotic discoloration of the visible mucous membranes of muzzle (Bayer AG, 2000).

Human experience

In 7 out of 14 workers exposed to concentrations below 8 mg aniline/m^3 in an aniline factory measurements at the end of the work-day revealed a mean Met-Hb content in blood of 0.9%. The MetHb content in the normal population is lower than 1% due to reduction by NADH dependent reductase (Rapoport, 1983). In urine 0.34 mg acetanilide/g creatinine and aniline released from haemoglobin conjugates < 10 μg/l blood were found. In the other 7 workers the following

figures were determined: Met-Hb content of 1.4%, 27 μg acetanilide/g creatinine, and released aniline of 123 μg/l blood (Lewalter and Korallus, 1985, see **Table 4.9**). Genetically caused 50% of the Europeans reveal a lower activity of N-acetyltransferase; these are called "slow acetylators" in contrast to "fast acetylators".

4.1.2.1.3 Dermal exposure

Studies in animals

No information is available.

Human experience

An investigation of liquid aniline absorption through the skin and urine excretion of the metabolite 4-aminophenol in man was carried out (Piotrowski, 1957). 10 mg/cm^2 (25 cm^2 gauze) of freshly destillated aniline was spread on the forearm of 11 volunteers. The gauze was isolated from the outside. The absorption period was 5 h. To investigate the aniline excretion in exhaled air two connected laboratory bubblers were used (absorption efficiency was about 95%). Possible excretion on this way does not exceed 0.5%. The absorption velocity from the layers of the gauze varied from 0.18 to 0.72 mg/cm^2/h at skin temperatures from 29.8 to 35°C. When the gauze was moistened, the absorption velocity increased rapidly (3.8 mg/cm^2/h, figure from only one experiment with an exposure time of 1 h). Statistical examination was made in order to estimate the amount of aniline absorbed on the basis of 4-aminophenol excretion in the urine. Using the linear curve regression, the amount of the aniline absorbed can be determined with an exactness of ± 35%.

Experiments in human volunteers revealed a similar intake of airborne aniline (5-30 mg/m^3) via respiratory tract (2-11 mg/h) and skin (3-11 mg/h) (Dutkiewicz, 1961). A pulmonary retention of more than 90% was observed. However, at lower concentrations of aniline (< 10 mg/m^3) a higher absorption via the skin was calculated. Furthermore increased intake of aniline was observed in enhancing moisture and temperature of air (Dutkiewicz, 1961; Dutkiewicz and Piotrowski, 1961).

The dermal absorption of aniline, aniline with 3% water and aqueous aniline (1 or 2%) was investigated in 10 healthy male and female volunteers (who had no professional contact with aniline) over 30 or 60 min. The exposed area of the hands (347-459 cm^2 immersed in aqueous solutions) or the forearm (liquid aniline and aniline with 3% water: 0.25 ml aniline was introduced under the watch-glass on a limited area calculated to be 26.3 cm^2) was geometrically measured and calculated for each person separately. In all experiments the absorbed amount of aniline was calculated on the basis of the amount of 4-aminophenol excreted in the urine during 24 h from the beginning of the exposure. The maximum excretion rate of 4-aminophenol in the majority of experiments was between 4 and 6 h from the beginning of the exposure. The ratio between the amount of aniline absorbed and the amount of 4-aminophenol excreted was calculated by the method of Piotrowski (1957). The absorption rates were reported with 2.5 and 3.0 mg/cm^2/h for aniline with 3% water and liquid aniline, resp., determined with an exposure time of 30 min., whereas absorption from aqueous solutions occurred with a lower rate (0.2-1.2 mg/cm^2/h, exposure time 30 or 60 min.) (Baranowska-Dutkiewicz, 1982).

Summarising the results of these studies with different experimental design and conditions with respect to exposure time, temperature, and moisture a dermal absorption of up to 38% can be estimated.

Table 4.9 Biological monitoring on workers being exposed to aniline under workplace conditions (Lewalter and Korallus, 1985)

Group	Met-Hb*	Compounds in urine				Aniline from Hb-adduct
		p-amino-phenol	p-acet-aminophenol	aniline	acet-anilide	
	[%]		[mg/g creatine]			[µg/l blood]
Fast acetylators**	0.9	3.6	3.4	0.38	0.34	10
Slow acetylators**	1.4	3.9	1.2	0.40	0.03	123

* Hb: haemoglobin
** n = 7

4.1.2.1.4 Other routes of administration

Following a single i.v. injection of 3, 30 or 100 mg/kg ^{14}C aniline in male Fischer 344 rats, the highest initial concentrations of radioactivity were found in blood, liver, kidney, bladder and gastro-intestinal tract. After 0.5 and 6 h the highest concentrations were found in stomach and small intestine. At the 100 mg/kg dose, the spleen was the only organ that did not show a time-dependent decrease in radioactivity over 24 h. The authors conclude from the i.v. data on the existence of an enterogastric cycle for aniline and its metabolite acetanilide (Irons et al., 1980).

Aniline is able to pass the placental barrier. This was demonstrated in a study with pregnant Sprague-Dawley rats (gestational days (g.d.) 10-12) which had been treated with a single dose of 1.3 mg ^{3}H aniline/kg bw subcutaneously (Maickel and Snodgrass, 1973). At 1, 2, and 4 hours after application fetal ^{3}H plasma concentrations were slightly higher (10 to 15%) than maternal plasma concentrations. A similar plasma half-life of 1.5 h was reported for fetal as well as for maternal plasma. Aniline was also rapidly recovered in fetal brain and heart at clearly lower than maternal concentrations, and in liver tissues at half of the maternal concentrations.

4.1.2.1.5 Metabolic transformation

Studies in animals

In laboratory animals aniline is metabolised by following principle metabolic pathways: N-acetylation, aromatic hydroxylation, N-hydroxylation and conjugation (glucuronides and sulfates) or combinations of these reactions (shown in Figure 1, see Appendix E).

With the rabbit, an average of 70% of the aniline (single oral dosage of 160 to 500 mg/kg bw) is eliminated in the urine in 3 days as 4-aminophenol. At an oral dose of aniline of 200 mg/kg rabbits yield 9% of 4-aminophenyl glucuronide and 17% of 4-acetamidophenyl glucuronide; at a dose of 500 mg/kg the corresponding figures are 22% and 25%, respectively, 2-aminophenol (9%), 3-aminophenol (0.1%), phenylsulphamic acid (5.5%), aniline-N-glucuronide (3.5%) and acetanilide (0.2%). The isomeric dihydroxyanilines and the free 2- and 4- acetamidophenols do not occur in the urine. With the dog (single oral dosage of aniline of 175 and 200 mg/kg,

respectively) about 20% of the aniline is eliminated via the urine in 16 h and about 50% in 2 days. The principal metabolites are 2-aminophenol (25%), 4-aminophenol (11%) and conjugates of aniline (5%). In both rabbit and dog there was no evidence for a N-methylation or desamination from the metabolites in the urine. The ratio of 4- to 2-aminophenol excreted in the urine of various animal species dosed with aniline was: gerbil, 15; guinea pig, 11; golden hamster, 10; rabbit, 6; rat, 6 (male) and 2.5 (female); chicken, 4; mouse, 3; ferret, 1; dog, 0.5; cat, 0.4 (Parke, 1960).

The *in vivo* metabolism of orally administered aniline (50 mg/kg) was investigated in the sheep, the pig, and the rat. N-acetylated derivates were identified as the major 24-h metabolites of aniline, representing 82%, 85%, and 76% of the urinary metabolites from the sheep, the pig, and the rat, respectively. The conjugate N-acetyl-4-aminophenyl glucuronide was the major metabolite in the sheep and in the pig (60% and 66%, respectively), whereas N-acetyl-4-aminophenyl sulfate was the major metabolite in the rat (56%). Minor urinary metabolites of aniline from these species included O-conjugates of 2- and 4- aminophenol (about 20%), acetanilide (about 3%), and N-acetyl-4-aminophenol (about 10%). N-Glucuronides and sulfates of aniline and free aniline were not detected as urinary metabolites (Kao et al., 1978).

In rats the main metabolite N-acetyl-4-aminophenol is predominantly excreted as sulfuric acid conjugate (at dosage up to 50 mg/kg bw). In higher dosages saturation is observed, leading to formation of 4-amino-phenylsulfate and N-acetyl-4-aminophenylglucuronide. Conjugation with glucuronic acid represents the main pathway in mice, sheep and pigs, however, saturation was not detected (Kao et al., 1978; McCarthy et al., 1985). By maintaining this elimination route also at higher dosages a more effective excretion of aniline and its metabolites is possible in mice compared to rats. Mice produced more 2-hydroxylated aniline derivates than rats. The ratio of 4-to 2- aminophenol was 8.1 for rats and 1.6 for mice. Predosing of rats and mice did not change the kinetic values for liver aniline 4-hydroxylase or N-hydroxylase but increased the amount of mouse liver cytochrome P-450 from 0.23 to 0.49 nmol/mg protein. Based upon the results of quantitative analyses of activity of hepatic enzymes, radioactivity associated with macro-molecules in various tissues, and metabolites of aniline eliminated in the urine, McCarthy et al. (1985) concluded that in mice aniline is metabolised (via N-acetylation) and detoxified to a greater extent than in rats, that its metabolism is not limited (in contrast to that of rats) at elevated levels of exposure, and that quantitatively fewer "reactive metabolites" are formed. Studies in Sprague-Dawley rats also indicated that the greater sensitivity of male rats to the effects of aniline may be related to quantitative differences in the metabolism of aniline (aniline hydroxylase activity, level of cytochrome-P 450, aniline induced binding spectrum in microsomes) in males and females (Pence and Schnell, 1979).

In rats no changes were found in microsomal liver enzymes after gavage of 50 and 100 mg/kg aniline during 7 days (McCarthy et al., 1985). Investigations on binding of aniline on macromolecules resulted in kidney, spleen, liver and gut being targets with binding to proteins, RNA, and to a lower, but significant extent to DNA. Macromolecular binding was lower in mice than in rats (cf. Section 4.1.2.7). Also Hb-binding index is lower in mice (2.2) than in rats (22.0) (Albrecht and Neumann, 1985; Birner and Neumann, 1988).

The N-acetylation of aniline to acetanilide (N-acetyl-aniline) is catalyzed by hepatic N-acetyl-transferase, while the aromatic hydroxylation of aniline to 2- or 4-aminophenol involves the cytochrome P-450 enzyme system (aniline hydroxylase). The metabolic pathway in which aniline is N-hydroxylated by the cytochrome P-450 enzyme system produces N-phenylhydroxylamine. It is believed that the N-acetylation pathway is an important route by

which aniline is detoxified, while N-hydroxylation is the principal route by which aniline produces toxic effects, including methaemoglobinaemia.

Eyer et al. (1980) studied aniline N-hydroxylation in the isolated perfused rat liver (haemoglobin-free). N-hydroxylation was not found in single-pass perfusions, however to a low extent in the recirculating perfusion, provided that the perfusion fluid contained red cells.

Methaemoglobin forming ability of aniline is based on formation of phenylhydroxylamine, but on 2- and 4-aminophenol metabolites, too; whereby phenylhydroxylamine reveals strongest activity. In presence of oxygen haemoglobin is oxidised to methaemoglobin and the metabolite phenylhydroxylamine to nitrosobenzene ("coupled oxidation"). Nitrosobenzene is enzymatically reduced and the cycle starts again in erythrocytes. The extent of the coupled oxidation is different in various species. By this every molecule arylhydroxylamine can produce several equivalents of methaemoglobin (Kiese, 1974). The relative potencies for methaemoglobin production *in vitro* (rat erythrocyte suspensions) after phenylhydroxylamine, 2-aminophenol, and 4-aminophenol were about 10:5:1. Compared with the *in vitro* data, the relative potencies of the aminophenols for methaemoglobinemia in rats after intraperitoneal injections are lower with respect to phenylhydroxylamine to 100:4:1, apparently as a result of rapid *in vivo* clearance of the aminophenols (Harrison Jr. and Jollow, 1987).

Using the ESR spin trapping technique, the *in vitro* and/or *in vivo* formation of 5,5-dimethyl-1-pyrroline-N-oxide (DMPO)/haemoglobin thiyl and DMPO/glutathiyl free radical adducts in RBCs and blood of male Sprague Dawley rats and humans was studied. Results indicated that aniline, phenylhydroxylamine, and nitrosobenzene are all metabolised *in vivo* to yield the same metabolite, most probably the phenylhydronitroxide radicals (produced from the reaction of phenylhydroxylamine with oxyhaemoglobin), which is responsible for the oxidation of thiols within RBCs (Maples et al., 1990).

Human experience

Genetically caused 50% of the Europeans reveal a lower activity of N-acetyltransferase; these are called "slow acetylators" in contrast to “fast acetylators”. In slow acetylation the reaction of aniline to acetanilide is retarded in favor to formation of phenylhydroxylamine, nitrosobenzene and aminophenol and hereby formation of methaemoglobin (Lewalter and Korallus, 1985, see **Table 4.9**).

4.1.2.1.6 Conclusion on toxicokinetics, metabolism and distribution

Aniline is well absorbed after oral, dermal and inhalation exposure. The extent of absorption after oral intake amounts 89-96% for rats. The corresponding figures for mouse, sheep and pig are 72%, 80% and 56%, respectively. Dermal absorption in humans was estimated to amount up to 38%. After metabolic transformation the metabolites are predominantly excreted via urine. The formation of methaemoglobin after single oral administration to dogs is one to six times higher than after inhalation exposure.

In rats treated for one day with radioactively labelled aniline the distribution of radioactivity in different tissues showed highest concentration in RBCs, followed by plasma, spleen, kidney, lung, heart, brain and fat. Repeated administration leads to accumulation of radioactivity in spleen.

The major contributors to aniline clearance appear to be a combination of acetylation and hydroxylation reactions. Acetanilide may be either deacetylated back to aniline or 4-hydroxylated to 4-hydroxyacetanilide. The glucuronide and sulfate conjugates of 4-hydroxyacetanilide represent the major urinary metabolites of aniline. The N-acetylation of aniline is catalyzed by hepatic N-acetyl-transferase, while the aromatic hydroxylation of aniline involves the cytochrome P-450 enzyme system. N-hydroxylation of aniline to N-phenylhydroxylamine (which may be further oxidised to nitrosobenzene, conjugated with glutathione, or re-reduced back to aniline) is the principal route by which aniline produces toxic effects, including methaemoglobinaemia.

4.1.2.2 Acute toxicity

4.1.2.2.1 Studies in animals

In experiments with rats and rabbits the acute toxicity of aniline is severe, independent of the route of application. Cats react much more sensitively because of severe formation of methaemoglobin in this species. Aniline is absorbed through the skin and the lungs, with formation of methaemoglobin leading to cyanosis, tremors, lacrimation and respiratory problems being the main toxic effects.

Oral exposure

In rats oral LD_{50} values of 442 mg/kg bw (Bio-Fax Industrial Bio-Test Laboratories, 1969) to 780 mg/kg bw in female and 930 mg/kg bw in male rats (Bier and Oliveira, 1980) were determined. In cats however, severe formation of methaemoglobin caused death of one of two animals after oral application of 50 and of 100 mg/kg bw.

An oral LD_{50} of 442 mg/kg body weight was determined when undiluted aniline (no data on purity) was tested in male rats at doses of 215, 316, 464, 681 and 1,000 mg/kg. Deaths occurred after oral administration of 464 mg/kg (4/5 rats died), 681 mg/kg (4/5 rats died) and 1,000 mg/kg (5/5 rats died). Clinical signs observed included tremors, fibrillation, hyperpnea, cyanosis, convulsions, hypothermia, salivation and prostration. Necropsy revealed inflammation of gastrointestinal tract in survivors, hyperemia of lungs and haemorrhage of gastrointestinal tract in descendants. No more details are reported (Bio-Fax Industrial Bio-Test Laboratories, unpublished report 1969).

In a test according to EPA guideline of 1978 oral LD_{50} values of 780 mg/kg body weight for female and of 930 mg/kg bw for male rats were calculated. Undiluted aniline (no data on purity) was tested in rats (at least 5 female and 5 male rats per dose) at doses of 500, 622.9, 775.9, 866, 966.6, 1,204.1 and 1,500 mg/kg. Deaths occurred after oral administration of 622.9 mg/kg (1/5 females and 0/5 males died), 775.9 mg/kg (4/5 females and 2/5 males died), 866 mg/kg (5/10 females and 2/10 males died), > 966.6 mg/kg (all rats died). Mortality was observed within the first 24 hours post-treatment with delayed mortality scattered over the first 7 days post-treatment. Clinical signs included cyanosis, lacrimation, tremors, tachypnea and lethargy. At necropsy, gastric haemorrhage, distention of urinary bladder and signs of irritation of the ileum were detected (Bier and Oliveira, 1980).

In a study on detection of methaemoglobin formation after oral administration, one out of two male cats died after gavage application of 102.2 mg/kg aniline p.a. (MERCK). Aniline was

orally administered to 10 cats using doses of 1.0, 2.6, 5.1, 10.2, 25.6, 51.1 and 102.2 mg/kg (as 0.05-1% aqueous emulsion in traganth). Light cyanosis was seen at day 1 after application of 2.6 mg/kg, 51.1 mg/kg caused severe cyanosis with staggering gait, atony and vocalisation, 102.2 mg/kg caused death of one out of two male cats (BASF AG, unpublished report, 1970).

In a similar test on the detection of methaemoglobin after oral application, one out of two cats died after oral administration of 51.1 mg/kg (administered as 0.5% aqueous emulsion of aniline p.a. (MERCK) in tylose). Approximately 2 hours after dosing the cats demonstrated panting and cyanosis, one animal additionally exhibited vomiting and salivation. The cat that died 4 days after administration demonstrated cyanosis over 2 days, followed by atonia, occasional vocalisation and mydriasis. Pathological examination revealed acute pneumonia probably due to gavage into the lungs. Both cats exhibited methaemoglobin formation of > 80% within 4 hours decreasing to normal percentages within 2-3 days (BASF AG, unpublished report 1971).

Formation of methaemoglobin and of Heinz bodies after oral administration of aniline (99% in polyethyleneglycol 400) was tested in a further study with cats. Doses of 10 mg/kg and of 50 mg/kg caused 60% enhancement of methaemoglobin contents and 55% enhancement of the amount of Heinz bodies when two cats were dosed orally. Methaemoglobin levels and Heinz bodies amount were assessed at 0, 3, 7, 24 and 30 hours after administration demonstrating maximum levels at 24 hours after administration that decreased after 30 hours. The only clinical sign reported after dosing with 50 mg/kg was loss of body weight (Bayer AG, unpublished report 1984).

Four Beagle dogs (2 males and 2 females; age: approx. 1 year, weight about 11-18 kg) received an oral dose of 15 mg aniline (purity > 98%) per kg body weight in isotonic saline. Methaemoglobin levels were determined before treatment, five times after treatment (from 45 minutes to 4:30 hours) and 24 hours after treatment. The highest levels were measured after 3 hours. At this time Met-Hb concentrations ranged from 19–29%. The following day concentrations were in the same range as before treatment (0.8% versus 0.6%). The visible mucous membranes appeared to be cyanotic with onset shortly after the administration of aniline. On the first post-exposure day all dogs appeared to be normal. There was no test substance induced mortality. Two weeks earlier the dogs had been subjected to a 4-hour inhalation exposure at a concentration of 0.174 mg/l of aniline. The dose of 15 mg/kg was selected for direct comparison between this dose and an inhalation concentration of 0.174 mg/l (Bayer AG, 2000).

Male rats (not fasted) were treated with a single oral dose of Aniline (redistilled) in isotonic saline. A dose of 20 mg/kg resulted in a slight increase in Met-Hb levels 3.3% versus 2.4% in control rats. Doses of 20, 40, 100 or 200 mg/kg resulted in increases from 12% to 16%. A dose of 300 mg/kg resulted in a Met-Hb level of 18%, and a dose of 1,000 mg/kg of 48%. These maximum values were usually observed within 1-4 hours of treatment. There are no data on the reversibility of Met-Hb levels. Mortality was not reported. The authors conclude that the no-effect dose after a single oral treatment of aniline is 20 mg/kg in rats (Jenkins et al., 1972).

Inhalation exposure

Inhalation LC_{50} values in rats are different depending on the kind of exposure. For head-only exposure 3.3 mg/l/4 hours and for whole-body exposure 1 mg/l/4 hours (Carpenter et al., 1949) resp. 1.9 mg/l/4 hours (Du Pont de Nemours and Co., 1982) were detected.

A LC_{50} of 839 ppm/4 hours (3.27 mg/l/4h) was determined in a study with rats according to EPA guideline of 1982 by head-only exposure of groups of 10 rats/group to aniline (no data on purity)

vapour/aerosol for a single 4-hour period at concentrations of 681, 790, 834 and 896 ppm. Aniline atmospheres were generated by passing nitrogen over the liquid contained in a 3-neck round-bottom flask heated from 80 to 100°C. The vapour/aerosol was diluted with humidified and oxygen-enriched houseline air and passed into the exposure chamber. Chamber samples were analyzed every 30 minutes. Deaths occurred at concentrations of 790 ppm (2/10 rats died), 834 ppm (5/10 rats died) and 896 ppm (8/10 rats died). Clinical signs at exposure included cyanosis, tremors and prostration up to 48 hours post-exposure, corneal clouding up to 14 days post-exposure, reddish brown nasal discharge and chromodachryorrhea. Clinical signs post exposure were pallor and rales, head and facial hair loss; after 4 hours exposure to 790 ppm mild to severe corneal damage was detected (DuPont de Nemours and Co., unpublished report 1982).

Whole-body exposure of groups of 10 rats/group to aniline vapour/aerosol for a single 4-hour period at concentrations of 359, 400, 453, 530 and 786 ppm revealed a LC_{50} of 478 ppm/4 hours (1.86 mg/l/4hours) in a study according to EPA guideline of 1982. Aniline (no data on purity) atmospheres were generated by passing nitrogen over the liquid contained in a 3-neck round-bottom flask heated from 80 to 100°C. The vapour/aerosol was diluted with humidified and oxygen-enriched houseline air and passed into the exposure chamber. Chamber samples were analysed every 30 minutes. Deaths occurred at concentrations of 400 ppm (2/10 rats died), 453 ppm (4/10 rats died), 530 ppm (7/10 rats died) and 786 ppm (10/10 rats died within 24 hours). Clinical signs at exposure were cyanosis, tremors, lacrimation and salivation up to 48 hours post-exposure, prostration and rales. Clinical signs post-exposure included pallor, head and facial hair loss, reddish-brown stained mouth, nasal and perinasal area (DuPont de Nemours and Co., unpublished report 1982).

An approximate LC_{50} value for rats of 250 ppm/4 hours (ca. 1 mg/l/4 hours) was detected after whole-body exposure of 6 rats to aniline vapour/aerosol (no data on purity) for a single 4-hour period. A concentration of 250 ppm killed 2-4/6 rats. No more data are reported (Carpenter et al., 1949).

Four Beagle dogs (2 males and 2 females, weight: approx. 11–18 kg; age: about 1 year) were exposed for 4 hours to a concentration of 0.174 mg/l aniline (nose-only exposure). The dog was chosen because this species has a breathing pattern more similar to man. The concentration was selected for a direct comparison between a 4-hour exposure of 0.174 mg/l and an oral dose of 15 mg/kg. Thus, the calculated exposure total dose was 14.6 mg/kg. A respiratory minute volume of 0.35 $l \cdot min^{-1} \cdot kg^{-1}$ was assumed. A mean body weight of 8.5 kg was used for calculation. The concentration of 0.174 mg/l is approximately 50 times higher than the current MAK value of 7.7 mg/m^3 (= 0.0077 mg/l). Based on the physico-chemical properties of aniline (a moderately soluble, lipophilic gas/vapour and inhaled as a vapour) the substance is retained within the respiratory tract and subsequently absorbed by perfusion rather than by ventilation controlled mechanisms. A coefficient of retention is in the range of 20% (this means 80% of the inhaled aniline is exhaled again). The exposure to 0.174 mg/l was tolerated without specific clinical signs by 3 dogs. One dog experienced signs of stress, including a marked hyperventilation, which subsided entirely on the first post-exposure day. The respective Met-Hb levels paralleled with the duration of exposure. Peak levels ranged in 3 dogs from 3-7% (average maximal Met-Hb formation of about 5%) and in one dog (which demonstrated clinical signs) up to 24%. This variability is apparently related to differences in respiratory minute volume. Following inhalation the Methaemoglobin was restituted at a half-time of 100 minutes. This restitution of Met-Hb appears to occur immediately after the end of exposure. This is taken as an indirect evidence that there is a limited solubility of aniline in blood and that the substance is eliminated from blood to a large extent by exhalation. It is concluded that conversion of oral doses to inhalation exposure concentrations is subject to significant errors. The apparent

discrepancies between these routes of uptake appear to be related to the physico-chemical characteristics of Aniline vapour which is likely to behave as a gas in the respiratory tract. When a conversion from oral to inhalation route is attempted for steady state exposure a retention factor of 0.2 should be considered. For this type of study no specific guideline can be applied. However, the exposure technology was carried out in compliance with OECD TG 412 (Bayer AG, 2000).

Dermal exposure

Acute dermal toxicity of aniline is characterised by LD_{50} values of 1,540 mg/kg bw for rabbits (Bio-Fax Industrial Bio-Test Laboratories, 1969), 1,290 mg/kg bw for guinea pigs (Roudabush et al., 1965) and 254 mg/kg bw for cats (Kondrashov, 1969).

Undiluted aniline (no data on purity) was tested in rabbits at doses of 1,000, 1,470, 2,150 and 3,160 mg/kg. Deaths occurred after dermal application of 1,000 mg/kg (1/5 rabbits died), 1,470 mg/kg (1/5 rabbits died), 2,150 mg/kg (5/5 rabbits died) and 3,160 mg/kg (5/5 rabbits died), resulting in a dermal LD_{50} of 1,540 mg/kg. Clinical signs included hypoactivity, hypersensitivity and salvation. Subdermal haemorrhages, severe edema and erythema were reported as local findings. Necropsy did not reveal any significant findings in survivors, but demonstrated hyperemia of liver and kidneys in decedents. No more details are reported (Bio-Fax Industrial Bio-Test Laboratories, unpublished report 1969).

An experiment according to regulation 21 CFR 191.11 with occluded abraded skin using undiluted aniline (no data on purity) and 4 rabbits/dose (no information on doses) gave a dermal LD_{50} of 820 mg/kg. No further data are mentioned (Roudabush et al., 1965).

A dermal LD_{50} of 1,290 mg/kg resulted for male guinea pigs in a study according to regulation 21 CFR 191.11. This experiment was conducted with occluded intact skin using undiluted aniline (no data on purity) and 4 guinea pigs/dose (no information on doses). A similar experiment with abraded skin of guinea pigs gave a dermal LD_{50} of 2,150 mg/kg. No further data are reported (Roudabush et al., 1965).

Application of aniline (no data on purity) to the skin of cats resulted in a dermal LD_{50} of 254 mg/kg bw. No further data on test performance are given (Kondrashov, 1969).

4.1.2.2.2 Studies in humans

Acute intoxication of humans with aniline/aniline vapours is reported frequently. In humans 60 ml of orally administered aniline causes death. This corresponds to about 876 mg/kg bw, based on a body weight of 70 kg (Janik-Kurylcio et al., 1973).

Benzene amines are all very readily absorbed through the skin as well as through the lungs. Aniline poisoning is first manifested as an intense cyanosis, the victims of poisoning being referred to as “blue boys”. In an aniline plant visited during war, cases of poisoning occurred daily and blue boys were a common sight. Following cyanosis they develop headache, dizziness, dysphagia, nausea, vomiting, chest and abdominal pain or convulsions, weakness, restlessness, palpitation, and irregular slow respiration with rapid feeble heart action. Pupils are contracted but respond to light. Temperature is subnormal. There is an aniline odor on the breath and on the sweat. The urine is dark in color owing to the presence of haemoglobin. In severe cases there may be a loss of sphincter controls (unvoluntary urination and defecation) and also pulmonary oedema. 0.4-0.6 mg/l air may be borne without much harm for 0.5-1 hour, but 0.1-0.25 mg/l for

several hours produces slight symptoms. Average lethal inhalation concentration for humans is reported to be 25 mg/l air or 0.35-1.43 g/kg body weight. Workers may develop a degree of tolerance but the cyanosis may persist (Smyth, 1931).

Suicidal oral intake of 60 ml aniline resulted in death at day 4 after intake. Initially, methaemoglobin formation had increased to 85% and decreased to 27% at day 4. During this time interval p-aminophenol excretion was approximately 8.4 mg/hours. Pathologic assessment revealed degenerative changes in myocard, liver and kidney, oedema in lung and brain and haemorrhages in medulla oblongata (Janik-Kurylcio et al., 1973).

Documentation of 13 accidents at the workplace demonstrate cyanosis, nausea, dizziness, respiratory problems and heart pains after accidental exposure to aniline between 1967 and 1992. The maximal percentage of methaemoglobin formation reported was 60% (BASF AG, unpublished reports 03.02.1993).

In older publications (Fairhall, 1957; Kiese, 1974; Sekimpi and Jones, 1986) aniline poisoning of workers was described with low till moderate cyanosis, anaemia with Heinz bodies, general weakness, mental disturbances, convulsions and dyspnea.

Methaemoglobin levels were determined in the blood of 20 volunteers (17 males and 3 females) after a single oral dose of 5, 15, and 25 mg aniline/volunteer on three consecutive days. The mean maximum increase in percentage of methaemoglobin was obtained in less than 4 hours after intake. Doses of 5 and 15 mg aniline produced no significant increase of methaemoglobin but the dose of 25 mg raised the level to 2.5% versus 1.2/1.8% in the lower doses. The dose of 45 mg raised the level to 7% (5 volunteers) and one volunteer who received a dose of 65 mg had a level of 15% Met-Hb. The blood samples taken 24 hours after each dose revealed no adverse effects upon packed cell volume, reticulocyte count, bilirubin or urobilinogen, except for a slight increase of serum bilirubin in two male volunteers following the administration of 45 and 65 mg. Aniline had no adverse effects on serum proteins, serum enzymes (not specified), blood urea and thymoturbidity test. No Heinz bodies were detected. The authors conclude that this investigation supports the view that the production of methaemoglobin is due to a metabolite of aniline, namely phenylhydroxylamine (measured in the blood *in vitro*), and that the catalytic effect of phenylhydroxylamine is promoted by glucose. Aniline is less toxic in the rat after a single oral dose (20 mg/kg) than in man and the no-effect dose of aniline in adult man is in the region of 15 mg/man (about 0.21 mg/kg body weight). The time intervals between treatment of different doses were not specified (Jenkins et al., 1972).

Three cases of intoxication have been detected by contact with shoes that had been dyed with a preparation which composition is the following: naphtha (50-100%), ethyl alcohol (25-50%), and aniline (10-25%). The cases showed as the main symptom a grave methaemoglobinemia which required urgent assistance in hospitals (National Network of Vigilance, Control and Santion of Chemical Products, 1999).

4.1.2.2.3 Conclusion on acute toxicity

Acute intoxication of humans with aniline/aniline vapours is reported frequently. In humans 60 ml of orally administered aniline causes death. 0.4-0.6 mg/l air may be borne without much harm for 0.5-1 hour, but 0.1-0.25 mg/l for several hours produces slight symptoms. Average lethal inhalation dose for humans is reported to be 25 mg/l air or 0.35-1.43 g/kg body weight. With respect to methaemoglobin formation the no-effect dose of aniline in adult man is in the region of 15 mg/man (about 0.21 mg/kg body weight). In animal experiments the acute toxicity

of aniline demonstrates significant species differences, independent of the route of application (oral LD_{50}, rat: 442-930 mg/kg; dermal LD_{50}, rabbit: 1,540 mg/kg; inhalation LC_{50}, rat: 1-3.3 mg/l/4h). Cats react much more sensitive, with a dermal LD_{50} of 254 mg/kg bw and death following oral application of as low as approximately 50-100 mg/kg. Aniline is absorbed through the skin and the lungs. In dogs 24 hours after oral treatment with 15 mg aniline/kg methaemoglobin levels are in the normal range of approximately 0.7% (being in the range of 19-29% after 3 hours). In an acute inhalation test with the same species peak methaemoglobin levels were from 3–24% within 3 hours after the start of the exposure and declined to normal levels (< 1%) after approximately 20 hours. Methaemoglobin was restituted at a half time of 100 minutes. In rats an oral dose of 20 mg aniline/kg resulted in a small increase of Met-Hb levels (3.3% versus 2.4% in controls). In adult man the no-effect dose after oral treatment for three consecutive days resulted in a no-effect dose in the region of 15 mg/man (about 0.21 mg/kg). Taking into account all available data on animals and humans aniline is classified as "T, toxic" and labelled as "R 23, 24, 25, toxic by inhalation, in contact with skin and if swallowed".

4.1.2.3 Irritation

4.1.2.3.1 Studies in animals

Skin

Primary skin irritation after application of 0.5 ml of undiluted aniline (no data on purity, exposure period not given) revealed erythema grade 1 in 6/6 rabbits for more than 3 days. No oedema were detected (Bio-Fax Industrial Bio-Test Laboratories, unpublished report 1969). In a second skin irritation test application of undiluted aniline to the skin of rabbits revealed slight erythema which were reversible within 8 days (BASF AG, unpublished report 1972).

However, in a test with undiluted aniline performed with rabbits aiming at the assessment of acute dermal toxicity (a 24-hour exposure period can be assumed) the animals exhibited subdermal hemorrhages and severe erythema (Bio-Fax Industrial Bio-Test Laboratories, unpublished report 1969).

Eye

Irreversible irritation revealed in a Draize test after instillation of 50 mg of undiluted aniline (no data on purity) into the eyes of rabbits: Severe corneal opacity and severe conjunctival erythema and oedema were detected (no scoring system used) which were not reversible within 8 days. Eight days after instillation of the test liquid pannus formation was determined (BASF AG, unpublished report 1972).

In eye irritation tests conducted in 1949 application of undiluted aniline (no data on purity) to the cornea of rabbits produced some lacrimation, some inflammation of the conjunctiva and damage to the cornea. These effects appeared maximal after approximately 1 hour and disappeared completely within 24-48 hours. The maximum corneal injury amounted to alteration of about half the corneal surface so that it stained rather deeply with fluorescein. The area affected varied with the amount applied indicating that the material had not spread evenly over the cornea by mixing with lacrimal fluid. Saturated aqueous solutions seemed to produce effects comparable to equivalent amounts of undiluted material. The authors concluded that eye damage can be caused

by small quantities entering the eye (Medical Division Army Chemical Center, report of 30.06.1949).

No data on reversibility are reported in an eye irritation test with rabbits using 0.1 ml aniline (no data on purity) and 6 animals. Mean scores of ca. 52/110 for effects on cornea, iris and conjunctivae were detected within the first 3 days after instillation. No more details are reported (Bio-Fax Industrial Bio-Test Laboratories, unpublished report 1969).

In a Draize eye irritation tested conducted in 1957, 0.1 ml aniline (no data on purity) was instilled into the eyes of each of the rabbits. Corneal opacity was reversible within 2 days, maximal conjunctival irritation was reached within 2 days after instillation, conjunctival irritation did not reverse within an observation period of 96 hours (Sziza and Podhragyai, 1957).

In an acute inhalation test with rats exposed to aniline vapours (head-only exposure) at an average concentration of approximately 3 mg/l/4 hours, in addition to numerous other clinical effects, eye damage was reported as follows. Mild to severe corneal damage up to corneal clouding was observed until day 14 (DuPont de Nemours, 1982).

Respiratory tract

No data are available.

4.1.2.3.2 Studies in humans

Human data on local irritant properties of aniline are not available.

4.1.2.3.3 Conclusion on irritation

Human data on irritation are not available. Aniline causes only weak irritation to the skin of rabbits if the test is conducted according to appropriate skin irritation/corrosion testing (Bio-Fax Industrial Bio-Test Laboratories, 1969; BASF AG, 1972), but long lasting severe irritation to the eye (Bio-Fax Industrial Bio-Test Laboratories, 1969; Sziza and Podhragyai 1957; BASF AG, 1972; Medical Division Army Chemical Center, 1949). In rabbit eyes, long lasting severe corneal opacity and severe conjunctival irritation were detected; eight days after instillation of the substance pannus formation was detected. Accordingly, aniline is classified as "Xi, Irritant" and labelled with "R 41, Risk of serious damage to eyes".

4.1.2.4 Corrosivity

4.1.2.4.1 Studies in animals

Aniline is not corrosive to the skin of rabbits if the normal exposure period of appropriate skin irritiation/corrosion testing is observed (Bio-Fax Industrial Bio-Test Laboratories, 1969; BASF AG, 1972), but eye damage can be caused by small quantities entering the eye (Bio-Fax Industrial Bio-Test Laboratories, 1969; Sziza and Podhragyai; 1957; BASF AG, 1972; Medical Division Army Chemical Center, 1949).

However, in a test with undiluted aniline performed with rabbits aiming at the assessment of acute dermal toxicity (a 24-hour exposure period can be assumed) the animals exhibited subdermal hemorrhages and severe erythema (Bio-Fax Industrial Bio-Test Laboratories, unpublished report 1969).

4.1.2.4.2 Studies in humans

Human data on local corrosivity are not available. Based on animal data, it is concluded that although eye damage may not be permanent, it may be painful enough to make a man unfit for work for several days (Medical Division Army Chemical Center, 1949).

4.1.2.4.3 Conclusion on corrosivity

Aniline is not corrosive to the skin as judged on the basis of appropriate skin irritation/corrosion testing with rabbits, but may cause serious damage to eyes. On the basis of the data on local irritant properties of aniline, the substance is not to be classified as corrosive to skin according to EU legislation.

4.1.2.5 Sensitisation

4.1.2.5.1 Studies in animals

Skin sensitisation potential of aniline was studied in three procedures in guinea pigs. For these procedures the optimum concentrations were tested (Goodwin et al., 1981):

1) In a Magnusson Kligman test with ten guinea pigs in the test group, (intradermal induction with 1.5%, topical induction with 25% and challenge with 10% aniline) positive reactions were reported for 1/10 animals (10%).

2) In a Single Injection Adjuvant test (SIAT) induction was performed by a single intradermal injection with 1.5% aniline in 0.9% physiological saline. The guinea pigs were challenged for the first time twelve to fourteen days later by a 6-hour occluded chamber application with 20% aniline. Following repeated challenges, positive reactions were reported for 5/10 guinea pigs (50%). Thus, the response rate in the SIAT test was higher than in the more sensitive Maximisation test.

3) No skin sensitising effects of aniline were observed in a third procedure with a Modified Draize test. Induction was performed by four simultaneous injections of 2.5% aniline at sites which overlay the axillary and inguinal lymph nodes. Challenge was made by intradermal injection and open topical applications on opposite shaved flanks fourteen days later with 1% aniline.

There is no information available on the potential for aniline to produce respiratory sensitisation in animals.

4.1.2.5.2 Studies in humans

With the use of the Maximisation test (topical induction 20% and challenge 10%) 7/25 volunteers showed positive reactions to aniline (purity not mentioned) which were judged by the author as a mild reaction rate (Kligman, 1966).

Sensitisation in humans has been reported. However, these results are often associated with para group cross reactivity (Schulz, 1962). The group allergy with aromatic amino compounds has been investigated experimentally. Patch-tests were performed during a monitoring survey in 181 persons with a confirmed group sensitisation to aromatic amino compounds. A test concentration of 1% aniline was mixed with an ointment of unknown components. Twenty-four of the persons showed mild to moderate positive reactions (13%). It is indicated that aniline is structurally related to para-substituted aminoaromatic compounds (e.g. p-phenylendiamin, p-toluylendiamin), that also cause contact allergy (Düngemann and Borelli, 1966).

In 67/1,377 patients (4-9%) suffering from occupational eczematous contact dermatitis of unknown origin reacted positive to aniline. Test method and aniline concentration were not reported (Meneghini et al., 1963).

306 (187 men and 119 women) patients suffering from stasis dermatitis with or without ulceration and/or with or without allergic contact dermatitis of eczematous type were patch tested with 63 substances. A rate of 8.8% was reported positive with aniline and related compounds. The patients were patch-tested with 5% aniline in petrolatum. Substances involved in cross sensitisation and belonging to the para-group are benzocaine, aniline and diaminodiphenylmethane. In a majority of cases these substances should be considered as indicators of cross-sensitivity (Angelini et al., 1975).

Patients of a dermatological hospital showed reaction rates between 5.1% and 13% (10% aniline in sweet almound oil, no further details). The percentage of patients reactive to patch tests of aniline decreased significantly between the years of 1956-1965 (Scarpa and Ferrea, 1966).

Positive patch test results are reported for 8 out of 200 patients suffering from leg ulcer and dermatitis for more than 6 months. Patch tests were performed with 5% aniline in petrolatum (Ebner and Lindemayr, 1977).

4.1.2.5.3 Conclusion on sensitisation

Animal data revealed a mild to moderate sensitisation rate. In 2/3 guinea pig tests a positive rate of 10% and 50% are documented. In the test revealing a 50% positive result 20% aniline was used for challenge, while the tests demonstrating weak or negative results used very low challenge concentrations (challenge with 10% aniline resulted in 1/10 sensitised animals, challenge with 1% aniline in no sensitisation at all). In humans positive reactions have also been reported, mainly in patients suffering from eczematous dermatitis. The positive reactions are often associated with para-group compound cross reactivity. Respiratory sensitisation has not been observed. However, based on the observed skin sensitisation, the occurrence of respiratory sensitisation cannot be ruled out.

In addition, in humans aniline shows cross-reactivity to substances of the para-substituted compound group which has to be considered as a hazard by itself. Based on animal and human data, aniline is labelled with the R-phrase R 43 “May cause sensitisation by skin contact”.

4.1.2.6 Repeated dose toxicity

4.1.2.6.1 Studies in animals

Main toxic effect: haemotoxicity

Rats

Irrespective of the application route, repeated inhalation exposure or oral administration of aniline to rats caused main toxic effects in the haematopoietic system with corresponding changes of the spleen, the bone marrow, the kidneys and the liver. Clinical symptoms in repeatedly dosed rats were cyanosis, reduced body weight gain and food consumption and at high-doses premature deaths. Aniline treatment damaged erythrocytes resulting in an haemolytic anaemia. Higher methaemoglobin levels than in controls and Heinz bodies were observed. The damaged red blood cells were scavenged predominantly in the red pulp of the rat spleen followed by increased haemosiderin accumulation, sinusoidal congestion, higher organ weight and darkened appearance of the spleen. Prolonged exposure to aniline resulted in a continuous damage of erythrocytes. Multifocal perisplenitis was seen after 4 weeks of oral administration. After chronic administration also stromal hyperplasia and fibrosis, and chronic capsulitis with papillary projections occurred in the spleen. Occasionally haemosiderosis of the kidneys and liver were observed. In response to the haemolytic effect reticulocyte counts, serum transferrin and total iron binding capacity were increased and erythropoietic activity was elevated in the bone marrow and at extramedullary sites (mainly in the spleen). The potential for carcinogenic effects is discussed in Section 4.1.2.8.

Mice

Long-term treatment to mice revealed lower body weights but no indication on haemotoxicity from histopathological examinations. Mouse data on haematology, methaemoglobinemia and clinical chemistry were not available. The single observation which possibly gives information on splenic effects was the occurrence of black discoloration of the spleen in mice from the 8-week dose finding study (NCI, 1978). Unfortunately, this study did not include haematology or histopathological data.

Repeated dose toxicity data with emphasis on red cell parameters were reported in tables. **Table 4.10** presents the systemic effects of aniline after repeated inhalation exposure to rats. Up to now, no reliable inhalation study on mice or other species is available. Short- and long-term studies with oral administrations to rats and mice were reported in **Table 4.11**. In addition to the tabulated rat data, Oberst et al. (1956) reported that female albino mice (20/group), Guinea pigs (10 or 9/group, no data on sex) and male Beagle dogs (2/group) were exposed for 6 hours/day, on 20 weeks (mice, Guinea pigs), resp. 26 weeks (dogs) to a chamber vapour concentration of 0 or 5 ppm aniline. The parameters examined and results observed were described only fragmentarily, especially for the mice and Guinea pigs. No toxic sign was observed in these species. As no deviation from normal values was detected, no (quantitative) haematological (including methaemoglobin levels) or biochemical results were documented. There were no exposure-related pathological findings in any of the animals (no data on methods of macroscopic or microscopic examination).

Figure. 2 Primary and secondary toxic effects of repeated aniline administration: see Appendix F.

Table 4.10 Repeated inhalation of aniline vapour to rats/non neoplastic lesions

Study design	MetHb ↑	Heinz bodies	Reticuloc-ytes↑	RBC↓	Hb↓	HCT↓	MCV↑	MCH↑	Clinical /post mortem findings	NOEC [LOAEC]	Reference
Rat, male, Sprague-Dawley 5/group, 5 d, 8 h/d 10, 30, 50, 150 ppm#	≥30 ppm	ND	ND	ND	ND	≥30 ppm	ND	ND	ND, maximum metHb levels at 30 ppm: 5%, 50 ppm: 13%, 150 ppm: 55%, daily metHb levels prior and after exposure increased with number of exposure days indicating a cumulative effect, decrease of methb level during the nonexposure time •	NOEC 10 ppm	Kim and Carlson (1986)
Rat, male, Sprague-Dawley 5/group, 4 d, 12 h/d 10, 30, 50, 150 ppm#	≥30 ppm	ND	ND	ND	ND	≥30 ppm	ND	ND	ND, maximum metHb levels at 30 ppm: 5%, 50 ppm: 14%, 150 ppm: 60%, daily metHb levels prior and after exposure increased with number of exposure days indicating a cumulative effect, decrease of metHb level during the nonexposure time •	NOEC 10 ppm	Kim and Carlson (1986)
Rat, 14 males / group (strain?) 2 wk, 3, 6, or 12 h/d, 5 d/wk 0, 10, 30, 90 ppm, with/witout 14d recovery	≥30 ppm (no quantitative data)	ND	ND	≥30 ppm	ND	ND	≥30 ppm non reversible at 90 ppm	≥30 ppm non reversible at 90 ppm	≥30 ppm (at 3, 6 and 12 h): splenic congestion/ haemosiderosis, spleens appeared nearly normal at 14 d post exposure, metHB levels plateaued after 4 exposures, remained at steady-state concentration until the 10th exposure, decreased to normal 14 days post exposure••	NOEC 10 ppm	Burgess et al. (1984a; b)
Rat, 16 males/group,.Crl:CD, 2 wk, 6 h/d, 5 d/wk + 13-d recovery, head-only exposure to analytical concentrations of 0, 17, 45, 87 ppm	≥45 ppm (2-8 fold increase, max. at day 5 of exposure)	ND	≥45 ppm	≥45 ppm @ persistent in 87 ppm group	≥45 ppm	≥45 ppm	≥45 ppm@	≥87 ppm	≥17 ppm: dose-dependent effects in spleen (minimal at 17 ppm): RES hypertrophy, hemosiderosis @persistent in all dose groups, hematopoiesis↑, ≥45 ppm: MCHC ↓@, urine: bilirubin ↑, spleen: weight ↑, congestion, bone marrow myeloid:erythroid ratio ↓@ persistent in 87 ppm group 87 ppm: cyanosis, diuresis ↑, Ly ↑, PMN ↓, platelets ↓@, Liver: haematopoeisis↑;••	NOEC not estimated [LOAEC 17 ppm]	EPA (1981)

Table 4.10 continued overleaf

Table 4.10 continued Repeated inhalation of aniline vapour to rats/non neoplastic lesions

Study design	MetHb ↑	Heinz bodies	Reticuloc -ytes↑	RBC↓	Hb↓	HCT↓	MCV↑	MCH↑	Clinical /post mortem findings	NOEC [LOAEC]	Reference
Rat, male, Wistar (9 rats for 26 wk + 11 rats intercurrently sacrificed), 5 d/wk, 6 h/d, 5 ppm (≙19mg/m³)	5 ppm (no quantitative data, described as slight and mild)	ND	ND	ND	NCE	NCE	ND	ND	5 ppm: cyanosis, no pathological (macro finding) ●§	NOEC not estimated [LOAEC 5 ppm]	Oberst et al. (1956)

ND not done, NCE no change evident, ● Histopathology not performed, ●● Histopathology from selected organs.
Blood parameters: MetHb↑ increased methaemoglobin concentration, RBC↓ reduced red blood cells, Hb↓ reduced haemoglobin, HCT↓ reduced haematocrit, MCV↑ increased mean corpuscular volume, MCH↑ increased mean corpuscular haemoglobin, PMN polymorphnuclear granulocytes, Ly lymphocytes, RES retikuloendothelial system in the spleen

\# no controls

∞ 6 rats/group exclusively used for MetHb determinations with 1 h following exposures on day 1,3,5,6,8, 10 and on recovery days 3,5,7,11,

@ not reversible

Table 4.11 Repeated oral studies of aniline in rats and mice/non neoplastic lesions

Study design	MetHb↑	Heinz bodies	Reticuloc-ytes↑	RBC↓	Hb↓	HCT↓	MCV↑	MCH↑	Clinical/post mortem findings	NOAEL [LOAEL]	Reference
Rat, 5 males/group Sprague-Dawley 4 d/ 0.25, 0.5, 1, 2 mmol/kg in drinking water* by gavage (≅23, 46, 93, 186 mg/kg bw/d aniline)	2mmol/kg sign.↑ (no quantitat-ive data)	ND	ND	2 mmol	≥1 mmol	≥1 mmol	ND	ND	≥0.5 mmol/kg: dose-related sign, higher iron content of spleen (+72%, 172%, and 325% in spleen homogenates at 0.5, 1, and 2 mmol/kg, iron not characterised), increased lipid peroxidation (+ 24%, 32%, resp. 44%) - histopathology/spleen: expansion of red pulp ≥1mmol/kg: increased spleen weights (rel/abs), histopathology/spleen: increased red pulp cellularity, cellular fragmentation, vascular congestion, stainable iron (Fe3+) deposits in phagocytes, erythropathocytosis - increased protein oxidation 2 mmol/kg: increased No. of WBC	NOAEL 0.25 mmol ≅23 mg/kg bw/d [LOAEL 0.5 mmol ≅46 mg/kg bw/d]	Khan et al. (1997)
Rat, F-344 0 (on 5, 10, or 20 d, each 10 males) or 110 mg/kg bw/d, on 5, 10, or 20 d (6, 8, resp. 8 males/group) gavage	ND	ND	ND	ND	ND	ND	ND	ND	110 mg/kg bw/d: premature deaths (4 before d 5, 2 on d 5-10, 2 on d 10-20), transient cyanosis after dosing, body weight gain↓ (only at 5 d), spleen: weight ↑, mild-moderate congestion, severe graded haemosiderosis (only at 20 d), mild-severe haematopoiesis↑, bone marrow: moderate-severe hypercellularity, kidneys: mild haemosiderosis●●	NOAEL not estimated [LOAEL 110 mg/]	Short et al. (1983)
Rat, F-344, 12 male/group, 4 wk; 10, 30, 100 mg/kg bw/d in diet* (≅ 4, 12 and 41 mg/kg bw/d aniline$)	100 mg: non-sign.↑	≥10 mg	≥30 mg	≥30 mg	≥30 mg	≥100 mg	≥30 mg	≥30 mg	cf. summary Table 4.12 and Table 4.13	NOAEL not estimated, [LOAEL 10 mg ≅4 mg/kg bw/d]	BASF (2001)
Rat, 6 animals/sex/group, Coworth-Wistar 13 d/0 or 0.093% in diet (≅65.1 mg/kg bw/d)	ND	ND	ND	ND	ND	ND	ND	ND	spleen: weight ↑, erythropoiesis↑, sinusoidal engorgement, haemosiderosis (no data on grading) liver: slight erythropoiesis	NOAEL not estimated [LOAEL 0.093%]	Jenkins et al. (1972)

Table 4.11 continued overleaf

Table 4.11 continued Repeated oral studies of aniline in rats and mice/non neoplastic lesions

Study design	MetHb↑	Heinz bodies	Reticul-ocytes↑	RBC↓	Hb↓	HCT↓	MCV↑	MCH↑	Clinical/post mortem findings	NOAEL [LOAEL]	Reference
Rat, 15 male/group, Sprague-Dawley 14 d/0 or 0.7 mmol/ kg bw/d (≙65 mg/kg bw/d aniline) in mineral oil by gavage, groups of 5 rats each were killed on d 1, 7, and 28 post exposure	0.7 mmol on day 1**	ND	ND	0.7 mmol on day 1 and 7	0.7 mmol on day 1	0.7 mmol on day 1	ND	ND	WBC cell counts increased on day 1, no effect on differential WBC counts, lower concentrations of serum transaminases AST and ALT on day 7, increased IgA levels on day 1, spleen: increased rel. weights on day 1 and 7, increased abs. weights on all time points, higher iron content at all time points in spleen homogenates, histopathology: day1: vascular congestion, heavy iron deposits in macrophages (haemosiderin) of the red pulp sinoids, day 7 decreased vascular congestion, iron deposition remained indistinguishable from day 1, day 28: congestion disappeared, iron deposition (haemosiderin) remained high, focal fibrosis, especially in the capsule, no changes found in heart, livere, lung, brain, kidneys, testes, thymus, pancreas●●	NOAEL not estimated [LOAEL 0.7 mmol/kg≙65 mg/kg bw/d]	Khan et al. (1995b)
Rat, 10 males/group (strain?) 28 d/0, 25, 150, 600 ppm (≙2.22, 13.5, 48.5 mg/kg bw/d) in diet	ND	ND	ND	ND	ND	ND	ND	ND	≥150 ppm: enlarged, blackened spleen●	NOAEL 25 ppm ≙ 1.75 mg/kg bw/d [LOAEL 150 ppm ≙ 10.5 mg/kg bw/d]	Anonymus (1969)
Rat, 10 males and 10 females/group F344, 30 d/0, 30, 100, 300, 1,000 mg/kg bw/d in diet*,	≥30 mg/kg	≥30 mg/kg	≥30 mg/kg	ND	ND	ND	ND	ND	1,000 mg/kg: 10 premature deaths/moribund sacrifices of females on d 24-27, body weight gain↓, black discolored cortex of kidneys, enlarged pancreatic lymph nodes, food consumption ↓ ≥300 mg/kg: cyanosis, ≥100 mg/kg: spleen and liver: irregular surface + enlargement, discoloration of spleen and kidneys●	NOAEL not estimated [LOAEL 30 mg/kg]	CIIT (1977)

Table 4.11 continued overleaf

Table 4.11 continued Repeated oral studies of aniline in rats and mice/non neoplastic lesions

Study design	MetHb↑	Heinz bodies	Reticul-ocytes↑	RBC↓	Hb↓	HCT↓	MCV↑	MCH↑	Clinical/post mortem findings	NOAEL [LOAEL]	Reference
Rat, 5 males and 5 females/group F344 8 wk/0, 0.01, 0.03, 0.3, 1% (≅5, 15, 151, 504 mg/kg bw/d aniline⊕) and Mouse, 5 males and 5 females/group B6C3F1 8 wk/0, 0.01, 0.03, 0.3, 1% (≅10.8, 32.4, 324, 1080 mg/kg bw/d aniline⊕) 8 wk/0.01, 0.03, 0.3, 1.0% in diet*	ND	ND	ND	ND	ND	ND	ND	ND	≥0.3%: black granular enlarged spleen in rats and mice, 1%: body weight gain↓ in rats• (dose finding studies)	NOAEL 0.03% in rats and mice	NCI (1978)
Rat, 15 males/group, Sprague-Dawley 0, 600 ppm in drinking water, 5 rats/ group sacrificed on day 30, 60 or 90 * (≅43 mg/kg bw/d aniline)	43 mg/kg on all time points**	ND	ND	43 mg/kg all time points	43 mg/kg on 30 d and 90 d	43 mg/kg on 30 d and 90 d	43 mg/kg on 60 d and 90 d	43 mg/kg on 60 d and 90 d	increased relative spleen weights on all time points; rel. liver weights decreased on day 30 and increased on day 60, increased WBC counts on day 30 only, increased IgA levels on days 60 and 90, decreased CD4+/CD8- T-helper cells at 90 days. Reduced AST activity on day 30 and 60. Spleen histopathology findings with time-related progression: marked red pulp expansion due to increased sinusoidal cells, fibroblasts, marked-massive iron (haemosiderin) deposition in macrophages/ red pulp, pericapsular fibrosis••	NOAEL not estimated [LOAEL 43 mg/kg]	Khan et al. (1993)
Rat, male and female (no./ group?, control?), F344 ≤ 1 y/ 10, 30, 100, 300, 1,000 mg/kg bw/d n diet*, abstract	≥30 mg/kg	≥30 mg/kg	≥30 mg/kg	≥30 mg/kg	ND	ND	ND	ND	≥30 mg/kg: spleen: enlarged, thickened capsule, fibrous hyperplasia of white pulp, red pulp hyperplasia, haematopoiesis, bone marrow: erythroid hyperplasia, 10 mg/kg: little toxic effects without further details••	NOAEL not estimated [LOAEL 10 mg/kg]	Gralla et al. (1979)

Table 4.11 continued overleaf

Table 4.11 continued Repeated oral studies of aniline in rats and mice/non neoplastic lesions

Study design	MetHb↑	Heinz bodies	Reticul-ocytes↑	RBC↓	Hb↓	HCT↓	MCV↑	MCH↑	Clinical/post mortem findings	NOAEL [LOAEL]	Reference
Rat, male (15, 10, 18, resp. 16 rats) Wistar 80 wk/0, 0.03, 0.06, 0.12% aniline in drinking water	ND	ND	ND	≥0.03 %	≥0.03 %	NCE	ND	ND	≥0.03%: dose-related increased incidence of slight-moderate bile duct proliferation, ●● (no data on spleen effects)	NOAEL not estimated [LOAEL 0.03% in water]	Hagiwara et al. (1980)
Rat, 130 males and 130 females/group F344 104 wk/ 10, 30, 100 mg/kg bw/d in diet*, (≅7, 22, 72 mg/kg bw/d aniline), 10 rats/sex/ group killed at wk 26 and 52, 20 rats/sex/ group killed at wk 78	≥30 mg/kg	100 mg/kg	≥10 mg/kg	≥10 mg/kg	≥10 mg/kg	≥10 mg/kg	≥10 mg/kg	100 mg/kg	100 mg/kg: survival rate↓, spleen: firm, enlarged, discolored, irregular surfaces, chronic capsulitis (at wk 26 and thereafter), stromal hyperplasia + fibrosis (wk 104), lymphoid athrophy (wk 104), fatty metamorphosis, bone marrow activity↑, pigment accumulation in pancreatic lymph nodes, adrenals and liver; liver weight↑ ≥30 mg/kg: spleen weight↑, dose-related increase of extramedullary haematopoiesis (at wk 26) ≥10 mg/kg: spleen: increased mean severity grades of pigment accumulation (haemosiderosis) and haematopoiesis (at wk 52 and thereafter)	NOAEL not estimated [LOAEL 10 mg/kg of aniline hydrochloride ≅ 7 mg/kg bw/d aniline]	CIIT (1982)
Rat, F344 103 wk/0.3, 0.6% in diet* (≅174.4 or 350.5 mg/kg bw/aniline)	ND	ND	ND	ND	ND	ND	ND	ND	≥0.3%: spleen: fibrosis of capsule and trabeculae, fatty metamorphosis, papillary hyperplasia of capsule kidneys: tubular haemosiderosis 0.6%: liver: Kupffer cell haemosiderosis	NOAEL not estimated [LOAEL 0.3%in diet]	NCI (1978)

Table 4.11 continued overleaf

Table 4.11 continued Repeated oral studies of aniline in rats and mice/non neoplastic lesions

Study design	MetHb↑	Heinz bodies	Reticul-ocytes↑	RBC↓	Hb↓	HCT↓	MCV↑	MCH↑	Clinical/post mortem findings	NOAEL [LOAEL]	Reference
Mouse, male and female, B6C3F1 103 wk/ 0.6 1.2% in diet*, (aniline ≅737 or 1,510 mg/kg bw/d in males and 733 or 1,560 mg/kg bw/d in females) both species: 25 animals/ sex/control group and 50 animals/sex/ dose groups	ND	ND	ND	ND	ND	ND	ND	ND	1.2%: body weight gain↓, ≥ 0.6%: liver: bile duct inflammation in males	NOAEL not estimated [LOAEL 0.6% in diet]	NCI (1978)

* Aniline hydrochloride as test substance, 100 mg of aniline hydrochloride is equivalent to 72 mg aniline

** Analysis of MetHb was performed 24 hr after collection of blood

$ Calculation based on limited stability of test substance (56.6%) in diet (6, 17, 56 mg/kg bw/d aniline hydrochloride), test substance concentration was weekly adjusted, aniline content was 72% of test substance (see *)

⊕ Calculations based on relative food consumption of 7% of body weight for rats and 15% for mice

ND not done, NCE no change evident, • Histopathology not performed, •• Histopathology from selected organs, MetHb↑ increased methaemoglobin concentration, RBC↓ reduced red blood cells, Hb↓ reduced haemoglobin, HCT↓ reduced haematocrit, MCV↑ increased mean corpuscular volume, MCH↑ increased mean corpuscular haemoglobin, WBC white blood cells, AST aspartate aminotransferase, ALT alanine aminotransferase

No controls

Repeated dose study on the mode of action

In a recent study (BASF, 2001) on the mode of action of aniline toxicity, aniline hydrochloride was administered to groups of 12 male F344 rats in the diet for one and four weeks. The test substance was administered in three dose groups (nominal dosages 10, 30 and 100 mg/kg bw/d). Another group of 12 rats served as controls. All animals were sacrificed on day 28/29 after study begin. Due to insufficient stability of the test substance in the diet (56.6% after 1 week), the concentrations of test substance were adjusted weekly. The corrected test substance intake was 6, 17 and 57 mg/kg bw/d aniline hydrochloride (≅ 4, 12 and 41 mg/kg bw/d aniline).

The study design was not fully compliant to OECD 407. Test parameters such as clinical signs of toxicity, food consumption, body weight, and gross oberservation were in accordance to the standard requirements. However, males being the most sensitive sex were only treated, examinations on clinical chemistry were not included, organ weight determination and histopathology were limited to possible target organs. Six rats of each dose group were used for histolopathologic examinations and blood examinations. Cell replication rate and immunohistology on filaments were examined in spleen sections of the other six animals of each group.

Study results were summarised in **Table 4.12**.

Table 4.12 Results from an oral 28-day study with 1 week or 4 week administration of aniline

Test design	Treatment period*	1 week application	1 week application	4 week application	4 week application	Assessment
	Dose groups (6 males/group):	4A (10 mg), 5A (30 mg), 6A (100 mg)	4B (10 mg), 5B (30 mg), 6B (100 mg)	0A (control), 1A (10 mg), 2A (30 mg), 3A (100 mg)	0B (control) 1B (10 mg), 2B (30 mg), 3B (100 mg)	
	Examinations	Hematology@, Pathology#, Histopathology§	Pathology#, Spleen histopathology* in 6B, Cell replication© in 6B	Hematology@, Pathology#, Histopathology§	Pathology#, Spleen histopathology* in 0B, 3B; Cell replication in 3B	
Results	Hematology	≥ 10 mg: Hb adducts↑, Heinz bodies in 2/6 m ≥ 30 mg: Hb↓, MCHC↓, PLT↑, Heinz bodies↑, serum iron (non-sign)↑ 100 mg: RBC↓, anisocytosis, polychromasia, hypochromasia of RBC, HCT↓, WBC↑, Reticulocytes↑, Methb (nonsign.)↑, transferrin ↑, total iron binding capacity↑		≥ 10 mg: Hb adducts↑, Heinz bodies in 4/6 m ≥ 30 mg: RBC↓, Hb↓, MCV↑, MCH↑, Reticulocytes↑, hypochromasia of RBC, Heinz bodies↑, serum iron↑ 100 mg: normoblasts↑, polychromasia, RBC, HCT↓, MCHC↓, WBC↑, Methb (nonsign.)↑, transferrin↑, total iron binding capacity↑		Onset of erythrotoxicity at 10 mg, dose and time related significant macrocytic hemolytic anemia ≥ 30 mg, hypo/anisochromasia indicated unstable/degenerated haemoglobin, acclerated iron metabolism, tendency for methaemoglobinemia, leucocytosis
	Organ weight/spleen	≥ 30 mg: ↑ of abs/rel weight	100 mg: ↑ of abs/rel weight	≥ 30 mg: ↑ of abs/rel weight↑	≥ 30 mg: ↑ of abs/rel weight	Dose-related effects on spleen weight and spleen enlargement were attributed to vascular congestion
	Macroscopy/spleen	100 mg: enlargement in 6/6 m	100 mg: enlargement in 6/6 m	100 mg: enlargement in 6/6 m	100 mg: enlargement in 6/6 m	

Table 4.12 continued overleaf

Table 4.12 continued Results from an oral 28-day study with 1 week or 4-week administration of aniline

Results	Treatment period*	1 week application	1 week application	4 week application	4 week application	Assessment
	Histopathology/spleen	30 mg: minimal vascular congestion in 6/6 m 100 mg: moderate vascular congestion in 6/6 m, single focus of perisplenitis in 1/6 m	100 mg: moderate vascular congestion in 6/6 m, single focus of perisplenitis in 1/6 m	10 mg: minimal vascular congestion in 4/6 m 30 mg: slight vascular congestion in 6/6 m 100 mg: moderate vascular congestion in 6/6 m mild-moderate multifocal perisplenitis in 6/6 m	100 mg: slight-moderate vascular congestion in 6/6 m mild-moderate multifocal perisplenitis in 6/6 m	Dose- and time related increase of incidence and severity of vascular congestion $\geq$ 10 mg, time-related increase of incidence and multiplicity of perisplenitis at 100 mg,
	Histopathology/ bone marrow			100 mg: slight hypercellularity in 6/6 m		
	Histopathology/liver			100 mg: minimal hemosiderin deposition in 6/6 m minimal extramedullary hematopoiesis in 1/6 m		
	Immunohistology/ spleen				No treatment-related effects on the content and distribution of actin, desmin, vimentin, VEGF	
	Cell replication/spleen		100 mg: lymphoid, polymorphnuclear or solitary fibroblastic cells invading the perisplenic foci positive for BrdU		100 mg: lymphoid, polymorphnuclear or fibroblastic cells invading the perisplenic foci positive for BrdU	higher mitotic activity in persplenic plaques relecting proliferation of inflammatory cells and fibocytic cells

* Animals of all groups were necropsied on day 28/29, ↑↓ increase or decrease of values, significant if not indicated as nonsignificant (nonsign)
\# Pathology included macroscopy and weight of anestetized animal, liver, spleen, kidneys
* Histopathology in 0B, 3B and 6B: HandE, Perl's Iron reaction, Elastic van Giesion and immunohistology on actin, desmin, vimentin and vascular endothelial growth factor (VEGF) on spleen sections
§ Histopathology in groups 0A, 1A, 2A, 3A, 4A, 5A, 6A: HandE, Perl's Iron reaction: spleen, bone marrow, liver, kidneys, mandibular and mesenteric lymphnodes, Elastic-van Gieson: spleen
@ Hematology: Standard blood parameters according to OECD 407 plus hemoglobin adducts, methaemoglobin, serum transferrrin, iron, total iron-binding capacity (TIBC)
© Cell replication: S phase response using BRDU in corporation

The data clearly indicated that hemolytic anemia was associated with hemoglobin disorders. Heinz bodies were seen in animals of all dose groups composed of denaturated protein that primarily consists of hemoglobin. They are absent in normal healthy individuals and were not seen in the control animals (group 0A). Although the increase in Heinz bodies gained significance at dose groups ≥ 30 mg/kg (see above), formation of Heinz bodies was also observed in a number of animals in the 10 mg groups. The mean counts and lower/upper limits increased with dosage (see **Table 4.13**). Appearance of Heinz bodies at the 10 mg dosage can be interpreted indicative for differences in the susceptibility between individual animals.

Hypochromasia of erythrocytes indicated reduced haemoglobin concentration, haemoglobin distribution was abnormal (anisochromasia) in animals of the 100 mg dose groups.

MCV, MCH, and MCHC are average quantities and therefore may not detect abnormalities in blood with mixed-cell populations of normal and abnormal erythrocytes. Grade 1 hypochromasia was seen with dose-related increased incidences in all test groups (≥ 10 mg/kg). However, statistically significant decrease of MCHC was only seen at 100 mg/kg.

Appearance of erythrocytes with abnormal morphology, size or staining properties were known to be more sensitive than significant changes of average means indicating anemia.

Anisocytosis, an early marker of morphologic change of erythrocytes in iron deficiency anemia, was only seen in the 100 mg dose groups with 1 week administration, but not after 4 weeks of treatment.

Table 4.13 Erythrocyte morphology in blood film examination

Treatment period	**1 week**			**4 weeks**			
Dose groups (6 males/group)	4A (10 mg)	5A (30 mg)	6A (100 mg)	0A (control)	1A (10 mg)	2A (30 mg)	3A (100 mg)
Heinz bodies(‰ RBC · 10^{-3})							
No. of males/group	2/6	6/6	6/6	0/6	4/6	6/6	6/6
Range within group (mean)	1-6 (4)	15-47 (27)	420-548 (473)	0	4-9 (4)	32-54 (44)	276-315 (289)
Hypochromasia							
No. of males/ group (total)	0/6	2/6	6/6	0/6	1/6	3/6	6/6
Grade 1	0/6	2/6	6/6		1/6	3/6	5/6
Grade 2							1/6
Anisochromasia							
No. of males/ group (total)	0/6	0/6	6/6	0/6	0/6	0/6	6/6
Grade 1			1/6				5/6
Grade 2			3/6				1/6
Grade 3			2/6				

Table 4.13 continued overleaf

Table 4.13 continued Erythrocyte morphology in blood film examination

Treatment period	1 week			4 weeks			
Anisocytosis							
No. of males/ group (total)	0/6	0/6	6/6	0/6	0/6	0/6	0/6
Grade 1			3/6				
Grade 2			3/6				

Study defaults

Positive control tissues used for the immunohistological examinations on actin, vimentin, VEGF and BrdU were not evaluated. Methods applied and results (positive for BrdU or negative for others) were not documented in this report. A specific reaction for the VEGF examined was neither seen in the spleen sections nor in the lung tissue that served as positive control. Therefore validity of these examinations is restricted.

Hemosiderin deposition was expected to be increased in the spleen of treated animals with hemolytic anemia. However, the Prussian blue reaction failed to demonstrate any difference of spleen hemosiderosis in treated animals compared to control animals.

Conclusion

On the basis of dosages chosen in this study, a NOAEL was not estimated. The low dosage of 10 mg (4 mg aniline) (LOAEL) was clearly toxic to erythrocytes, and induced formation of haemoglobin adducts in peripheral blood. Vascular congestion of spleen known as early lesion in the cascade of events leading to chronic spleen toxicity has been observed at 10 mg. The above reported adverse effects on red blood parameters and spleen increased dose-related.

Significant changes of red blood cell parameters characterising a hypochromatic hemolytic anemia were seen at a dose of 30 mg/kg and above. Although mean counts for red blood cells in animals that received 10 mg/kg were similar to that of control, low numbers of Heinz bodies in 4/6 males and a single animal with hypo/anisochromasia were considered as early indicators of erythrotoxicity. Increased formation of haemoglobin adducts compared to background levels was observed from the 10 mg dose upwards.

Reticulocytosis at 30 mg and above, higher transferring levels and total iron binding capacity, bone marrow hypercellularity and extramedullary hematopoietic activity in animals receiving 100 mg/kg reflected the attempts to compensate the anemic status.

A tendency towards higher concentrations of methaemoglobin was found in the high-dose group. In contrast to the expected finding of methaemoglobin formation being significantly raised, this finding may be attributed to high methaemoglobin reductase activity in rats, the aniline intake via feed during night and the long fasting period before blood sampling.

Unless expected from other aniline studies, this study did not confirm significant increases of hemosiderin deposition and hematopoietic activity in the spleen. The authors explained the absence of differences between treated and control animals by the insensitivity of the test methods and high physiological background in control animals. The presence of both findings in the liver clearly indicated that hemosiderin deposition and extramedullary hematopoiesis were induced by treatment. From other studies on hemolytic agents it is well known that the spleen is

the primary location for erythrocyte cleavage and commonly shows hemosiderin deposition and compensatory hematopoietic activity at earlier onset and at lower dosage compared to those inducing accessory effects in liver and kidneys.

Gross enlargement of the spleen and increased spleen weight were contributed to the vascular congestion that has been observed in all dose groups with dose-related increase in incidence and mean severity. Microscopy of the altered red pulp was more sensitive than macroscopy and weight determination. Differences in the content and distribution of actin, desmin and vimentin filaments were reported to be absent at the end of the study. This indicated that there was no treatment-related increased production of filaments by muscle or fibroblastic cells or increased proliferation of these cells within this study. That means that structural changes, e.g., hypertrophy or hyperplasia of stromal or endothelial compartments could not be detected and was not responsible for sinusoidal congestion.

A relationship between leucocytosis and perisplenitis may exist. Due to the authors opinion, the occurrence of perisplenitis was associated to increased numbers of WBC. However, increase of WBC was most pronounced after the 1-week administration when perisplenitis was occasionally found as solitary young plaques. There was no progression in the amount of WBC increase after 4 weeks of treatment, whereas the severity and multiplicity of perisplenitic plaques were raised. It appears that the course of leucocytosis reflected the time course of acute to subacute nature of the perisplenic lesion.

After 4 weeks of treatment, the perisplenitis was described as flat, fungiform or papillary cell accumulations on the surface of the spleen between splenic capsule and mesothelium. The majority of cells were fibroblasts and fibrocytes wrapped in collagen rich matrix and a central core of connective tissue. Other cells were lymphoid cells, a few polymorphnuclear granulocytes, and iron-laden macrophages. Higher cell replication rates in foci of perisplenitis were attributed to mitotic activity of lymphoid cells and fibroblastic cells.

Cellular accumulations on the spleen surface were also observed in animals receiving aniline hydrocloride for one week. In contrast to those seen after 4 weeks of treatment they content only individual fibroblasts with very few collagen fibers, embedded in amorphous intercellular ground substance and intermingled with few erythrocytes and very few and very small iron-laden granules indicating small hemorrhages.

Comparing both treatment schedules, the nature of the plaques became more fibrotic and phagocytosis of erythrocytes or intercellular iron-positive granules appeared after 4 weeks of treatment. Interestingly, the lymphoid cells were reported for both time intervals as poorly differentiated cells with a round to ovoid faint to dark blue nucleus with absent or poorly visible cytoplasm. The role of these cells in the inflammatory and fibrotic responses is currently unknown. Further studies are necessary to identify the fate of the perisplenic plaque formation. Whether the perisplenitis and the fibroblastic proliferation is related to tumorigenicity of aniline hydrochloride representing the initial lesions in the development of a variety of sarcomas has to be clarified, too.

The authors concluded that aniline-related spleen tumores are late-onset sequelae of a long lasting inflammatory spleen toxicity caused by a preceeding hematoxic mechanism. They concluded that two dose ranges may be identified: a low dosage of 10 mg/kg limited to the haematotoxic effects and without adverse effect on the spleen and higher dosages (≥ 30 mg/kg) that causes spleen toxicity, hemotoxicity and related tumor formation. In contrast to this opinion, this study does not allow to identify a dose without spleen toxicity because the low dosage of 10 mg showed clear evidence on hemotoxicity and spleen toxicity.

Other treatment-related effects

Treatment-related adverse effects were also observed in the adrenals and the ovaries. Repeated subcutaneous applications of 300 mg/kg of aniline on 6 days to female rats resulted in hypertrophia of the adrenals cortex and marked increase of adrenal weight. Weight increase was inhibited by glucocorticoid pretreatment and accelerated by estradiol pretreatment (Lefebvre and Szabo, 1971). Enlargement of the adrenal cortex was also found in rats after receiving 7 or 14 subcutaneous injections of 30 mg/kg bw/d of aniline. Histochemistry revealed decreased activities of succinate dehydrogenase, lactate dehydrogenase and steroid-3ß-ol dehydrogenase indicating an inhibition of steroid synthesis.

In six female rats after subcutaneous injection on 7 days of 50 mg/kg bw/d of aniline, ovaries had lower weights and corpora lutea exhibited numerous large lipid-storing clear cells and reduced activity of steroid-3ß-ol dehydrogenase compared to six control rats. Ovary weight reduction was slightly and non significant (means of 19.3 ± 1.0 mg versus 17.5 ± 0.9 mg in controls). Ultrastructurally, the endoplasmic reticulum disappeared partly or totally (Hatakeyama et al., 1971). Significantly lower ovary weights (absolute and relative) were reported in rats after 104 weeks of aniline hydrochloride treatment at a dose of 100 mg/kg bw/d (CIIT, 1982), but no weight change was obvious after week 26, 52 and 78. No treatment-related microscopic lesion was observed at the end of study or at interim sacrifices.

Aniline treatment also affected the weight of testes. Male rats which were administered to 600 ppm of aniline hydrochloride in the drinking water (equivalent to 43 mg/kg bw/d of aniline) showed lower weights at days 60 and 90 of treatment (Khan et al., 1993). No indication on microscopic lesions of the rat testis was reported in this study. Examination of testis weight and microscopy was also conducted in the 14-day study of Khan et al., 1995b. No treatment-related changes were reported for these parameters in the rats which received 0.7 mmol kg bw/d (65mg/kg bw/d).

Comment on aniline effects on the ovaries (study of Hatakeyam et al., 1971): The number of animals treated and the treatment duration was considered to be insufficient to clearly demonstrate an atrophy of the rat ovaries. Lipid-storing cells of the corpora lutea and disappearance of the endoplasmatic reticulum are also normal features seen in untreated animals during luteal regression phase. To the rapporteur's opinion, no conclusion can be drawn from this study. In summary, there is no clear indication of adverse effects of aniline on the ovaries as well as on the testis.

Higher levels of serum immunoglobulines IgA and IgG and decreases in splenic T-helper (CD4+/CD8-) population after oral administration for 90 days were also observed as a result of aniline hydrochloride treatment in Sprague-Dawley rats (Khan et al., 1993). Whether these changes reflect an immunotoxic potential will need further studies.

Biomarkers of aniline toxicity

From the results of the subacute/subchronic studies, formerly it has been concluded that parameters like methaemoglobinemia and Heinz bodies were sensitive to indicate aniline-induced toxicity. Changes of these parameters occurred at the same dose level (Burgess et al., 1984a; b; Oberst et al., 1956) or at lower dose level (CIIT, 1977) compared to the dosage which induces morphologic lesions. However, this comparison is incomplete as far as none of these studies included the full spectrum of parameters/organs on haematology, clinical chemistry, gross pathology and microscopic examinations according to the current guideline test protocols. In contrast to this, the 104-week study (CIIT, 1982) revealed that methaemoglobinemia (> 30 mg/kg

dose groups) and Heinz bodies (100 mg/kg dose group) occurred at higher doses than the changes of other red blood cell parameters (RBC, HCT, Hb, MCV), reticulocyte counts, and pigment accumulation and haemopoiesis in the spleen. These represent the most sensitive indicators of haemotoxicity.

The reversibility of increased methaemoglobin concentration demonstrated by some studies as a decline after maximum levels cannot be interpreted indicating that the damage of erythrocytes was also restricted to some hours analogous to time course of methaemoglobin concentrations. Associated to the methaemoglobinemia each affected erythrocyte presumably is irreversibly damaged and undergoes the degradation by macrophages which are primarily active at the spleen. The haematopoietic system shows increased haematopoiesis as a response to the higher demand secondarily to erythrotoxicity. As no sign of bone marrow damage, e.g. irreversible damage of stem cells, was evident from the studies available, the erythrotoxicity was reversible in that sense that the haematopoietic system was able to regenerate.

Based on the data available and unless the time point of maximum formation of methaemoglobin formation is not considered, information on methaemoglobin concentration and Heinz bodies from the rat are not considered to be appropriate as most predictive indicators of aniline-related toxicity. For quantitative risk calculations dose limits, e.g. a NOAEL, should not be derived from it. Additionally it is well known that the rat has relatively low sensitivity of methaemoglobin formation (Jenkins et al., 1972).

4.1.2.6.2 Studies in humans

Investigations on the effects of aniline on human health were of limited quality. References contain no or only limited exposure data (e.g. air/liquid concentration of aniline, duration of exposure, determination method). People were simultaneously exposed to other compounds or possible carcinogens. Mostly exposure was qualitatively reported only by separating exposed from unexposed groups. Therefore only one report on effects on exposed humans was cited.

In a clinical study by Jenkins et al. (1972) aniline was administered orally to 20 volunteers at doses (total amount) of 5, 15, or 25 mg on three successive days. Higher doses were then administered to some of these volunteers. Five of them received higher doses of 35 or 45 mg, two received 55 and one 65 mg on successive days (no further details on total number of days available). Methaemoglobin was estimated in blood obtained from a pricked finger, 1, 2, 3 and sometimes 4 hours after administration of aniline. Blood was obtained by venepuncture 24 hours after each dose of aniline and was subjected to the following tests: Staining for Heinz bodies; examination of blood films and buffy coat preparations; estimation of methaemoglobin, packed cell volume, percentage reticulocytes, total serum proteins, serum albumin, globulin, activities of ASAT, ALAT and alkaline phosphatase, total serum bilirubin, direct-reading bilirubin and blood urea; thymol turbidity test. Erythrocyte sedimentation rate, PCV, percentage of reticulocytes and the differential white cell count were also investigated. After voiding the bladder at 2 pm., urine was obtained at 4 pm. and tested for urobilinogen, glucose and protein. Methaemoglobin increased significantly at doses of 25 mg or higher in comparison to the methaemoglobin level at 5 mg dose. Maximum level of 16.1% methaemoglobin was reached 2 hours after administration of 65 mg aniline, the level was within normal limits one hour later. The mean maximum increase of methaemoglobin concentrations were 2.46%, 3.68%, 7.08% and 5.17% at doses of 25, 35, 45, resp. 55 mg aniline (without any data when the maximum was reached). A slight increase of serum bilirubin in two males (without any quantification) following the uptake of 45 and 65 mg of aniline (0.6, resp. 0.9 mg/kg bw/d) give indication on an increased haemoglobin catabolism.

No other adverse effect was observed in selected parameters of haematology, clinical chemistry and urinalysis. It was not checked in these studies whether the volunteers were slow or fast acetylators. In slow acetylators increased sensitivity to aniline has to be expected.

No clear evidence on aniline-related bladder tumors was seen in a cohort study on cancer morbidity and mortality of male workers from a factory in the rubber industry (Sorahan et al., 2000). Among 385 workers with a minimum exposure of 6 months there was a slight excess of cancer-related mortalities (observed 4, expected 2) without gaining significance. However, data on smoking habits were not available and a number of workers were coexposed to other chemicals with significantly increased incidences of bladder cancer.

4.1.2.6.3 No observed adverse effect level (NOAEL)

Oral exposure

The combined chronic and carcinogenicity study in rats (CIIT, 1982, **Table 4.11**) was accepted for the minimum requirements of the regulation 793/93/EEC. Although clinical chemistry parameters did only include alkaline phosphatase, blood urea nitrogen and serum alanine aminotransferase, this study showed the best concordance to the standard test parameters of the OECD Test Guideline 407. This was the primary reason to consider this study the most relevant with regard to the establishing of a N/LOAEL. Secondarily, it was also selected because of the chronic administration of aniline. A NOAEL was not estimated in the CIIT study, the LOAEL of systemic toxic effects (non neoplastic lesions) was 7 mg/kg bw/d of aniline.

Other studies on subacute and subchronic toxicity of aniline (**Table 4.11**) were not in (full) compliance to the current test guidelines. Most of them have been focused on selected parameters. Main findings of these studies showed good consistency and therefore they were also considered for the effect assessment.

Inhalation exposure

The existent studies with repeated inhalation exposures were not in accordance to the test design of the current guidelines. Therefore no valid data on effects via inhalation of aniline are available. None of the existent studies investigated local effects on the respiratory tract. With respect to systemic effects, the most reliable study seems to be the subacute inhalation study on rats (EPA, 1981) due to its haematological, clinical chemical and pathomorphological examinations. The discrepancies of this study compared to the actual standards were the lack of female animals at test, the limited exposure period (14 days instead of 28 days), the limited spectrum of organs examined and lack of a full report, since the EPA document was a summary only. The LOAEC derived from this study was 17 ppm (2 -week study, exposure on 5 days/week, 6 hours/day). This is in line with sparce data from a 26-week rat study (Oberst et al., 1956) on which no further detail than the occurrence of methaemoglobinemia and cyanosis was reported in rats at doses of 5 ppm (19 mg/m^3).

4.1.2.6.4 Conclusion on repeated dose toxicity

Overt haemotoxicity or indication on this is the relevant toxic effect consisting of haemolytic anaemia and its consequential alterations. This was seen in repeated dose studies with oral administration at dosages, which need classification as toxic and labelling with T, R 48/25.

Although the parameters examined, the sensitivity of examination and the quantification of the results were very different between the studies performed, the toxic profile observed after prolonged aniline exposure was very consistent within the rat studies. Experience from humans after repeated oral uptake also give indications on haemotoxicity (besides methaemoglobin formation) at dosages from 0.4 mg/kg bw/d (Jenkins et al., 1972). Database on the inhalation route is insufficient. However, the limited studies give also indications that aniline is haemotoxic at very low concentrations (5 ppm (19 mg/m^3)/26 weeks (Oberst et al., 1956, ≥ 17 ppm (approx. 66 mg/m^3)/2 wk (EPA, 1981). Although no dermal study is available, the dermal route was also included in labelling because aniline is well absorbed after all exposure routes.

Aniline is classified as toxic and labeled with T, R 48/23/24/25.

4.1.2.7 Mutagenicity

4.1.2.7.1 *In vitro* studies

Bacterial mutation assays

A bacterial gene mutation test was negative in *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 for doses up to 6,666 µg/plate without S-9 mix or in the presence of Aroclor-induced S-9 mix from rat or Syrian hamster livers; the highest dose produced toxicity in all strains with and without S-9 mix (Haworth et al., 1983). Negative bacterial mutation tests with aniline were also reported by other authors (e.g. Simmon et al., 1979; de Flora, 1981; Dunkel et al., 1984; Nakamura et al., 1987; Jung et al., 1992).

Incubation of aniline and the comutagen norharman in the presence of S-9 mix results in a dose-dependent increase of revertants of *S. typhimurium*. TA98 from 20 to 200 µg/ml aniline (Nagao et al., 1977). Just recently, a highly potent direct mutagen aminophenylnorharman was identified as the reaction product of aniline and norharman (Totsuka et al., 1998).

Mammalian cell gene mutation assays

In five mice lymphoma assays aniline led to controversial findings. A clear positive effect was obtained by Wangenheim and Bolcsfoldi (1988) with S-9 mix in the dose range 0.5 to 5.0 mmol/l (46.5 to 465 µg/ml) and without S-9 mix in the dose range 10 to 15 mmol/l (930 to 1,395 µg/ml). Weak positive effects with and without S-9 mix were reported for high-doses by Mitchell et al. (1988), Myhr and Caspary (1988) and McGregor et al. (1991). A negative result-only tested in presence of S-9 mix-is published by Amacher et al. (1980) for doses up to 1,100 µg/ml. Colony sizing was conducted in none of these investigations.

In a poorly documented HPRT assay with V79 cells aniline was positive in the presence of S-9 mix for the extremely high-doses of 50 and 60 mmol/l (4,650 and 5,580 µg/ml), and it was negative without exogenous activation for doses up to 20 mmol/l (1,860 µg/ml); in the presence of primary rat hepatocytes an equivocal result was obtained (Fassina et al., 1990).

In vitro chromosomal aberration assays

In the presence of S-9 mix *in vitro* chromosomal aberration tests were positive for high-aniline doses in CHO cells (Galloway et al., 1987; 1,600 and 5,000 µg/ml), in CHL cells (Ishidate,

1988; 1,000 and 2,000 µg/ml) and V79 cells (Miltenburger, 1986; 4,300 µg/ml). Cytotoxicity data were not reported.

Without S-9 mix negative results were established in CHO cells by Galloway et al. (1987; highest dose 1,600 µg/ml) and in CHL cells by Ishidate and Odashima (1977; highest dose 250 µg/ml). Positive findings are described for doses of 1,000 and 2,000 µg/ml in CHL cells by Ishidate et al. (1988) and for a dose of 4,300 µg/ml in V79 cells by Miltenburger (1986).

Micronucleus assay

Aniline was reported to be negative on an inadequately documented *in vitro* micronucleus assay with Syrian hamster embryonic (SHE) cells without S-9 mix (Fritzenschaf et al., 1993).

Sister chromatid exchanges (SCE)

Aniline (as hydrochloride) induced SCE at concentrations of 5 and 10 mmol/l (465 and 930 µg/ml) in human fibroblasts which were described as lacking significant levels of cytochrome P-450 and NADPH-cytochrome P-450 reductase (Wilmer et al., 1981). In this study a series of different aniline metabolites were also tested from which o-aminophenol and N-phenylhydroxylamine were the most effective SCE-inducers already at 0.1 and 0.05 mmol/l aniline, respectively.

In concanavalin A induced T lymphocytes from whole blood culteres aniline induced SCE in a dose-dependent manner in a concentration range from 0.1 to 1.0 mmol/l (9.3 to 93 µg/ml) (Wilmer et al., 1984); this effect was not seen in cultures with purified lymphocytes whereas a marginal increase of SCE was observed when purified lymphocytes were exposed to aniline in the presence of 1,000 µg/ml haemoglobin. From these results it is concluded that erythrocytes contribute to the transformation of aniline into genotoxic intermediates.

In CHO cells aniline induced SCE at concentrations from 50 to 500 µg/ml without additional metabolising system. In the presence of S9-mix the effective concentrations were 3,000 to 5,000 µg/ml. Although the increase of SCE was marginal, it was reproducible with and without S9-mix. The different effective concentrations with and without S9-mix reflect the long-term exposure, without, and short-term exposure, with S9-mix (Galloway et al., 1987).

Unscheduled DNA synthesis (UDS)

Aniline did not induce DNA repair (unscheduled DNA synthesis, UDS) in primary human (Butterworth et al., 1989) or rat hepatocytes (Yoshimi et al., 1988) in doses up to 1.0 mmol/l (93 µg/ml); data on cytotoxicity were not given.

DNA strand breaks

In the presence of S-9 mix an increase in DNA strand breaks was found in mouse lymphoma cells at the extremely high-aniline dose of 21.5 mmol/l; according to the authors this result is equivocal (Garberg et al., 1988). Without S-9 mix negative results were obtained (Garberg et al., 1988; Kozumbo et al., 1992). Slight toxicity was reported with and without S-9 mix by Garberg et al. (1988).

4.1.2.7.2 *In vivo* studies

Host mediated assay

Urine samples of about 10 rats (figures given for different groups: 8-12 animals) receiving 300 mg/kg aniline p.o. were collected during 24 hours after application. The ether extracts of the urine probes were tested for mutagenicity with *S. typhimurium* TA 100 and TA98; a clear dose-dependent effect was obtained with TA98 in the presence of S9-mix (Tanaka et al., 1980).

Micronucleus assays

There are several independent investigations on the capacity of aniline to induce micronuclei in bone marrow cells of mice or rats after oral or intraperitoneal application. In general, the tests were conducted in line with recent guidelines. For details see **Table 4.14**.

Table 4.14 Aniline: Overview on *in vivo* micronucleus tests with mouse and rat bone marrow cells

Species	Sex	Type of exposure	Sampling times (h)	Dose range (mg/kg)	LOED (mg/kg)	Max. MN freq. (neg.co)	Genetic effect	Decrease in P/N ratio	Reference
Mouse	m	2 · i.p.	6 to 48	100-380	380	1.43% (0.27%)	positive	no	Ashby et al. (1991)
Mouse	m	1 · i.p.	24	380	380	0.31% (0.03%)	positive	inconcl.	Westmoreland and Gatehouse (1991)
Mouse	m	1 · p.o.	24 to 48	400-1,000	1,000	0.61% (0.04%)	positive	inconcl.	Westmoreland and Gatehouse (1991)
Mouse	m + f	2 · i.p.	24 to 48	30-300	300	2.58% (0.16%)	positive	yes	Vlachos (1989)
Mouse	m	1 · p.o.	24	125-250	-	-	negative	no data	Harper et al. (1984)
Mouse	m + f	1 · p.o.	24, 48, 72	610			negative	yes	BG Chemie (1985)
Rat	m	1 · p.o.	24	215-500	287	0.36% (0.17%)	positive	yes	George et al. (1990a)
			48	215-500	400	0.41% (0.17%)	positive	no*	
Rat	m	1 · p.o.	48	500			negative	no	Bayer AG (2001a)

LOED lowest observed effective dose
P/N ratio, PCE/NCE ratio, increase in P/N ratio
f females
m males

In mice, positive bone marrow micronucleus tests were reported by Ashby et al. (1991), Vlachos (1989) and Westmoreland and Gatehouse (1991). Micronuclei were only induced by the highest doses tested which also produced severe toxicity. After intraperitoneal administration the effective doses were 300 mg/kg (Vlachos, 1989) and 380 mg/kg (Ashby et al., 1991; Westmoreland and Gatehouse, 1991), the latter corresponding to 80% of the LD-50 (Ashby et al., 1991). After oral administration a positive response was found after administration of 1,000 mg/kg by Westmoreland and Gatehouse (1991). Harper et al. (1984) reported on a negative finding, however, relatively low doses of 125 and 250 mg/kg were used since their goal was to investigate the effect of co-exposure of benzene and aniline. In a well performed

micronucleus test BG-Chemie (1985) estimated the oral MTD of 610 mg/kg as 80% of the LD_{50} and none of the animals treated with 610 mg/kg aniline showed signs of toxicity and only male mice showed slight reduction in PCE/NCE ratio. Frequencies of micronuclei were similar for controls and treated animals of both sexes.

The positive mouse micronucleus data were extensively discussed in the literature because mice are non-responsive to aniline-induced carcinogenicity. Ashby et al. (1991) discussed the possibility that the induction of micronuclei may be the indirect result of erythrocyte degeneration/iron precipitation due to methaemoglobinaemia. This idea, however, was not supported by data from other inducers of methaemoglobinaemia. Westmoreland and Gatehouse (1991) emphasised the occurrence of abnormally shaped micronuclei which may be induced by a usual mechanism. Furthermore, they state that the use of high-doses could render less meaningful data.

In rats a weak induction of micronuclei in bone marrow cells was reported by George et al. (1990a) for single oral doses of aniline in doses ranging from 215 to 500 mg/kg. At a sampling time of 24 hours the genetic effect at 287, 400 and 500 mg/kg was paralleled by decreasing P/N ratios. At a sampling time of 48 hours a genetic effect was observed at the two highest tested doses of 400 and 500 mg/kg; at all tested doses an increase of P/N ratios was observed. No clinical signs were seen. A problematic aspect of this study is that micronucleus scoring might be of low reliability due to low DNA specificity of the staining (haematoxylin and eosin). The positive result reported by George et al. (1990a) was checked by a re-investigation carried out by Bayer AG (2001a), which resulted in a negative response. However, only the effect of 500 mg/kg at a sampling time of 48 hours was investigated. The treated animals showed clinical signs; no cytotoxic effects were observed. The negative result of the re-investigation is not fully reliable due to methodological insufficiencies, especially only 1 dose and 1 late sampling time.

Chromosomal aberration assay

An *in vivo* chromosomal aberration assay with mouse bone marrow cells led to a negative result (Bayer AG, 2001b). Two doses of 220, 300 and 380 mg/kg were given intraperitoneally with an interval of 24 hours sampling times were 16 hours, 20 hours and 24 hours after second treatment. All doses induced clinical symptoms, no cytotoxic effects were induced.

Induction of sister chromatid exchanges

In mouse bone marrow cells a weak dose-dependent induction of sister chromatid exchanges (SCE) was obtained after intraperitoneal administration of aniline doses ranging from 61 to 420 mg/kg (Parodi et al., 1982). Three to 9 animals were used per experimental group; sampling was done 24 hours after treatment.

DNA strand breaks

Induction of DNA strand breaks was investigated by the alkaline elution technique in various tissues of rats and mice after intraperitoneal administration of aniline. Parodi et al. (1982) reported positive findings for rat livers (from 105 mg/kg upwards) and kidney (210 mg/kg) but a negative finding for rat spleen (210 mg/kg) and livers, kidney and bone marrow of mice (210 mg/kg). According to Cesarone et al. (1982) DNA strand breaks were induced in mouse kidneys by intraperitoneal administration of 300 mg/kg aniline, in livers a negative finding was obtained.

DNA binding

After single intraperitoneal injection of aniline to rats, relatively low DNA binding capacities were 25 and 60 hours later in liver, kidney and spleen (Roberts and Warwick, 1966). In this study tritium-labelled aniline was used. Since nucleotides were not analysed, unspecific incorporation of tritium in DNA cannot be excluded. The covalent binding index (CBI) according to Lutz (1979) was as low as 3.7 for the liver.

McCarthy et al. (1985) compared DNA-binding after single application of doses up to 250 mg/kg ^{14}C-aniline with that after seven pre-doses, in the maximum seven pre-doses of 50 mg/kg plus a final dose of 250 mg/kg were given. In rats of the highest dose group, pre-dosing led to higher DNA-binding in kidneys (CBI 14.2 as compared to 7.4 without pre-dosing). In spleen and large intestine CBIs of 3.7 and 4.3 were measured. No substantial DNA binding was found in rat livers and in mice organs. With respect to the spleen CBI it has to be considered that spleens are composed by various cell types including a high percentage of lymphocytes.

Germ cell mutagenicity

A dominant lethal assay with Wistar derived rats was conducted according to recent guidelines (CTL, 1998). Groups of 40 male rats proven of fertility were dosed with 47, 150 or 200 mg/kg aniline/day i.p. for 5 consecutive days. Appropriate negative and positive controls were included. The males were mated 1:1 with females of the same strain for ten consecutive one-week periods.

In general, findings were negative. However, in the 200 mg/kg/day aniline group at week 3 the number of live implants was decreased slightly but statistically significant; furthermore, the number of early deaths was increased. These findings were triggered by a sub-group of 7 out 40 animals with clearly reduced numbers of live implants and elevated number of early dead implants. The overall result cannot be judged as negative nor as positive (due to the weakness of the effects). Therefore, the test result is considered to be inconclusive.

Table 4.15 Aniline - Dominant lethal assay with rats: relevant data from negative controls and from animals treated with 200 mg/kg aniline

	No. of implants per female		Live implants per female		Early deaths per female	
	neg. contr.	200 mg/kg aniline	neg. contr.	200 mg/kg aniline	neg. contr.	200 mg/kg aniline
Pre-exptl.	14.3	14.3	13.8	13.8	0.5	0.5
Week 1	14.4	13.3	13.5	12.2	0.7	0.9
Week 2	15.1	13.8	14.6	13.0	0.6	0.7
Week 3	14.9	13.8	13.9	12.3	0.8	1.4
Week 4	15.2	14.4	14.1	13.9	1.0	0.5
Week 5	14.4	14.7	13.4	13.9	0.7	0.6
Week 6	14.4	13.8	13.7	13.5	0.6	0.8
Week 7	15.6	15.9	14.6	13.5	0.9	0.7
Week 8	14.9	14.2	14.2	13.9	0.6	0.8
Week 9	14.0	13.4	13.4	14.6	0.6	0.7
Week 10	15.2	14.0	14.5	13.4	0.6	0.6

Sperm head abnormalities

Groups of 5 male mice received 5 daily i.p. injections of doses ranging from 17 to 200 mg/kg aniline hydrochloride. Five weeks after the last dose there was no increase in the frequency of sperm head abnormalities (Topham, 1980a).

In vivo mutagenicity data on compounds structurally related to aniline

Relevant *in vivo* mutagenicity data are available for 4-aminophenol, a main metabolite of aniline which is systemically available. 4-Aminophenol is positive in *in vivo* micronucleus tests with mice in various tissues. In bone marrow cells increased micronucleus frequencies were found after two oral doses ranging from 109 to 436 mg/kg (Wild et al., 1980a). In liver cells two intraperitoneal doses of 107 or 214 mg/kg induced micronuclei (Cliet et al., 1989). Benning et al. (1994) reported that increased frequencies of micronucleated cells were found in mouse splenocytes by oral doses ranging from 53 to 214 mg/kg. The authors discuss that 4-aminophenol is an indirect clastogen with short-lived metabolites, and they speculate that either the compound is activated in the liver and the locally generated metabolites enter circulating lymphocytes when they pass through the liver or it may be metabolised in the splenocytes.

Azobenzene-a "dimmer" of aniline which is metabolised into two molecules of aniline-induces micronuclei in bone marrow cells of rats and mice (George et al., 1990a; 1990b). As for aniline, the effect is far more pronounced in rats than in mice.

Furthermore, data from *in vivo* micronucleus tests are available for two classes of structurally related compounds, amino-anilines and alkyl-anilines.

The amino-aniline, 1,2-phenylendiamine is positive in mice, Chinese hamsters and guinea pigs (Wild et al., 1980b), whereas negative results are reported for 1,3-phenylenediamine (Fraunhofer-Institut-ITA, 1994).

Four alkyl-anilines gave negative results in micronucleus tests with mice: 2,6-xylidine (Parton et al., 1988; 1990), o-toluidine (McFee et al., 1989; Morita et al., 1997) and 2,4-diaminotoluene (Morita et al., 1997). In rats, 2,4-diaminotoluene led to an inconclusive finding (George and Westmoreland, 1991) and for 2,6-diaminotoluene a negative and an inconclusive finding are reported (Allavena et al., 1992; George and Westmoreland, 1991).

4.1.2.7.3 Conclusion on mutagenicity

Aniline is negative in routine bacterial mutation tests. In mammalian cell cultures positive effects were obtained with respect to chromosomal effects, SCE and possibly for gene mutations. In general, stronger effects are induced in the presence of an exogenous metabolic activation system than in the absence. *In vivo*, in bone marrow assays with mice a negative response was found in one investigation on chromosomal aberrations, whereas weak positive effects were found in micronucleus tests, which, however, were limited to high-doses in the toxic range. In rats induction of micronuclei was found at several doses. A re-analysis led to a negative response is not fully reliable. The mutagenicity *in vitro* and *in vivo* of aniline is supported by *in vivo* studies showing DNA strand breaks and DNA adducts in different organs.

Furthermore mutagenicity data of a metabolite (4-aminophenol) and a structurally related substance (azobenzene) strengthen the evidence for mutagencity of aniline in somatic cells of animals. Available data on germ cell mutagenicity, which are negative (sperm head anomalies)

or equivocal (dominant lethal assay), are of limited predictive values due to relatively poor sensitivities of the test systems. A chromosomal aberration test on spermatogonia was not conducted.

The available data of mutagenicity are not sufficient to classify aniline as a category 2 mutagen, however, due to the positive findings in several *in vitro* and *in vivo* tests, especially in the bone marrow micronucleus test with rats aniline is classified as a category 3 mutagen and labelled with "R 68, possible risk of irreversible effects".

4.1.2.8 Carcinogenicity

4.1.2.8.1 Studies in animals

Cancer studies with oral administration of aniline hydrochloride

Rats

In a CIIT study (1982) dietary intake of aniline hydrochloride for 104 weeks at levels of 10, 30, 100 mg/kg bw/d (equivalent to aniline doses of 7, 22, 72 mg/kg bw/d) to F 344 rats (a total of 130 animals per sex and group) was associated with an increased incidence of primary splenic sarcomas. At 26 and 52 weeks, ten rats of each sex in each group, and at week 78, twenty rats of each sex in each group were sacrificed intermittently. The remaining 90 animals per sex and group were treated until the termination of the study. Summary tables on non neoplastic findings and statistical evaluation on overall mortality rates and on pathological findings including tumor incidences were absent in the study report. Individual finding tables data revealed that mortalities were more frequent in the high-dose males from the week 78 to the end of the study where 20 high-dose males died premature or were sacrificed moribund compared to 13 males in the control group and 14 and 11 males in the low-dose and mid-dose groups, respectively. (The total number of mortalities was included in **Table 4.16**) Although cause of deaths were not discussed in the report, premature deaths in high-dose males were obviously not related to toxicity, several of these were tumor bearing animals. Whereas control animals had no primary spleen tumors, 35 high-dose males had spleen tumors (27%), nine of which were reported in the group of premature deaths (**Table 4.16**). In the mid-dosegroup, a spleen tumor (stromal sarcoma) was found in a single male. Females had no spleen tumor other than a haemangiosarcoma in a high-dose female. Other findings of non neoplastic nature were also related to the treatment. Stromal hyperplasia were characterised by morphologic similarities to the cell type of the stromal sarcoma possibly representing pre-neoplastic lesions. Other treatment-related findings were chronic capsulitis, fatty morphosis (incidences given in **Table 4.16**). Also, severity of haemosiderin and severity of extramedullary haematopoiesis in high-dose males, incidence of splenic atrophy in high-dose males and females, severity and frequence of splenic congestion in treated males and high-dose females were reported to be increased (no summary data available in the study report). Other non neoplastic findings are cited in **Table 4.17**.

A dose-related increase of tumors of the spleen was also observed in an earlier 103-week study with aniline hydrochloride (NCI, 1978). Groups of 50 F-344 rats received a diet with 0.3 and 0.6% of aniline hydrochloride, control groups contained 25 males and females (see also **Table 4.17**). Dosages were equivalent to aniline doses of 174.4 and 360.5 mg/kg bw/d in male and female rats. There were no significant positive associations between the administered dietary

concentrations of aniline hydrochloride and mortality in either sex of rats. The incidences of several types of mesenchymal tumors (haemangiosarcoma, fibrosarcoma, fibroma and sarcoma) were increased in treated male rats (**Table 4.17**).

Haemangiosarcoma, the most frequent spleen tumor was evident with an incidence of 19/50 (38%) in low-dose males and of 20/46 (43%) in high-dose males. Splenic fibrosarcomas were described as invasive with widespread extension into the abdominal cavity. Only few tumors were evident in the spleen of treated female rats (haemangiosarcoma in one low-dose female and two high-dose females, sarcoma in three high-dose females). Additionally mesenchymal tumors were more often observed in multiple organs of the pleural and abdominal cavities for dosed males and high-dose females (**Table 4.17**). Pheochromocytomas were increased dose-dependently in male animals (12% and 27% in low - high-dose males (both nonsignificant) vs. 8% in controls). In females, this tumor occurred in 4% of the controls and 8% of the high-dose. According to the NCI report, the increases of haemangiosarcomas of the spleen and the combined incidence of fibrosarcomas and sarcomas NOS of the spleen were each statistically significant in male rats. The combined incidence of fibrosarcomas and sarcomas NOS of multiple body organs was also significantly increased in male rats. The number of female rats having fibrosarcomas or sarcomas NOS of either the spleen alone or multiple organs of the body cavity was significantly associated (in the Cochran Armitage test) with increased dietary concentration of aniline hydrochloride. This result was not supported by Fisher exact tests, but because of the rarity of these tumors, the observed incidences in the low - high-dose groups were considered indicative of a compound-related carcinogenic effect. The National Cancer Institute concluded that aniline hydrochloride was carcinogenic to male and female F 344 rats.

Additional information

Weinberger and coworkers (1985) reviewed the spleen sections from the NCI study (1978) and found statistically significant increased tumor rates in the spleen of low - high-dose males (27% and 60%, resp.). A total of two splenic tumors was evident in low-dose females (4%) and 5 (10%) tumors were seen in high-dose females. No spleen tumor was seen in control animals. Non neoplastic lesions in spleen as fibrosis, capsular hyperplasia and haemorrhage were seen with significantly higher incidence in males and females of each dose group. The incidence of fatty metamorphosis was significantly increased in male groups. Splenic fibrosis and fatty metamorphosis showed a strong statistical correlation with tumor incidence.

Mice

Neoplastic lesions in mice were not related to the feeding of aniline hydrochloride (NCI, 1978). Groups of 50 B6C3F1 mice were supplied with feed containing aniline hydrochloride at concentrations of 0, 0.6 and 1.2 percent for 103 weeks followed by a 4-week observation period. Test parameters included body weight, food consumption, clinical observations on mortality and presence of tissue masses and lesions, necropsy and histopathologic examinations of > 32 organs/tissues. Test substance concentrations are equivalent to doses of 737 and 1,510 mg/kg bw/d in male mice and 733 and 1,560 mg/kg bw/d in female mice. Mean body weight was depressed during the study and at the end of treatment (without any data that the depression gained significance). No treatment-related effect on survival rates was observed compared dose and control groups. Survival rates were 82% and 84% for high-dose males and females, 86% and 74% for low-dose males and females, and 66% and 60% for control males and females. None of the observed tumors were considered to be test-substance related as they were found in approximately equal number in control and dosed groups.

With respect to the spleen, the target organ in rats, there was a single hemangioma in high-dose males (1/49 = 2%), a hemangiosarcoma in a low-dose male (1/49 = 2%) and a malignant lymphoma in a control male (1/38 = 3%). In the spleen of females, one control animal (1/45 = 2%) had a hamangiosarma and malignant lymphomas were seen in three low-dose females (3/48 = 6%).

Table 4.16 Incidence of primary spleen tumors and other spleen findings in a 104-week study in F344-rats treated with aniline hydrochloride (CIIT, 1982)

	Males				Females			
Dose group (mg/kg) $	0	10	30	100	0	10	30	100
No. of animals examined# No. of spleen examined Premature deaths (total No.)	130 123 16	130 129 14	130 128 15	130 130 26	130 129 20	130 129 21	130 130 14	130 130 13
Tumor type*								
Fibrosarcoma	0	0	0	3 (1*)	0	0	0	0
Stromal sarcoma	0	0	1	21 (1*)	0	0	0	0
Capsular sarcoma	0	0	0	1	0	0	0	0
Haemangiosarcoma	0	0	0	6 (4*)	0	0	0	1
Osteogenic sarcoma	0	0	0	3 (3*)	0	0	0	0
Lymphoreticular neoplasm	0	0	0	1	0	0	0	0
Other findings at week 104 (90 animals/sex/group)**								
Stromal hyperplasia	1	0	0	31	0	0	0	9
Chronic capsulitis	1	1	2	62	0	4	4	70
Fatty metamorphosis	0	0	0	14	0	0	0	0

$ Equivalent to aniline doses of 0, 7, 22, and 72 mg/kg bw/d

* In parentheses: tumors in premature deaths

** Treatment-related non neoplastic findings of the spleen were also observed at week 26, 52, and 78, but were not reported here.

\# No. of animals including interim sacrifices at weeks 26 (10/sex/group), 52 (10/sex/group), and 78 (20/sex/group).

Table 4.17 Incidence of mesenchymal tumors in the spleen and body cavities/other organs of rats of a 103-week study with aniline hydrochloride (NCI, 1978)

	Males			Females		
Dose groups	**control**	**0.3%**	**0.6%**	**control**	**0.3%**	**0.6%**
Tumors in spleen and capsule						
No. of animals with tissue examined	25	50	46	23	50	50
Sarcoma NOS	0	4	2	0	0	3
Fibroma	0	7	6	0	0	0
Fibrosarcoma	0	3	7	0	0	0
Haemangiosarcoma	0	19	20	0	1	2
Lipoma	0	0	0	0	0	1
Haemangioma	0	0	0	0	0	1
Tumors in body cavity/multiple organs						
No. of animals with tissue examined	25	50	43	24	50	50
Fibrosarcoma	0	2	8	0	1	3
Leiomyosarcoma	0	0	2	1	0	0
Haemangiosarcoma	0	0	1	0	0	0
Sarcoma NOS	0	0	1	0	0	1

Other studies on carcinogenic effects

In vitro studies: Cell transformation assays

A series of transformation assays were performed with aniline. In primary cells from Syrian Hamster embryos (SHE-cells; 0.01-10.0 µg/ml; without S-9 mix) and with mouse derived C3H/10T1/2 cells (0.8-100 µg/ml; without S-9 mix) negative results were obtained (Pienta and Kawalek, 1981; Dunkel et al., 1988). In mouse Balb/3T3-cells (0.8-100 µg/ml; without S-9 mix) a positive effect without dose-response was reported (Dunkel et al., 1981).

Experimental animals

There were further studies of limited validity on the carcinogenic effects of aniline. Data on test substance purity were not given. Testing procedures and documented results were not comparable to testing efforts according to the current regulatory test protocols. Some of the observed effects were at least partly consistent to the results of more recent studies. To complete the database these studies were reported here:

Concerning the spleen as a target organ of the carcinogenic activity of aniline furthermore limited studies should be cited. In an early study on 43 male and female Osborne-mendel rats (White et al., 1948) cirrhosis (18 animals) and hepatomas (4 animals) in the liver and haemorrhage (23 animals), fibrosis (14 animals) and sarcomas (3 animals) in the spleen were reported to occur in rats fed with diet with 0.033% of aniline hydrochloride for an exposure period of 420 to 1,032 days (average 654). No data were given on test substance purity, impurities by other substances, or distributions of lesions in between the sexes.

In contrast to this, no tumor response was seen in an other early study by Druckrey (1950) where each rat received 22 mg aniline hydrochloride per day (calculated to be 120-220 mg/kg bw/d by the author) with the drinking water for up to 750 days of treatment. 25 of the 50 animals of the study survived 425 days of treatment.

Some less reliable studies mainly focused on the urinary bladder:

In the study of Hagiwara et al. (1980) with male Wistar rats receiving 0.03, 0.06, 0.12% of aniline (28 animals/group) with the drinking water for a treatment period of 80 weeks selected parameters on haematology and clinical chemistry were investigated and histopathology from kidney, liver, and inflated urinary bladder was performed. No increase of proliferative changes of the urinary bladder or other organs was observed. There was a dose-related decrease of the mean RBC counts and of the haemoglobin levels at the end of treatment. The total incidence of bile duct proliferation increased with the dose level in comparison to the control. No treatment-related effect was observed in the kidneys.

None of eleven rats fed with a diet containing 5-7.5 mg aniline per animal on 144-256 days had proliferative changes of the bladder epithelium (Ekman and Strömbeck, 1949). Some of them showed hyperemia and haemorrhages in the urinary bladder.

52 subcutaneous injections of 1.9 mmol/kg bw/wk aniline in oil to Syrian hamsters failed to induce proliferative lesions of the urinary bladder when the experiment was terminated after 87 weeks (Hecht et al., 1983).

Perlmann and Staehler (1932) subcutaneously injected aniline at doses of 1 mg/day to 10 rabbits and of 10 mg/day to 20 rabbits (6 or 60 mg/week) and observed a single urinary bladder tumor in a low-dose rabbit which died after four weeks of treatment.

Proliferative lesion was not observed in an other rabbit study injecting intraperitoneally once a week 15 ml of a 2.5% aniline solution to 12 rabbits which survived more than six months (Berenblum and Bonser, 1937).

Summary of studies in animals

From long-term studies applicating aniline hydrochloride it was demonstrated that aniline treatment is carcinogenic in rats inducing spleen sarcomas. The increase of tumor incidence was marked in males, splenic sarcomas occurred in only few females.

A carcinogenic effect could not be demonstrated in mice.

4.1.2.8.2 Studies in humans

Epidemiology data

Tumors of the urinary bladder have been discussed in numerous reports since the late 18th and early 19th century to be associated with the occupational exposure of workers to aniline. The database on the exposure to aniline was considered to be insufficient; therefore these early reports were not taken into account.

In 1990, the NIOSH (NIOSH Alert, 1990; Ruder et al., 1992; Ward et al., 1991) studied the incidence of bladder cancer at a plant that had used o-toluidine, aniline, hydroquinone and other chemicals since 1957. Air concentrations of o-toluidine and aniline measured by NIOSH were below 1 ppm, a concentration consistent with company data available since 1975. Workers occasionally came into skin contact with liquid processing chemicals including o-toluidine and aniline.

Among 1,749 individuals who were ever exposed at the plant, 13 cases of bladder cancer were observed, whereas 3.61 cases were expected on the basis of New York State incidence rates. Among 708 workers considered definitely exposed to o-toluidine and aniline, 7 cases of bladder cancer were observed and 1.08 cases were expected. Among 288 workers who were possibly exposed, 4 cases of bladder were observed and 1.09 was expected. No significant difference of the incidence was seen in 753 workers who were probably unexposed.

Smoking without occupational exposure to o-toluidine and aniline would account for less than a twofold increase in bladder cancer compared with the greater than sixfold increase observed among (smoking) workers definitely exposed to o-toluidine and aniline. Bladder cancer risk increased with duration of work in the area. The preshift and postshift mean concentrations of aniline (and o-toluidine) of exposed workers were significantly higher than the levels of unexposed workers.

It was concluded that the occupational exposure to o-toluidine and aniline was associated with an increased risk of bladder cancer among workers. The effects of o-toluidine and aniline could not be separated epidemiologically, because workers were exposed to both of these chemicals.

The correlation between o-toluidine or aniline and bladder cancer was doubted by Freudenthal and Andersen (1994) who argued that employees investigated were also exposed to other substances with possible carcinogenic activity on the urinary bladder which were not taken into account in the study from Ward et al. (1991).

A mortality survey on 139 and 48 employees assigned to two aromatic amine-based dye production areas (manufacture of indigo and potassium phenyl clycine and acetanilide) in the period from 1940 to 1975 using aniline (and other process chemicals) as raw material did not show increases in mortality by work area or duration of exposure within work area. No deaths due to bladder cancer were observed (Ott and Langner, 1983).

Summary of studies in humans

There are data on increased incidence of bladder cancer in workers exposed to aniline and other chemicals. Although aniline is suspected to induce cancer, aniline could not be identified as the responsible agent.

4.1.2.8.3 Other information from structurally similar substances

Malignant splenic tumors were not only restricted to aniline. Looking for other structural related chemicals with the spleen as a target organ of carcinogenicity and similar database with respect to the erythrocyte-directed toxicity and genotoxicity, there are some aromatic amines found suitable for a comparison to aniline (azobenzene and o-toluidine). Other SAR-related substances were not included into the comparison because of the data lack on carcinogenicity and/or toxicity or a different tumor spectrum from that of aniline. The compounds azobenzene and o-toluidine induced aniline-like effects as fibrosis, unusual mesenchymal proliferation and sarcomas after long-term exposure. Data from azobenzene are summarised in **Table 4.18**. The azobenzene data showed coincidental evidence of erythrocytoxicity and methaemoglobinemia. Similar to aniline, spleen tumor incidences were higher in male rats than in female rats. Comparable to cancer studies with aniline, azobenzene induced a variety of fibrosarcomas, osteosarcomas and haemangiosarcomas in the rat spleen giving suggestion that primitive/multipotential mesenchymal cells are the target cells.

Azobenzene like aniline was negative in the mouse cancer study and induced bone marrow micronuclei (rats > mice).

Differences in the structure as well in tumor data demonstrated a lower similarity of o-toluidine to aniline. Unlike aniline, o-toluidine is carcinogenic in mice, too (NTP, 1979a, TR 153). Not a variety of sarcomas, but predominantly haemangiosarcomas in the spleen as well as in the liver were evident in mice treated with o-toluidine. Besides of splenic sarcomas, o-toluidine induced a broader spectrum of tumors in several other organs in rats. The significance of multiple organ carcinogenicity of o-toluidine in comparison to aniline data is unclear. Therefore o-toluidine data were considered less reliable with respect to aniline toxicity.

No carcinogenicity data are available for p-aminophenol (aniline metabolite). Toxicity is different to aniline in that p-aminophenol is nephrotoxic in rats and humans (for review, BG Chemie 27b, 1995).

Table 4.18 Data from azobenzene

Haemotoxicity	Genotoxicity	Tumor response	Other morphologic lesions (non-tumor)	Reference
Rat: erythrotoxic, methaemoglobinemia	rat: bone marrow micronucleus positive in single + multiple dose studies	rat: variety of sarcomas in spleen in m > f, malig. haemangiopericytomas in spleen + other abdom. organs of f	rat: mesenchym.proliferations in spleen in m and f, chronic spleen capsulitis, haemosidero sis in spleen, liver and kidney [2)]	[1)] George et al.(1990b) [2)] NTP (1979a)
Mouse: erythrotoxic, methaemoglobinemia [1)]	mouse:bone marrow micronucleus marginal positive at high dosages in single dose studies, no multiple dose study[1]	mouse: no tumor response [2)]		

In conclusion, data from azobenzene give support that, male and female rats are susceptible to aniline carcinogenicity. Males are more sensitive than females. Tumor and genotoxicity data from azobenzene confirm the aniline effects. Both substances were not able to induce tumors in mice.

4.1.2.8.4 Discussion on carcinogenicity

Interspecies comparison: methaemoglobinemia

Humans are expected to be more sensitive to aniline toxicity than rodents are.

Sensitivity of rats to form methaemoglobin compared to humans was reported to be low. Oral administration of aniline to volunteers on three or some more successive days in the study of Jenkins et al. (1972) showed that dosages of 25 mg or higher induced methaemoglobinemia. Calculated on a mean body weight of 70 kg, the LOAEL in humans is 0.36 mg/kg bw/d. In rats treated orally with aniline the authors found increased methaemoglobin levels at doses from 40 mg/kg with maximum levels within 1-4 hours after treatment (16.6% vs. 2.4% in untreated volunteers), and 20 mg/kg bw/d was considered as the NOAEL in this study. Higher susceptibility of humans is discussed to be likely because of the interspecies differences in the activity of methaemoglobin reductase, which reduces methaemoglobin to haemoglobin. The activity of this enzyme is five and ten times higher in rat erythrocytes and mouse erythrocytes, respectively than in human erythrocytes (Smith, 1986).

The interspecies comparison of methaemoglobin levels from other studies is restricted due to methodical defaults. Most of the studies with repeated exposure to aniline did not give information on the time point of methaemoglobin analysis. It cannot be excluded that the results on methaemoglobin levels from the studies of Khan et al. (1993; 1995b) were false negative or incorrect as the authors did the analysis at 24 hours after blood collection. Methaemoglobin levels fall rapidly during blood storage even at cold temperatures and meaningful results are obtained only on freshly drawn blood (Beutler et al., 1995).

Comparison of aniline-induced rat methaemoglobin levels to mouse methaemoglobin levels are not possible, because this parameter was not examined in mice repeatedly exposed to aniline. From single dose studies administering azobenzene there is evidence that mice show methaemoglobinemia, changes after multiple doses were not examined in mice (George et al.,

1990b). In earlier studies mice were also sensitive to methaemoglobin formation after a single administration of aniline with maximum increases to 5-15.4% at 10-20 min post-exposure after 0.1-5 mmol/kg bw and sudden deaths after 60 min at the highest dose (Smith et al., 1967).

Methaemoglobin production was considered as one possible, but not the most suitable indicator of erythrotoxicity for experimental animals. As humans showed a stronger increase of methaemoglobin levels it may be considered as a useful biomarker compared to the rat.

Interspecies comparison: erythrotoxicity

Although the aniline database for mice is yet very insufficient, the few data available did not indicate that aniline induces spleen tumors as it does in the rat. With relation to haemotoxicity, there are some data which may indicate a possible erythrotoxicity of aniline in mice, too.

The appearance of black enlarged granular spleens described in the feed dose-finding study in (rats and) mice treated with dosages from 0.3% of aniline hydrochloride may indicate an adverse effect on the blood red cells or on spleen cells not specified. Clarification still remains open because of the incompleteness of theses studies. Long-term studies in the mouse revealed no indication on red blood cell toxicity by secondary morphologic effects on the spleen and other organs (NCI, 1978). Bile duct inflammatory changes occurred in the mouse ingested 0.6% aniline hydrochloride in feed (appr. 735 mg/kg bw/d of aniline) but not in the rat (NCI, 1978). The differences in toxicity seem to indicate a quantitative higher susceptibility of rats which may be explainable by differences in the metabolic pathways. In mice, aniline is metabolised mainly by N-acetylation which is not limited in contrast to N-hydroxylation pathway in rats and glucuronidation is the main elemination pathway in mice. (Kao et al., 1978; McCarthy et al., 1985). The detoxification rate is higher in mice compared to rats.

However, reduced peripheral red cell counts after a single dose of azobenzene to mice (George et al., 1990b) give further support to the hypothesis that aniline may be haematotoxic in mice. Additionally, mice may differ from the rat in their binding of aniline/-metabolites to erythrocytes and/or degradation of damaged erythrocytes in spleen differ qualitatively and/or quantitatively. A specific retention of ^{14}C-aniline in mouse erythrocytes was not detected and radioactivity did not accumulate in mouse spleen (Robertson et al., 1983).

Further appropriate studies in mice may clarify this. Based on the insufficient mouse data it remains unclear whether erythrotoxicity and methaemoglobinemia is species-specific for the rat.

Toxic effects to erythrocytes are seen in rats as well as in man. Like in other mammals, the clearance of damaged erythrocytes is also located in the spleen in humans. Although there are no data on splenic toxicity of humans exposed repeatedly to aniline, the spleen is considered as target organ in rats and humans.

Tumor biology

The majority of tumors which were seen in the rat spleen were fibrosarcomas and/or haemangiosarcomas (CIIT, 1982). Haemangiosarcomas and (fibro-) sarcomas were also described in the original report on the NCI study (1978). The review of this NCI study by Weinberger et al. (1985) revealed that with the exception of a single haemangioma all malignant tumors were classified as fibrosarcomas or variants of them. This can be explained in that the pluripotential mesenchymal stem cells constitute a common cell compartment of the histogenesis of both tumor types. A main diagnostic feature in the differential diagnosis may be the lack or presence of a vascular pattern with prominent endothelial cells (Mohr, 1992). As the spleen red

pulp by itself is characterised by a relative high amount of vascular structures, morphologic differentiation only by light microscopy may be difficult in case of highly malignant, less differentiated tumors. For this reason, splenic sarcomas in aniline carcinogenicity were interpreted as a common entity although they may show, at least in large areas, fibrous, vascular or even osteoid differentiation.

Spontaneously, sarcomas and fibrosarcomas derived from the capsular and trabecular smooth muscle and fibrous tissue are rare in rats. The incidence of primary splenic haemangiomas or haemangiosarcomas was cited between 0 and 1.5 percent in males and 0 and 0.5 percent in females of four rat strains, no case of primary fibroma or fibrosarcoma was reported (Losco, 1992). Primary tumors of the spleen are rare in humans without any history of exposure to xenobiotics. One of the most common benign tumor type is the haemangioma, other benign tumors as fibromas are extremely rare. Primary malignant tumors including haemangiosarcomas are also uncommon (Bonner, 1988). Of 11,166 men and 8,518 women who had sarcomas at any site, none of them had a fibrosarcoma in the spleen. Haemangiosarcomas were found in four men and four women out of these groups (Mack, 1995).

Although tumors in several organs were observed in long-term studies, aniline is not considered to a multiple site carcinogen inducing various primary tumors. Sarcomas observed in the body cavity and other organs may be caused by the invasion through the splenic capsule involving the abdominal viscera and adjacent organs directly or by haematogenous metastasing (NCI, 1978). In the reevaluation work, Weinberger et al. (1985) described that poorly differentiated fibrosarcomas were predominantly located on the capsular surface or extraspenically, metastasis was extensive, and metastatic sites included the pancreas, intestinal tract, liver, kidney, adrenal gland, intraabdominal fat, and the surfaces of the testis and epididymis.

Due to the morphologic similarity of splenic fibrosis and capsular hyperplasia to the induced splenic sarcomas it was suggested that these lesions are pre-neoplastic. Dysplastic cells were present in the capsular hyperplasia. (Weinberger et al., 1985). Occasionally fibrosarcomas are described to occur within areas of fibrosis and well differentiated areas of fibroblasts were found adjacent to less differentiated areas of tumor cells (Ward et al., 1980). This was interpreted that sarcomas arise from areas of pre-existing fibrosis (Popp, 1990).

Repeated dose studies demonstrated that splenic toxicity of aniline is clearly associated with the damage of erythrocytes in that the spleen is the organ responsible for the erythrocyte clearance. The clearance activities of the spleen are dependent, in part, on its structure of macrophage-augmented filtration beds that are interposed between arteries and veins or lymphatics. Myofibroblastic cells functioning as barrier cells of the fibroblastic stroma constitute part of these splenic filtration beds (Weiss, 1991). Hypothetically, these myofibroblastic cells may represent possible target cells when activated by the degradation process of damaged erythrocytes. However, this assumption is not proven, tumor cells have not been characterised by special techniques.

The clear increase of incidences of splenic tumors in male rats (CIIT, 1982; NCI, 1978) may be interpreted as a sex-specific reaction in males. However, a minor increase of splenic tumors and the high frequencies of splenic lesions considered as pre-neoplastic lesions confirm that female rats are also susceptible (CIIT, 1982; NCI, 1978). Female rats are less sensitive than males, possibly explainable due to a higher hydroxylation rate in males in comparison to females, which was found in Sprague-Dawley rats (Pence and Schnell, 1979).

Mechanisms of tumor development

Spleen tumors

The evidence of splenic sarcomas and the micronuclei formation of bone marrow in rats suggest that tumor response may be mediated by a direct interaction of aniline and the DNA of spleen cells. This can be interpreted as consistant relationship of *in vivo* genotoxicity and tumor production of aniline.

The DNA adduct formation in spleen cells strongly supports a direct action of aniline on spleen cells (McCarthy et al., 1985). Absolute binding index of spleen cells was relatively low in comparison to the kidney values. DNA adduct formation is determined in relation to all (not specified) spleen cells. Low absolute values may be explainable in that the cell compartment of mesenchymal cells assumed to represent target cells of aniline carcinogenicity is minor in relation to other nucleated cells. Depending on the age of animals, the fraction of other nucleated cells contains a large majority of lymphocytes, nucleated erythrocytes and granulocytes. These cells were shown to be 85 percent or higher of total number of nucleated cells in mice (Green et al., 1981). Similar values were expected for the rat. Assuming that DNA adducts were not formed in these cells, the mesenchymal cell fraction probably shows a higher DNA adduct formation. To clarify this, further studies are necessary to characterise cells with DNA adducts and to quantify the DNA adduct formation of splenic mesenchymal cells.

The existence of other mechanisms is also assumed to contribute to tumor development.

Whereas severity of spleen toxicity seems to be correlated to the grade and persistence of erythrocytic damage, it is uncertain, whether tumor development may be associated to a certain degree of erythrotoxicity. This hypothesis may be augmented by the observation that a low-dose of 10 mg/kg bw/d of aniline hydrochloride (appr. 7 mg/kg bw/d of aniline) was clearly erythrotoxic (CIIT, 1982), but did not produce increased numbers of spleen tumors in rats. Thus, a noncarcinogenic dose may be identifiable on the basis of a dose, which is clearly not toxic to erythrocytes.

However, there are arguments against this hypothesis. Sensitivity of female rats to erythrotoxicity was comparable to that of male rats (CIIT, 1982), but did not result in higher tumor rates at the low and mid-dose level. In consequence, erythrotoxicity by itself is not conclusively the key step of tumor development. Otherwise, every xenobiotic substance inducing haemolytic anaemia should be suspected to induce spleen cancer. Data from azobenzene showed that mice were anemic and produced methaemoglobin but, like in aniline studies, did not produce tumors in carcinogenicity study.

Haemosiderin deposition is not a critical step in the development of splenic tumors. The re-evaluation of the NCI study (Weinberger and coworkers, 1985) could not demonstrate a clear relationship between frequency of intrasplenic haemosiderin deposition and tumor development. They postulated the presence of acute vascular congestion as the initial alteration. Extravascular blood may have stimulated an extensive fibrous tissue activation and proliferation. It was suggested that following prolonged exposure to aniline fibrous tissue undergoes cellular transformation and dedifferentiation into multipotential mesenchymal cells.

However, no other splenic vascular congestion induced by nonxenobiotic processes is known to induce sarcomas of the spleen. Vascular congestion was considered to represent primarily an early phase reaction, which in 28-day studies was predominant on day 1 and regressed on day 7

of treatment (Khan et al., 1995b). Congestion was not reconfirmed in treated rats of the NCI study (Weinberger et al., 1985).

Excessive iron accumulation is discussed to contribute to the tumor development. Iron preservation for reuse in haemoglobin synthesis is a normal function of a healthy spleen. After repeated aniline treatment, splenic iron content increased up to day 7, and there was no further increase until day 28 of treatment (Khan et al., 1995b). This may indicate that after several days of exposure, the spleen adapted to the increased demand of erythrocytic clearance by an increased number of macrophages active sequestering the cellular debris. At the end of long-term studies, the incidence (number of animals) and mean severity score of haemosiderin deposits did not correlate to the treatment duration or dose (Weinberger et al., 1985). Deposition of erythrocyte debris containing iron has been discussed to mediate tissue damage by iron-catalyzed free radical creation possibly resulting in a variety of tissue-damaging reactions (Khan et al., 1993). In this study, lipid and protein peroxidation was shown to be higher in spleen homogenates of aniline-exposed rats. In contrast to Khan et al. (1993), others concluded that haemosiderin formation resulting in iron overload must be considered as a cytoprotective mechanism (O'Connell et al., 1986). Under physiological conditions iron in the form of haemosiderin is far less active in promoting lipid peroxidation than ferritin-bound iron.

In general, there is no evidence from other substances that haemolytic anaemia has any association to tumor development.

Up to now, there is no proof that methaemoglobinemia is necessarily linked to tumor development. Methaemoglobin concentrations were increased in rats during longer treatment periods, there was no further progress of increase with treatment duration. Even at later time points of treatment, the relative concentrations of methaemoglobin levels were lower (Khan et al., 1995b).

Erythrotoxicity and methaemoglobinemia were seen in mice after a single oral administration of azobenzene (George et al., 1990b). Assuming that aniline like azobenzene induces methaemoglobin formation in mice, negative cancer studies in mice would not give arguments for a contribution of methaemoglobin formation to the tumor development.

Extrasplenic sites

Additional to the major role of the spleen other tissues are also able to remove the damaged erythrocytes or are involved in the degradation process. Haemosiderosis of the kidney and the liver give indications on this (NCI, 1978). However, no increase of tumor incidences was observed in extrasplenic clearance sites. Results from the early study from White et al.(1948) revealing increased frequencies of cirrhosis and hepatomas were not confirmed by other studies. McCarthy et al. (1985) reported substantial DNA adduct formation in the rat kidney. Obviously erythrocytic degradation process and DNA binding at extrasplenic sites did not represent preconditions sufficient to succeed in tumor growth.

Overall, the role of damaged erythrocytes as carriers of aniline and/or its metabolites and their contribution with respect to the initiation and development of spleen tumors remains unclear.

4.1.2.8.5 Conclusion on carcinogenicity

In two carcinogenicity studies on the F344 rat, aniline produced dose-dependently higher incidences of spleen sarcomas in males. A few splenic tumors observed in female rats were also

considered to be related to aniline treatment. Aniline is genotoxic *in vivo* in rats and in mice. It can be assumed that the genotoxicity is responsible for tumor initiation and development, but this did not necessarily include a scientific plausible proof that the underlying mechanism of carcinogenicity is based on the genotoxic activity. Other mechanisms are also discussed to be involved in tumor development. Until now, it is not possible to demonstrate a plausible mode of action indicating the existence of a threshold mechanism.

Further studies are necthe Technical Meeting (TM) II/2000 that they have started a new research program to elucidate further the possible mechanisms of spleen tumours in rats.

As far as known aniline is metabolised similarly in rat and man. Therefore a certain carcinogenic risk for man cannot be excluded for all exposure scenarios. Although available human data do not support a carcinogenic risk to humans this holds true only for the described circumstances. From the limited human database a final assessment of human cancer risk is not possible.

In accordance to the EU criteria for classification and labelling of carcinogens, aniline is classified as carcinogenic, category 3 and labelled with R 40 “limited evidence of a carcinogenic effect”.

4.1.2.9 Toxicity for reproduction

4.1.2.9.1 Studies in animals

Fertility impairment

Multi-generation studies, respectively fertility studies are not available for aniline.

Data on organ weights for testes with epididymides and for ovaries as well as information on gross pathology and on histopathology of the high-dose treated animals for reproductive organs of both sexes can be derived from a 104-week chronic toxicity feeding study with Fischer 344 albino rats (CIIT, 1982; cf. Section 4.1.2.6), during which groups of 130 animals/sex had been treated with aniline hydrochloride at doses according to approximately 7, 22, and 72 mg aniline/kg bw/day. At 26 and 52 weeks on diet groups of 10 animals/sex/dose and at 78 weeks on diet 20 animals/sex/dose were sacrificed. The remaining animals were terminated at the end of the study after a total of 104 weeks. No apparent treatment-related effects were observed for the male reproductive system in comparison to the controls in terms of testes weight and testes histopathology at either time interval or at final termination. In the females absolute and relative ovary weight in treated animals were slightly higher but not statistically significantly different from that of the control groups after 26, 52 and 78 weeks on diet, whereas at termination after 104 weeks absolute and relative ovary weight was statistically significantly lower in the high-dose group (0.095 gram, resp. 0.36%) than in controls (0.190 gram, resp. 0.71%). An increased in the incidence of uterine endometrial polyps was recorded during histopathological evaluations in aniline treated dams after 78 weeks in diet only, with six high-dose, three mid-dose and two low-dose females afflicted, while only one case was observed in the control group. No such effect was recorded at the evaluation after 26 and 52 weeks on diet nor at the end of the study (104 weeks). Since uterine endometrial polyps are a common observation for this rat strain, the isolated observation at 78 weeks of exposure only of a treatment-related increase in the incidence of uterine endometrial polyps is considered an incidental finding. From the overall evaluations of this study a clear substance-related induction of uterine endometrial polyps could

not be established. Furthermore aniline hydrochloride had been used during a further 103-week feeding study (bioassay of aniline hydrochloride for possible carcinogenicity) in B6C3F1mice and F344 rats (NCI, 1978; cf. Section 4.1.2.6). Animals were feed two different dose levels. Groups of 50 mice each received a low-dose level of 0.6% in diet (according to approximately 737 mg aniline/kg bw/day) and a high-dose level of 1.2% in diet (according to approximately 1,510 mg aniline/kg bw/day). Groups of 50 rats each received a low-dose level of 0.3% in diet (according to approximately 174.4 mg aniline/kg bw/day) and a high-dose level of 0.6% in diet (according to approximately 350.5 mg aniline/kg bw/day). From this study it is not reported that weight determination of reproductive organs had been performed, whereas organs of the reproductive system had been monitored histopathologically for neoplastic as well as for non neoplastic lesions. In mice no treatment-related effects in any of the organs of the reproductive system of either males or females were reported for doses up to and including approximately 1,510 mg aniline/kg bw/day. Also, for male rats no effects in any of the organs of the reproductive system attributable to treatment were reported with doses up to and including approximately 350.5 mg aniline/kg bw/day. In female rats after 103 weeks on diet increased incidences of uterine endometrial polyps were reported with 15/48 (31%) animals of the low-dose group (approximately 174.4 mg aniline/kg bw/day) and with 7/50 (14%) animals of the high-dose group (approximately 350.5 mg aniline/kg bw/day) afflicted in comparison to 2/24 (8%) animals of the control group. The occurrence of endometrial polyps in untreated as well as in treated animals of this rat strain was also mentioned in the above cited CIIT study (1982).

Further information comes from investigations with an *in vivo* model proposed as a "rapid-test" to identify carcinogenic activity, where aniline hydrochloride failed to induce sperm-head abnormalities in a special inbred strain of mice (Topham 1980a, 1980b; cf. Section 4.1.2.7).

Developmental toxicity

Aniline hydrochloride was evaluated for teratogenicity as well as for postnatal effects in an oral study with Fischer 344 rats (Price et al., 1985). For the one part of this study 21 to 24 pregnant dams/group were treated by gavage with doses of 10, 30, and 100 mg aniline HCl/kg bw/d (purity and stability not further specified) at a volume of 2 ml/kg bw during gestational days 7-20. For the other part of this study additional groups of 12 to 15 pregnant dams per dose group were treated during gestational days 7 to parturition (p.n. day 0) for the evaluation of postnatal development. Concurrently to the vehicle controls (a. dest.) hydroxyurea (200 mg/kg bw/d) was used as a positive control for teratogenic effects. Dams were monitored for body weights and clinical signs daily until termination. At gestational day 20 dams were sacrificed and a minimum of 20 pregnant rats per treatment group were recorded for body weight, liver and spleen weights, gravid uterine weights, numbers of corpora lutea, implantations, early and late resorptions, and live and dead fetuses. Fetuses were sexed, evaluated for body weight and crown-rump length, for gross morphological as well as for visceral and skeletal abnormalities and for spleen and liver weights. Furthermore, hematological parameters were investigated in the dams and the fetuses of the high-dose group (100 mg/kg bw/d) as well as in vehicle controls.

With the prenatal part of the study all treated dams survived until termination on g.d. 20. Signs of maternal toxicity were observed in terms of dose-related lower mean absolute body weight gain (weight gain g.d. 0 to 20 minus gravid uterine weight), which was statistically significantly decreased to 19.4 $\pm$ 1.5 g in the high-dose group (100 mg/kg bw/d) in comparison to that of the control (26.7 $\pm$ 1.5 g), as well as a dose-dependent statistically significant increase in mean relative spleen weight in the 10, 30 and 100 mg/kg bw dose groups amounting to 24.3, 25.5 and 36.7% body weight in comparison to that of the controls (19.2% of body weight). Dams of the high-dose (100 mg/kg bw/d) group revealed signs characteristic of aniline toxicity, e.g. (2-3-

fold) increased methaemoglobin concentrations, decreased red blood cell counts and increased reticulocyte counts. No differences were observed among vehicle controls and aniline treated groups with respect to pregnancy rates, number of corpora lutea per dam or number of implantation sites per dam. There were also no differences for the numbers of live fetuses per litter, average fetal body weight, crown-rump length or relative fetal spleen weight. Fetal examination exhibited a slight but statistically significantly increased relative liver weight of 8.0 ± 0.15% of body weight for the 100 mg/kg dose group in comparison to the control (7.7 ± 0.12% of body weight), whereas no such changes were observed at the 10 and 30 mg/kg bw dose levels. A low incidence of malformations was observed in all treatment groups: 3/178 (1.7%) fetuses in the vehicle control, and 0/181 (0%), 7/210 (3.3%), and 4/190 (2.1%) fetuses in the 10, 30, and 100 mg/kg bw dose groups. No statistically significant differences were observed between vehicle and aniline-treated litters with regard to the proportion of litters with one or more malformed fetuses, or in the percentage of malformed fetuses per litter. The evaluation of hematological parameters of the litters from the high-dose treated dams did not reveal any effects on percentage of reticulocytes, white or red blood cell counts, hematocrit or platelets, and on methaemoglobin concentrations in comparison to the vehicle controls. However, a slight but statistically significantly smaller red blood cell distribution width of 14.83 + 0.16 versus 15.64 + 0.14 in the control and increased red blood cell size (MCV) of 162.6 + 0.55 μm^3 versus 158.8 + 1.15 μm^3 in the controls was observed.

For the postnatal part of this investigation dams sacrificed at p.n. day 30 exhibited a significantly elevated relative spleen weight, elevated methaemoglobin concentrations and an increased MCV in the high-dose group, whereas relative spleen weight was not increased in the 10 and 30 mg/kg/day treated groups. Litters born to aniline-treated dams did not differ from vehicle controls in live litter size, incidence of stillborn pups per litter, percentage of male pups per litter, average (male and female) pup weight or average (male and female) pup length per litter, average relative spleen weight, or average relative liver weight on p.n. day 0. Pups of the 100 mg/kg/day derived group exhibited a statistically significant increase in red blood cell size (MCV) of 141.3 ± 1.7 μm^3 versus 133.4 ± 0.72 μm^3 in the controls. It is reported that no statistically significant differences were noted among vehicle control and aniline-treated groups for other hematological endpoints on p.n. day 0, or for any parameter in the hematological profile on p.n. day 10, 25, and 50 (data not provided). After culling on p.n. day 0 the remaining offspring was raised up to p.n. day 60. During this period incidental and transiently lower body weights as well as incidental and transiently higher relative spleen and relative liver weights in comparison to the controls were recorded. Male offspring of the 100 mg/kg/day derived group exhibited body weights below those of controls on p.n. day 0, 2, and 10, but not on p.n. day 25, 50 or 60; female offspring of the 100 mg/kg/day derived group exhibited body weights below those of controls on p.n. day 2, but not on p.n. day 0, 10, 25, 50 or 60. Also, a significant dose-response trend for elevation of pup relative spleen weights with aniline treatment was observed on p.n. day 25, but not on p.n. day 10, 50, or 60. Statistically significantly different from controls, but not dose related higher pup relative liver weights were seen on p.n. day 25 in the 10 and 30 mg/kg/day derived groups and on p.n. day 50 in the 50 mg/kg/day derived group, but not on p.n. day 10 or 60. At termination on p.n. day 60, no differences among aniline-treated and vehicle control groups were found for male pup relative testis weight. When compared to the controls for the progeny of treated dams there were indications of some preweaning mortality reported in terms of a higher number of total deaths (6, 15, and 13 pups in the 10, 30, and 100 mg/kg dose groups in comparison to 3 pups in the controls; litter size or total number of pups not provided) and a slightly higher number of litters with one or more postnatal deaths (3/16, 4/15, and 5/16 in the 10, 30, and 100 mg/kg dose groups in comparison to 2/15 in the controls). The higher number of litters in which one or more postnatal deaths occurred (mostly before p. n. day

6 and not later than weaning) was not statistically significantly different in the aniline-treated groups in comparison to the controls. No deaths were observed after weaning up to p. n. day 60. The age of appearance for indices of physical development (pinna detachment, visible pilation, lower incisor eruption, eye opening, vaginal opening, and testis descent), behavioral development (surface righting, cliff avoidance, auditory startle, wire grasping, and mid-air righting), or behavior of offspring in an open field (p. n. day 30) were not affected by aniline treatment.

In summary, at daily doses of 100 mg aniline HCl/kg body weight signs of general toxicity (body weight gain reduction) and of substance-specific hematotoxicity (red blood cell counts ↓, reticulocyte counts ↑, metHb concentrations ↑, relative spleen weight ↑) were revealed for the dams. Moreover, after cessation of treatment aniline-specific toxic effects persisted in these dams continuously, at least for the period of nursing, as evidenced by aniline-specific toxic effects (metHb concentrations ↑, relative spleen weight ↑, MCV ↑) when animals were sacrificed on p.n. day 30. Since elevated relative spleen weight as an indication of increased erythropoietic activity is considered to reflect aniline induced hematotoxicity and based on the observation of a statistically significant and dose-related increase in relative spleen weight of the dams during the period of gestation, a NOAEL/maternal toxicity can not be derived from this study. Based on statistically significantly increased relative spleen weight during the period of gestation a LOAEL/maternal toxicity of 10 mg aniline HCl, corresponding to 7 mg aniline/kg bw/day is derived from this study. Treatment of dams during pregnancy with aniline HCl, even with doses that induced clear-cut aniline-specific hematotoxicity as well as signs of maternally toxic effects, however, did not give evidence for interference with the development of the conceptus. There were no indications for any impairment of prenatal viability of the embryo or of pre-/perinatal viability of the fetus/newborn. This was evidenced from the successful pregnancy outcome and unimpaired growth parameters of fetuses/newborns of both sections of the study. Also, there were no indications for a substance-specific induction of structural abnormalities during this study. Fetuses of the high-dose treated dams examined at g.d. 20 exhibited a slightly increased relative liver weight, however, no such increase was found for newborns examined at birth. Fetuses of the high-dose treated dams examined at g.d. 20 exhibited minor indications for changes in some hematological parameters, a similar finding was revealed in newborns. Both findings may be taken as some indication that at a dosage of 100 mg/kg bw/day there is probably some interference also with the hematopoietic system of the prenatal fetus, respectively the newborn. During the postnatal observation period there were no major differences in viability, growth and development as well as no indication for increased hematopoietic activity in the offspring of treated dams in comparison to that of the controls. Thus, no significant impairment of pre- or postnatal development could be revealed for aniline HCl at doses up to and including 100 mg/kg bw/day. However, taking into account slight indications for aniline-specific effects related to its hematotoxic properties to occur also in prenatal fetuses and in newborns as well as some indications for interference with postnatal viability it appears rather justified to estimate a more conservative NOAEL/developmental toxicity of 30 mg aniline HCl, corresponding to 21 mg aniline/kg bw/day from the overall results of this study.

In a screening assay covering a total of 60 chemicals a group of 50 CD-1 albino mice was treated orally by gavage with 560 mg aniline/kg bw/d in corn oil in a volume of 5 ml/kg bw on gestational days 6 to 13 (Piccirillo et al., 1983; Hardin et al., 1987). This dose was equivalent to the LD10 predicted from a preceding dose-finding study and in fact revealed clear-cut signs of maternal toxicity, since 6 out of 50 dams died and a significantly reduced mean body weight change was noted. In this study the treatment with aniline at a single, maternally toxic dose level had no apparent effect on the number of live litters produced and on liveborn pups per litter.

However, statistically significant reductions in birth weight (1.5 ± 0.1 g versus 1.6 ± 0.2 g in the control group) and statistically significant reductions in offspring weight gain (0.9 ± 0.2 g versus 1.1 ± 0.4 g in the control group) as well as nonstatistically significant lower offspring viability (94.1 ± 16.5% versus 99.3 ± 2.6% in the control) through the first three postpartum days were seen in the aniline-treated litters in comparison to the control group.

4.1.2.9.2 Studies in humans

Available information concerning the reproductive and developmental effects of exposure to aniline in humans is limited to one epidemiological study in which an increase in menstrual disturbances, ovarian dysfunction and spontaneous abortion was reported in an incomplete account of a study of Russian women occupationally exposed to aniline in addition to other chemicals (Podluzhnyi, 1979). Due to methodological pitfalls and missing exposure data this study was considered of insufficient value for assessment purposes.

4.1.2.9.3 Conclusion on toxicity for reproduction

There are no fertility studies available for aniline. Data from animal studies with lifetime repeated exposure did not reveal substance related and/or significant impairment of organs of the reproductive system of the male and the female sex. The reported observations from studies with rats concerning female sex organs (reduced ovary weight, uterine endometrial polyps) are not considered to be of significance in relation to female reproductive capacity and capability. Any such effects were observed, if at all, only in the later or beyond the reproductive phase and thus may be taken rather as an indication of possible impairment of general health conditions than as biologically meaningful for reproductive health conditions. Dietary exposure to aniline over periods of up to 52 weeks obviously did not interfere with uterine endometrium integrity. The available developmental studies did not reveal a potential for aniline to specifically interfere with prenatal or postnatal development at dosages that previously induced aniline-specific hematotoxicity in the dams. Thus, from the available animal data aniline is not assessed to be a reproductive toxicant.

For risk assessment of aniline with respect to toxicity of reproduction no studies with the inhalatory or the dermal route of administration are available.

In studies with repeated oral administration of doses of 7, 22 and 72 mg/kg bw/d (CIIT, 1982) organ weight determinations as well as histopathological evaluations had been performed for both sexes and at periods relevant for reproduction. Testes weights and histology had not been affected during this study. Also female reproductive organs were not affected by continuous aniline exposure up to the age of more than 52 weeks. At the highest dose severe chronic toxic effects and carcinogenicity has occurred in the study. The results concerning reproductive organs are interpreted as giving no indication for an impairment of fertility up to doses which induce toxic and tumorigenic effects. Thus, a NOAEL/reproductive organ toxicity of 72 mg/kg bw/day was determined. Based on some indications for interference with the hematopoietic system of the conceptus at the perinatal stage related to maternal dosages of 100 mg/kg bw/d and on some indications for interference with postnatal viability a NOAEL/developmental toxicity of 30 mg aniline HCl, corresponding to 21 mg aniline/kg bw/d was derived from the study of Price et al. (1985). Based on findings indicative for aniline-induced hematotoxic events in the dams, a NOAEL/maternal toxicity could not be established, but a LOAEL/maternal toxicity of 10 mg aniline HCl, corresponding to 7 mg aniline/kg bw/d was derived from the same study. The

qualification and quantification of this LOAEL/maternal toxicity is in good agreement with the L/NOAEL considerations for repeat dose toxicity, indicating that there is no significant difference in the sensitivity and susceptibility of the pregnant organism to aniline in comparison to that of non pregnant animals.

4.1.3 Risk characterisation

4.1.3.1 General aspects

Aniline is well absorbed after oral, dermal and inhalation exposure. The extent of absorption after oral intake amounts 89-96% for rats. The corresponding figures for mice, sheep and pigs are lower (72%, 80% and 56%, respectively). Dermal absorption in humans was estimated to amount up to 38%.

Aniline is metabolised to different metabolites by N-acetylation (acetanilide), aromatic hydroxylation (2- and 4-aminophenol) and N-hydroxylation to N-phenylhydroxylamine which is responsible for the formation of methaemoglobin. The metabolites are predominantly excreted in the urine. Repeated administration of radiolabelled aniline leads to accumulation of radioactivity in spleen.

Acute intoxication of humans with aniline/aniline vapours is reported frequently. Aniline is absorbed through the skin and the lungs with formation of methaemoglobin leading to cyanosis as main toxic effect. Workers handling with aniline may develop a degree of tolerance but the cyanosis may persist. 0.4-0.6 mg/l air may be borne without much harm for 0.5-1 hour, but 0.1-0.25 mg/l for several hours produces slight symptoms. Average lethal inhalation dose for humans is reported to be 25 mg/l air or 0.35-1.43 g/kg body weight. In humans 60 ml of orally administered aniline causes death. This corresponds to about 876 mg/kg bw, based on a body weight of 70 kg.

In experiments on rats and rabbits the acute toxicity of aniline is moderate, independent of the way of application: In rats oral LD_{50} values of 780 mg/kg bw in females and 930 mg/kg bw in males were determined. Inhalation LC_{50} values in rats are different depending on the kind of exposure: For head-only exposure 3.3 mg/l/4 hours and for whole-body exposure 1.86 mg/l/4 hours were detected. Acute dermal toxicity of aniline is characterised by LD_{50} values of 1,540 mg/kg bw for rabbits and 1,290 mg/kg bw for guinea pigs. Cats however, react much more sensitive, with a dermal LD_{50} of 254 mg/kg bw and death following oral application of as low as approximately 50-100 mg/kg.

Data on local irritancy to the skin and eyes of humans are not available. Aniline causes weak irritation to the skin but long lasting severe irritation with pannus formation to the eyes of rabbits.

Human data on local corrosivity of aniline are not available. Aniline is not corrosive to the skin of rabbits; eye damage can be caused by small quantities entering the eye. Although damage may not be permanent, it may be painful enough to make a man unfit for work for several days.

In humans aniline causes contact allergy, often associated with para-group cross re-activity. Aniline causes mild to moderate skin sensitisation in guinea pigs.

Repeated aniline administration to rats has been shown to damage erythrocytes followed by haemolytic anaemia, cyanosis and methaemoglobinemia at doses from 7 mg/kg bw/d in rats after oral administration or 5 ppm (19 mg/m^3) after inhalation exposure. Corresponding effects were haemosiderin deposits in the spleen and to a lower degree or at higher doses in the kidneys and the liver, respectively, increased erythropoeitic activity in the bone marrow and the spleen. The spleen of treated animals showed congestion of the red pulp sinuses and increased weight. Chronic testing resulted in excessive fibrosis and fatty metamorphosis of splenic stroma and chronic capsulitis.

Treatment-related adverse effects of minor relevance than the above mentioned effects were also reported to occur in the adrenals (cortical hyperplasia) and ovaries (reduced organ weights).

Aniline is positive in mammalian cell cultures with respect to chromosomal effects, SCE and possibly for gene mutations. In general, stronger effects are induced in the presence of an exogenous metabolic activation system than in the absence. *In vivo*, aniline is an inducer of micronuclei in mouse and rat bone marrow cells. Whereas in mice positive effects occur only in high-doses in the toxic range, in rats a positive dose-related response can be seen in non-toxic doses. The mutagenicity *in vitro* and *in vivo* of aniline is supported by *in vivo* studies showing DNA strand breaks and DNA adducts in different organs.

Furthermore mutagenicity data of a metabolite and structurally-related substances (4-aminophenol, azobenzene) strengthen the evidence for mutagenicity of aniline in somatic cells of animals. Unequivocal data on germ cell mutagenicity are lacking.

At this time data on carcinogenicity in humans are inadequate. No clear tumor response could be associated with aniline exposure to humans. Aniline is carcinogenic in rats, together with the knowledge on metabolism and positive *in vivo* genotoxicity a relevant concern on carcinogenicity in humans is concluded. Aniline is considered to be a non-threshold carcinogen.

Concerning reproductive toxicity, data from valid epidemiological studies are not available. Animal data on functional testing for fertility (e.g. generation studies) are not available. Data from other animal experimental studies (sperm-morphology, repeated exposure) did not give evidence of an impairment of parameters related to male/female fertility. Whether incidental findings concerning female sex organs (ovaries, uterus) are of significance for reproductive capacity and/or performance was not further evaluated. Rapid and complete transplacental transfer of aniline was demonstrated in studies with rats. The available developmental studies did not give evidence for a specific embryotoxic, fetotoxic or teratogenic potential of aniline. As far some effects on fetuses and on postnatal development were observed, these findings were associated with dose levels resulting in maternal toxicity over an extended period.

4.1.3.2 Workers

4.1.3.2.1 Introductory remarks

Aniline is a colourless liquid with a vapour pressure of 0.4 hPa at 20°C, which is soluble in water and organic solvents. Aniline is exclusively used as a chemical intermediate. About 71% of the handled aniline is processed to MDA (4,4'-methylenedianiline) as starting material for polyurethan plastics, approximately 15% is used in rubber chemicals as stabilisers, activators, etc. The occupational exposure scenarios have been described and discussed in Section 4.1.1.2. Exposure routes to be considered at the workplace are inhalation against aniline vapour and skin

contact with the liquid substance and its formulations. For workers risk assessment, either the average exposure levels as reported in **Tables 4.5** and **4.6** or the upper values of a given exposure range are taken forward to risk characterisation.

The toxicological data of aniline have been described and discussed in Section 4.1.2. Although some quantitative human toxicity data are available, several risk considerations and estimations have to be based on animal data. The experimental threshold levels identified during hazard assessment will be taken forward for occupational risk assessment. According to the toxicity profile of aniline the most prominent effects seem to be acute and repeated dose toxicity and carcinogenicity.

Contributions to occupational risks by different routes of exposure

Aniline seems to be readily absorbed via the oral, dermal and inhalation route. For certain toxicological endpoints aniline data originate from oral studies. In these cases route-to-route transformation is essential for workers risk assessment, since workers are exposed either by inhalation or by skin contact. It is recognised though, that route-to-route extrapolation is a difficult and contested issue in risk assessment.

In Section 4.1.2.2 an acute study with dogs is described which is used to assess the differences in methaemoglobin formation according to oral and inhalation application. Four dogs were exposed against 15 mg/kg aniline, applied either by 4 hours nose-only inhalation using an air concentration of 174 mg aniline/m^3 or as single oral dose. Three hours after oral application methaemoglobin reached maximum levels of 19–29%. During 4 hours of inhalation methaemoglobin levels steadily increased reaching maximum levels of 3–24% at the end of the inhalation period. The results indicate that concerning methaemoglobin formation in acute studies inhalation doses may be 1–6 times less effective than oral doses depending on the respiratory rate of the individual animal.

The situation to address dermal risks is even more difficult. There are no data available comparing directly the toxicity of aniline after oral and dermal application. From toxicokinetic data for oral application complete absorption might be assumed. From absorption experiments with human volunteer's dermal absorption rates in the range of 0.2–3.0 mg/cm^2/h are reported which lead under the specific conditions of the experiment to a dermal absorption of up to 38%. Among other factors dermal absorption seems to depend critically on the water content of aniline-solutions (Section 4.1.2.1). Under optimal conditions the maximal amount of aniline penetrating through 1 cm^2 of skin in 8 hours is approximately 24 mg (3.0 mg/cm^2/h $\cdot$ 1 $cm^2 \cdot$ 8 h).

For the purpose of risk assessment at the workplace in a first approximation similar availability of aniline by all routes will be assumed. In a second step inhalation or dermal scenarios leading to conclusion (iii) will be checked for plausibility if risks have been calculated on the basis of route to route extrapolation. For this evaluation dermal permeation data will be taken into account and the possibility that for single inhalation exposures risks may be 6 times lower as estimated.

Combined exposure

In order to assess risks by combined exposure a so-called "internal body burden" is identified. This parameter combines the different contributions of dermal and inhalation exposures to a total effective dose. For aniline an approach weighed by absorption is not necessary because systemic availability is assumed to be similar at both routes (see above).

In **Table 4.19** the route-specific exposure values are listed and the internal body burdens of workers as result of repeated combined exposure via inhalation and dermal exposure are identified. For aniline in most cases one exposure route has a major impact (in the range of 90% or above) on total body burden. The highest exposure level is obtained for Scenario 3b, with a total body burden of 540 mg/person/day.

Table 4.19 Occupational exposure levels and total human body burden

Area of production and use		Inhalation shift average (mg/m^3)	Dermal shift average (mg/p/d)	Internal body burden of workers[1] [2] (mg/p/d)			Contribution to total body burden	
				Inhalation	Dermal	Total body burden	Inhaltion	Dermal
Production and further processing in the large-scale chemical industry								
1a	Production, reduction of nitrobenzene by means of H_2	2.5	low	25	low	25 [3]	n.d.[3]	
1b		2.5	42-420	25	420	445	6%	94%
2a	Production, reduction of nitrobenzene by means of Fe	1.5	low	15	low	15 [3]	n.d.[3]	
2b		1.5	42-420	15	420	435	3%	97%
3a	Production by means of H_2 or by means of Fe	2-12	low	120	low	120 [3]	n.d.[3]	
3b		2-12	42-420	120	420	540	22%	78%
4a	Further processing to various products	2	low	20	low	20 [3]	n.d.[3]	
4b		2	42-420	20	420	440	5%	95%
Release of aniline as a decomposition product								
5	Vulcanisation of rubber plastics and rubber processing	0.8	low	8	low	8 [3]	n.d.[3]	
6	Iron, steel and aluminium foundries	6.4	low	64	low	64 [3]		
7	Different branches (e.g. plastics processing, electrical engineering)	0.1	low	1	low	1 [3]		

Table 4.19 continued overleaf

Table 4.19 continued Occupational exposure levels and total human body burden

Area of production and use			Inhalation shift average (mg/m^3)	Dermal shift average (mg/p/d)	Internal body burden of workers[1) 2)] (mg/p/d)			Contribution to total body burden	
					Inhalation	Dermal	Total body burden	Inhaltion	Dermal
Use of products with residual aniline									
8a	Use of dyes with residual aniline (2%), used e.g. in the textile industry	liquid dyeing formulations	0-0.08	17-84	0.8	84	84.8	1%	99%
8b		powdery dyes (+ LEV)	0-0.02	17	0.2	17	17.2	1%	99%
8c		powdery dyes (- LEV)	0-0.1	17	1	17	18	6%	94%
9	Use of adhesives (0.3%) engineering, device and tool construction industries		0-0.08	0.06-0.6	0.8	0.6	1.4	57%	43%

1) Shift average · 10 m^3
2) Upper value of a given range used for calculation
3) Inhalation is supposed to be the major exposure route at the workplace, total human body burden therefore is assumed to be almost similar to the internal body burden by inhalation. Contribution to total body burden in quantitative terms, however, cannot be determined because of missing quantitative data for dermal exposure

Default values for physiological parameters

body weight, rat	250 g
body weight, human	70 kg
respiratory rate, rat at rest	0.8 l/min/kg
respiratory volume worker during 8 hours of light activity)	10 m^3

Calculation of MOS and MOE values

For toxicological endpoints with relevant quantitative data MOS values are calculated as quotient of experimental NOAEL (or LOAEL) from animal or human studies and workplace exposure assessments. For MOE determination concerning carcinogenicity T25 is used instead of N/LOAEL. If the route of application in animal or human studies is different from the actual occupational exposure, dose units have to be adapted previously to MOS/MOE calculation. In case of aniline the relevant studies are dominated by oral application, the according effects data are given as dose in mg per kg bodyweight. The physiological default values from above are used to identify a so-called “starting point” for workers risk assessment.

MOS/MOE values for inhalation and dermal route are considered separately. The combined MOS/MOE value is calculated as quotient of the internal NAEL (or LAEL or T25) and total body burden. Because of a high degree of absorption at all routes for aniline (see above) the internal NAEL is supposed to be similar to the external NOAEL. With respect to the possible outcome of an assessment for combined risks, interest focusses on scenarios with conclusion (ii) at both exposure routes. It is recognised, that on that background for aniline combined risks only rarely will be able to decide concern. However, all combined MOS values are given in this report for matters of completeness.

Evaluation of MOS values

Risk assessment based on MOS values implies the identification of a minimal MOS as decision mark between conclusion (ii) and (iii). In order to obtain consistent results for different chemicals, substance-specific assessment factors are identified, which may vary depending on data availability and the specific toxicological endpoint to be evaluated. Scientifically based adjustment factors describe the extrapolation of animal data to the worker population. The uncertainties in the specific calculations are weighed by expert judgement and expressed as an additional "uncertainty factor". The value of the minimal MOS results from the multiplicative combination of the different assessment factors.

If the MOS value for a certain exposure scenario is below the minimal MOS for a specific endpoint, the corresponding risk situation is considered to be of concern. A MOS value higher than the minimal MOS indicates no concern.

In a parallel procedure, which gives identical but more direct results, the toxicological starting point taken forward to risk characterisation may be divided by the endpoint-specific assessment factors. As result an exposure level is identified which by direct comparison with the occupational exposure levels may serve as trigger for decisions. In the context of this risk assessment report it will be called "critical exposure level". Concern will be expressed for scenarios above this trigger value.

Interspecies extrapolation

Considerable species differences exist concerning the susceptibility for methaemoglobin formation and cyanosis, which are the main effects of acute aniline intoxication. Cats react about five to ten times more sensitive than rodents according to acute toxicity data. In humans the oral dose causing acute methaemoglobinemia seems to be 10 to 100 times less than that for rats or dogs calculated on a body weight basis (see Section 4.1.2.2). Differences in enzyme activities up to a factor of ten could partly explain a higher human susceptibility to methaemoglobin formation (Section 4.1.2.8).

Repeated administration has been shown to be haematotoxic too, followed by damage to the spleen and increased erythropoietic activity. Although probably not directly responsible methaemoglobin formation might be involved in chronic haematotoxicity. For both effects N-hydroxylation of aniline is supposed to be the critical metabolic step in toxification. Because, easily reversible methaemoglobin formation is not the most sensitive parameter in toxicity studies for aniline. However, with reservation it might be used as indicator for metabolic processes which after long-term exposure lead to chronic effects.

For repeated dose toxicity and cancerogenicity species extrapolation (rat to human) for the oral route will use an overall factor of 10. It is assumed that the differences in metabolic rate between rats and humans generally account for species differences on a bodyweight basis in the range of a factor of 4 (for calculation see NO_NL, 1999). Thus the specific human sensitivity to aniline toxicity compared to rats is only estimated with an additional factor of 2.5 in applying an overall factor of 10. Since for inhalation no metabolic correction is necessary species extrapolation following the inhalation route (from rat inhalation to human inhalation) will consequently use a factor of 2.5.

It has to be noticed that the scientific support for an overall interspecies extrapolation factor of 10 is weak. For acute methaemoglobin formation for instance the species differences between rats or dogs and humans seem to be clearly more pronounced and there is indication that the

specific susceptibility of humans for inhalation toxicity might be underestimated with a factor of 2.5. These aspects have to be considered in deciding on the endpoint specific uncertainty factors (see below).

Intraspecies extrapolation

Aniline is metabolised by different metabolic pathways, depending on the enzyme profile of the exposed individual. About 50% of the European population have a genetically caused lower activity of N-acetyltransferase. For the so called "slow acetylaters" increased sensitivity to aniline has to be expected.

No data are available which allow to quantify the sensitivity differences within the human population and thus a scientifically basis to introduce an intraspecies extrapolation factor is missing. In the following the assessments are not adjusted for intraspecies differences, which implicates that single individuals might react more sensitive than estimated. This aspect will be taken up in decision on the endpoint specific uncertainty factors (see below).

Duration adjustment

According to the fact that studies with suitable experimental design are available for aniline there is no need for a specific duration adjustment step in extrapolation. Where adaptation of daily or weekly doses is necessary, e.g. in the calculation of totally administered amounts of aniline, adjustment uses a linear regression.

Uncertainty considerations

The adjustment factors outlined above serve to adapt animal data to humans. They rely mainly upon general knowledge in physiology or toxicity. From a statistical point of view the individual parameters have to be understood as point estimates belonging to probability density functions. It is intended to take each factor from a point near the maximum of its distribution. The multiplicative combination of all factors therefore is supposed to result in a central tendency point estimate, addressing a situation which is likely to occur. However, the actual risks may either be less or more pronounced than estimated.

In practice for each toxicological endpoint an additional uncertainty factor is defined which is used to modify the initial data in terms of precaution (Delogu, 2000). This factor takes into account several aspects, which by their nature are not easy to quantify, as for instance the reliability of the database, the biological relevance of the observed effects, the slope of the dose response curve or the variability of the human population. Uncertainty factors therefore have to be based on expert judgement. To give some orientation it is proposed to use an uncertainty factor of 5 for the evaluation of repeated dose toxicity based on a subacute oral study (BAU, 1994). The uncertainty factor may be lower in case of additional relevant data or adverse effects that are not considered severe, as for instance in the case of acute toxicity or reproductive toxicity for aniline. Concerning carcinogenicity the uncertainty factor for aniline is 460 including risk extrapolation from T25 to low doses.

4.1.3.2.2 Occupational risk assessment

Acute toxicity

Local effects (inhalation, dermal)

See Irritation, no further information available.

Systemic effects (inhalation, dermal, combined)

Smyth (1931) reported that inhalation of 100 mg aniline/m^3 for several hours caused slight symptoms in humans. A singular oral study with human volunteers (Jenkins et al., 1972) indicates a significant increase in methaemoglobin level at doses as low as 25 mg aniline per person. The no effect dose concerning methaemoglobin formation by aniline was approximately 15 mg/person (i.e. 0.21 mg/kg, see Section 4.1.2.2). This dose could be taken in by inhaling air concentrations of 1.5 mg/m^3 for a working day (8 hours, inhalation volume 10 m^3) or a peak air concentration of approximately 70 mg/m^3 for 10 min (inhalation volume 0.2 m^3).

Lewalter and Korallus (1985) detected aniline-dependent modification of blood parameters (Met-Hb, Hb-adducts, see **Table 4.9**) in workers under conditions where the limit value for workplace air of 8 mg aniline/m^3 was kept. Even though the validity of the study is limited because of missing exposure measurements its results are interpreted as indication for the plausibility of a NAEC for humans as low as 1.5 mg/m^3.

In rabbits and guinea pigs dermal LD_{50} values in the range of 1,300-1,500 mg/kg were obtained. From cats, which seem significantly more sensitive towards acute aniline toxicity, a dermal LD_{50} of 254 mg/kg is reported. Three cases of intoxication in humans by contact with shoes being dyed with an aniline containing preparation have been reported (Section 4.1.2.2). No estimation of the relevant dermal dose is given.

As starting point for worker risk assessment the human NOAEL concerning methaemoglobin formation of 15 mg/person is chosen, the according air concentration at the workplace would be 1.5 mg/m^3 for exposure duration of 8 hours. For risk evaluation no further aspects have to be considered. A special uncertainty factor does not seem necessary since several precautious elements are already included in the assessment: human data include specific sensitivity of this species, similar availability of aniline is assumed for all routes, for inhalation risks calculation is based on shift average values in combination with 8 hours of exposure, the upper values of exposure ranges are selected for the assessment. In summary the minimal MOS simply is 1. The critical exposure levels are identified as 1.5 mg/m^3 for inhalation (8 hours) or 15 mg/person for dermal or combined exposure. It should be kept in mind, though, that exposure levels which may induce life threatening acute intoxication accompanied by severe cyanosis are expected to be about 100 times higher.

In **Table 4.20** MOS values concerning acute risks for exposures during a working day (8 hours) are calculated. For some inhalation exposures especially during production and further processing in the large-scale chemical industry concern is indicated. Dermal exposure scenarios being in the concern range are production and further processing if unsuitable glove material is provided and use of dyes with residual anilin. For none of the scenarios additional concern has to be expressed as result of combined inhalation and dermal exposure.

Short-term exposures are only identified for inhalation in the large-scale chemical industry during further processing of aniline with an exposure level of up to 12 mg/m^3 for about 10 min. The corresponding MOS value is 5.8 (70/12), which is clearly out of the concern region.

Table 4.20 MOS values for acute toxicity of aniline, systemic effects

			Inhalation			Dermal			Combined		
Starting point for MOS calculation			**1.5 mg/m^3**			**15 mg/p/d**			**15 mg/p/d**		
Minimal MOS			**1**			**1**			**1**		
Critical exposure level			**1.5 mg/m^3**			**15 mg/p/d**			**15 mg/p/d**		
			Exposure (mg/m^3)	**MOS**	**Conclusion**	**Exposure (mg/p)**	**MOS**	**Conclusion**	**Exposure (mg/p)**	**MOS**	**Conclusion**
Production and further processing in the large-scale chemical industry [1]											
1a	Production, reduction of nitrobenzene by means of H_2		2.5	0.6		low	high	ii	25	0.6	
1b			2.5	0.6		420	0.04	**iii**	445	0.03	
2a	Production, reduction of nitrobenzene by means of Fe		1.5	1		low	high	ii	15	1	
2b			1.5	1	**iii**	420	0.04	**iii**	435	0.03	[2]
3a	Production by means of H_2 or by means of Fe		12	0.1		low	high	ii	120	0.1	
3b			12	0.1		420	0.04	**iii**	540	0.03	
4a	Further processing to various products		2	0.8		low	high	ii	20	0.8	
4b			2	0.8		420	0.04	**iii**	440	0.03	
Release of aniline as a decomposition product											
5	Vulcanisation of rubber plastics and rubber processing		0.8	1.9	ii	low	high [3]		8	1.8	ii
6	Iron, steel and aluminium foundries		6.4	0.2	**iii**	low	high [3]	ii	64	0.2	[2]
7	Different branches (e.g. plastics processing, electrical engineering)		0.1	15	ii	low	high [3]		1	15	ii
Use of products with residual aniline											
8a	Use of dyes with residual aniline (2%), used e.g. in the textile industry	Liquid dyeing formulations	0.08	19		84	0.2		84.8	0.2	
8b		Powdery dyes (+ LEV)	0.02	75		17	0.9	**iii**	17.2	0.9	[2]
8c		Powdery dyes (- LEV)	0.1	15	ii	17	0.9		18	0.8	
9	Use of adhesives (0.3%) engineering, device and tool construction industries		0.08	19		0.6	25	ii	1.4	11	ii

1) In the chemical industry normally suitable gloves are worn (Scenarios 1a-4a), however it cannot be excluded that the use of unsuitable glove material provides only limited protection (Scenarios 1b-4b)
2) Conclusion (iii) already results from a single exposure component (inhalation or dermal), therefore it does not seem specific for combined exposure scenarios
3) According to a rough estimate in Table 4.6 exposure levels are supposed to lie < 1 mg/person/day, the respective MOS calculates to > 1,470

Discussion might be started whether the acute risks might be over estimated on the basis of the oral data (see Section 4.1.3.2.1). Furthermore it might be questioned whether a slight increase in methaemoglobin formation is of relevance for acute intoxication. On the other hand significant individual differences concerning susceptibility to aniline intoxication have to be assumed which are not accounted for in the evaluation. In addition on the background given above no uncertainty considerations have been included in the decision. Concerning dermal risks it is highly reasonable by permeation data that the amount of aniline applied on the skin (up to 1 mg/cm^2) might be completely absorbed. Thus in summary it is believed that for the exposure scenarios identified below, there is reason to rise concern with respect to the acute risks: **conclusion (iii)**.

(1)–(4) Production and further processing in the large-scale chemical industry, (inhalation and dermal contact in case of unsuitable gloves)
(6) Iron, steel and aluminium production (inhalation)
(8a,b,c) Use of dyes with residual aniline (dermal)

Irritation/Corrosivity

Dermal

Aniline causes weak irritation to the skin of rabbits. This was not sufficient for classification, no concern for humans is derived: **conclusion (ii)**.

Eyes

After instillation in the eyes long lasting severe damage was observed in rabbits, no data are available on the effects of dilutions. If the pure substance reaches the eyes of humans similar effects as in laboratory animals are expected to develop. The concentration of a dilution without eye irritating effects for humans cannot be estimated, but for risk assessment purposes it is assumed that preparations containing ≥ 5% aniline are irritating to human eyes.

Eye contact critically depends on the proper use of eye glasses. Even though suitable personal protective equipment (PPE) usually should be available at the working places in question unintended contact by non-proper use is considered to represent an incident, which may occur frequently in different exposure situations. Therefore a risk from eye irritation has to be considered.

On the grounds that control measures exist for aniline, which should be able to efficiently minimise exposure thereby similarly mitigating concern, **conclusion (ii)** is proposed. However, these control measures must be implemented and complied with to reduce the risk of damage to the eyes.

Inhalation

No studies are available concerning irritation potential of aniline after inhalation since the respiratory tract was not examined in the submitted inhalation studies. Nevertheless obvious clinical symptoms indicating severe damage were not observed. Other information which might be used for estimation comes from dermal and eye irritation studies: whereas weak effects at the application site were obtained by dermal application instillation in the eyes caused long lasting severe irritation.

However, although there is a lack of data concerning local effects in the respiratory tract **conclusion (i)** is not recommended since severe airway damage is not anticipated. In addition severe local toxicity in the respiratory tract is not expected at concentrations below those relevant for systemic toxicity. Thus specific risk reduction measures concerning this toxicological endpoint are not considered to be necessary: **conclusion (ii)**.

Sensitisation

Dermal

Animal data revealed mild to moderate skin sensitisation rates, sensitisation in humans has also been reported. From the available data the concentration of a dilution which will not be able to induce skin sensitisation in humans cannot be estimated but for risk assessment purposes it is assumed that preparations containing ≥ 1% aniline are sensitising to human skin.

As a rule in the large-scale chemical industry dermal contact is low because of technology in combination with the use of personal protective equipment. In several other scenarios dermal exposure is either expected to be low by technical reasons or because the aniline content of the formulation which leads to skin contact is below 1%. However, in the chemical industry it cannot be excluded that in rare cases unsuitable glove material might be used which only provides limited protection. In addition during use of dyes considerable dermal exposure might occur. For these scenarios, even if only occasional contact is assumed, the risk of workers to develop a contact allergy is of concern.

Allergic contact dermatitis is considered to be a severe health problem. For aniline positive reactions in reports on humans underline the fact that risk reduction measures beyond those already applied have to be considered: **conclusion (iii)**.

(1)-(4) Production and further processing in the large-scale chemical industry, unsuitable gloves
(8a,b,c) Use of dyes with residual aniline

Inhalation

Data on respiratory sensitisation in man (e.g. case reports) and in experimental animals are not available. Some potential of aniline to cause respiratory sensitisation cannot be excluded with certainty since in skin sensitisation the substance demonstrated allergenic properties. However at the background of occupational exposure in former years aniline seems at least not to be a strong respiratory sensitiser in humans. For the time being no animal model is available which would be able to verify the question of respiratory sensitisation. In summary concern cannot be expressed: **conclusion (ii)**.

Repeated dose toxicity

Local effects (inhalation, dermal)

See Irritation, no further information available.

Systemic effects (inhalation, dermal, combined)

Chronic application of aniline to rats caused main toxic effects in the haematopoietic system with corresponding changes of the spleen, the bone marrow, the kidneys and the liver. The

observed effects are believed to be relevant for humans. Quantitative information on aniline toxicity following repeated administration is available from inhalation and oral studies in rats and mice. The most reliable data are obtained from a 104-week oral study in the rat performed by CIIT (1982) with a LOAEL of 7 mg/kg/day (see Section 4.1.2.6). For comparison data from a 14-day inhalation study in rats (EPA, 1981) are also included in **Table 4.21**. In neither of the two experiments a NOAEC/NOAEL was identified. The effects observed in both studies are very similar. Based on the weekly doses corresponding to the LOAEL or LOAEC, calculated under the assumption of 100% absorption, the effect levels in both studies appear to differ by a factor of two only (see **Table 4.21**). Several aspects, like the short duration of the inhalation study, the comparison of LOAELs instead of NOAELs and a low number of animals in the inhalation test are supposed to contribute to this phenomenon. In addition absorption differences between oral and inhalation route cannot be excluded. However, summarising all arguments, the study results do not indicate relevant differences in route-specific potency. No quantitative dose effect studies with long-term dermal application are available. Risk assessment at the workplace will therefore be based on the oral study.

As starting point for MOS calculation the human dose corresponding to the rat LOAEL from the oral CIIT study is identified as 490 mg/person/day (7 mg/kg/day · 70 kg) the according air concentration at the workplace would be 49 mg/m^3 (490 mg/person / 10 m^3).

Table 4.21 Comparison of repeated dose toxicity by oral and inhalation application

Study	Critical effect	LOAEC/LOAEL	Weekly dose per rat	Source
Rat, diet, 104 weeks, 7 days/week	haematotoxicity splenic effects, increased haematopoiesis	7 mg/kg/d	12.3 mg [1)]	CIIT (1982)
Rat, inhalation, 14 days, 5 days/week, 6 hours/day	haematotoxicity splenic effects, increased haematopoiesis	17 ppm	23.5 mg [2)]	EPA (1981)

1) 7 mg/kg/day · 0.25 kg · 7 days/week
2) 7 ml/m^3 · 3.86 mg/ml · 0.8 l/min/kg · 360 min · 10^{-3} l/m^3 · 0.25 kg · 5 days/week

Evaluation of the MOS values has to account for the following aspects: 1) assessment starts from a rat dose scaled per body weight, thus for species extrapolation a factor of 10 is introduced, 2) study duration was 7 days per week compared to occupational exposure of 5 days per week, 3) from the shape of the dose-response curve it seems justified to use a factor of 3 to extrapolate from LOAEL(rat) to NAEL(rat), 4) an uncertainty factor of 5 is applied on the background that the assessment is based on a LOAEL, an assumption is used for sensitivity differences among species and intraspecies variability in humans is not accounted for. Altogether the minimal MOS for systemic effects after repeated exposure calculates to 107 (10 · 5/7 · 3 · 5). The according critical exposure level is 4.6 mg/person/day (490 mg/person/day / 107) or 0.5 mg/m^3 (49 mg/m^3/ 107).

The mechanism of spleen toxicity after prolonged exposure is not yet fully elucidated. However the following idea is supported by the available data (compare Section 4.1.2.6): prolonged exposure of aniline results in a continuous damage of erythrocytes, indicated by methaemoglobin formation. Presumably this damage is irreversible and affected erythrocytes have to be degraded by macrophages which are primarily active in the spleen. As a response increased hemosiderin accumulation, sinusoidal congestion, higher organ weight and darkened appearance of the spleen are observed. After chronic administration of aniline also stromal hyperplasia and fibrosis, and

chronic capsulitis with papillary projections occur in the spleen. According to this model small increases in methaemoglobin levels are of relevance concerning prolonged toxicity, although they are not considered to be in any case the most predictive indicator of aniline toxicity. However, if observed, elevated methaemoglobin levels are to be taken serious. Under this aspect the data on methaemoglobin formation in humans, outlined during discussion of acute toxicity, are of relevance. The NOAEL for humans after single oral application of aniline is 15 mg/person. For repeated dose toxicity the critical exposure level for humans is calculated to approximately 5 mg/person/day. Both values are in the same order of magnitude, the chronic NAEL being 3 times lower. This relationship seems to be plausible and consistent with the mechanistic ideas outlined above.

For the various exposures against aniline the resulting MOS values fall in a range between 1 and 2,500 indicating quite different levels of concern (see **Table 4.22**). Several inhalation or dermal MOS values are below the minimal MOS. For none of the scenarios additional concern has to be expressed as result of combined inhalation and dermal exposure.

Table 4.22 MOS values for repeated dose toxicity of aniline, systemic effects

		Inhalation			Dermal			Combined		
Starting point for MOS calculation		**49 mg/m³**			**490 mg/p/d**			**490 mg/p/d**		
Minimal MOS		**107**			**107**			**107**		
Critical exposure level		**0.5 mg/m³**			**5 mg/p/d**			**5 mg/p/d**		
		Exposure (mg/m³)	**MOS**	**Conclusion**	**Exposure (mg/p)**	**MOS**	**Conclusion**	**Exposure (mg/p)**	**MOS**	**Conclusion**
Production and further processing in the large-scale chemical industry [1]										
1a	Production, reduction of nitrobenzene by means of H_2	2.5	20	iii	low	high	ii	25	20	2)
1b		2.5	20		420	1.2	**iii**	445	1.1	
2a	Production, reduction of nitrobenzene by means of Fe	1.5	33		low	high	ii	15	33	
2b		1.5	33		420	1.2	**iii**	435	1.1	
3a	Production by means of H_2 or by means of Fe	12	4		low	high	ii	120	4	
3b		12	4		420	1.2	**iii**	540	0.9	

Table 4.22 continued overleaf

Table 4.22 continued MOS values for repeated dose toxicity of aniline, systemic effects

			Inhalation			Dermal			Combined		
Starting point for MOS calculation			**49 mg/m³**			**490 mg/p/d**			**490 mg/p/d**		
Minimal MOS			**107**			**107**			**107**		
Critical exposure level			**0.5 mg/m³**			**5 mg/p/d**			**5 mg/p/d**		
			Exposure (mg/m³)	**MOS**	**Conclusion**	**Exposure (mg/p)**	**MOS**	**Conclusion**	**Exposure (mg/p)**	**MOS**	**Conclusion**
4a	Further processing to various products		2	25		low	high	ii	20	25	
4b			2	25		420	1.2	**iii**	440	1.1	
Release of aniline as a decomposition product											
5	Vulcanisation of rubber plastics and rubber processing		0.8	61	**iii**	low	high (3)	ii	8	61	2)
6	Iron, steel and aluminium foundries		6.4	8	**iii**	low	high (3)		64	8	2)
7	Different branches (e.g. plastics processing, electrical engineering)		0.1	490	ii	low	high (3)		1	490	ii
Use of products with residual aniline											
8a	Use of dyes with residual aniline (2%), used e.g. in the textile industry	liquid dyeing formulations	0.08	610	ii	84	6	**iii**	84.8	6	2)
8b		powdery dyes (+ LEV)	0.02	2,450		17	29		17.2	28	
8c		powdery dyes (- LEV)	0.1	490		17	29		18	27	
9	Use of adhesives (0.3%) engineering, device and tool construction industries		0.08	610		0.6	820	ii	1.4	350	ii

1) In the chemical industry normally suitable gloves are worn (Scenarios 1a-4a), however it cannot be excluded that the use of unsuitable glove material provides only limited protection (Scenarios 1b-4b)
2) Conclusion iii already results from a single exposure component (inhalation or dermal), therefore it does not seem specific for combined exposure scenarios
3) According to a rough estimate in Table 4.6 exposure levels are supposed to lie < 1 mg/person/day, the respective MOS calculates to > 1,470

In case of dermal exposure during production and further processing of aniline it might be argued that the EASE model probably overestimates the exposure situation since it does not take into account protection measures applied. However, MOS values are so low that a limited protection by unsuitable gloves would not sufficiently reduce the risks with respect to chronic toxicity of aniline. Concerning use of dyes it is known as a result of a research project that in the dyeing industry PPE is not generally worn. Especially during preparatory works like dosing and filling of dyes extensive dermal contact with liquid or powdery dyes may occur (see Section 4.1.1.2.3).

Altogether for exposure scenarios with MOS values below 100 risk reduction measures should be initiated. Air concentrations at the workplace should be controlled so that they do not exceed the critical exposure level of 0.5 mg/m^3. Special emphasis should be given to reduce dermal risks.

(1)–(4) Production and further processing in the large-scale chemical industry, (inhalation and dermal contact in case of unsuitable gloves)
(5) Vulcanisation of rubber plastics and rubber processing (inhalation)
(6) Iron, steel and aluminium foundries (inhalation)
(8a,b,c) Use of dyes with residual aniline (dermal)

Conclusion (iii).

Mutagenicity

Several studies have been performed to investigate the mutagenic properties of aniline. The results show that under certain circumstances aniline is able to induce genotoxic effects in soma cells *in vivo*, but overall the findings are difficult to interpret. The available data on germ cell mutagenicity are of limited predictive value. With reference to Section 4.1.2.7 further studies cannot be recommended because it is not expected that further data will sufficiently clear the open questions.

The pre-requisites for an assessment concerning heritable genetic damage in germ cells of humans are therefore limited; especially no data are available which would allow dose-response considerations. However, the information on the genotoxic properties of aniline in soma cells has marked effects on the evaluation of carcinogenicity (see below).

In summary for aniline possible risks by heritable damage cannot be excluded. With the available data, a more differentiated risk estimation concerning different exposure situations is not possible. Since the nature of the effect in general is considered to be severe, concern is raised for all exposure scenarios even if only occasional. A high degree of uncertainty is associated with this decision. It should be evaluated whether the control measures applied for risk reduction can be accepted as sufficient for workers protection in the case of aniline.

Conclusion (iii).

Carcinogenicity

In two carcinogenicity studies aniline caused increasing incidences of spleen sarcomas in male F344 rats. **Table 4.23** summarises the tumour incidences for male rats obtained in the study by CIIT (1982), which is judged to be the most sensitive one (for details see Section 4.1.2.8). Selected non neoplastic lesions are also listed.

Table 4.23 Incidences of spleen sarcomas in male F344 rats (CIIT, 1982)

Rat, oral (104 wk, 7 d/wk)		**7 mg/kg/d**	**22 mg/kg/d**	**72 mg/kg/d**
Tumour incidence[1)]		**0%**	**1.1%**	**39%**
Non-Neo-Plastic Effects (selected)	mortality	-	-	reduced survival rate
	spleen	chronic capsulitis (1/129)	chronic capsulitis (2/128) increased weight	chronic capsulitis (62/130) stromal hyperplasia (31/130) fatty metamorphosis (14/130) atrophy
	blood	increased haematopoiesis MetHb: 1.89% [2)]	increased haematopoiesis MetHb: 1.40% [2)]	increased haematopiesis MetHb: 3.63% [2)]

1) Compare Table 4.16, incidences in male rats at terminal kill, including premature deaths or animals killed in extremis 0/90, 1/90, 35/90 for doses of 7, 22, 72 mg/kg/day, respectively expressed in %; control animals: 0%
2) Controls: 1.39%, values for female rats not reported

With reference to Section 4.1.2.7 aniline is genotoxic *in vivo* in rats and in mice. It can be assumed, that genotoxicity might be responsible for tumour initiation and development, but other mechanisms might also be involved. Until now a plausible mode of action supporting a threshold mechanism cannot be demonstrated (for detailed discussion see Section 4.1.2.8). Since the tumour incidence in the rat experiment is clearly non-linear, a multistage model according to EPA (1986) is judged to be appropriate for quantitative estimation of excess cancer risk at low doses. The model, which is described in detail by Anderson et al. (1983), includes the determination of the upper 95% confidence limit on the extra risk, which will be used as basis for risk evaluation. According to the multistage model an oral dose of 1 mg aniline/kg/day for rats is associated with a risk level of $9.1 \cdot 10^{-4}$. Since the model reveals a linear dose-response relationship for aniline at low doses, a risk level of $1 \cdot 10^{-4}$ corresponds to an oral dose for rats of 0.11 mg aniline/ kg/day. This risk level will be used as decision mark between situations for which immediate action is deemed necessary (conclusion (iii)) and those for which a low risk level should be taken into account when considering the adequacy of the existing control measures (conclusion (iii) (low)).

Risk assessment concerning carcinogenicity will use the T25 concept according to Dybing et al. (1997) to calculate MOE values. From the rat carcinogenicity data a T25 of 46 mg/kg/day is obtained (72 mg/kg/day·25% / 39%, no correction for spontaneous incidence or duration of experiment necessary). As starting point for MOE calculation the corresponding human dose is identified as 3,220 mg/person/day (46 mg/kg/day·70 kg), the according air concentration at the workplace calculates to 322 mg/m^3 (46 mg/kg·70 kg / 10 m^3).

In evaluation of MOE values the following aspects are of relevance: 1) from the results of the multistage model, risks in the range of $1 \cdot 10^{-4}$ are assigned to experimental doses which are 460 times lower than T25 (0.11 mg/kg/day compared to 46 mg/kg/day, see above), 2) interspecies extrapolation based on the oral route yields a factor of 10, 3) the correction factor for "standard life span humans" versus duration of exposure at work is 2.84 (75y·52w·7d / (40y·48w·5d), constants taken from DECOS (1995). Altogether the minimal MOE for carcinogenicity calculates to 1,620 (460·10 / 2.84). The according critical exposure level is 2 mg/person/day (3,220 mg/person/day / 1,620) or 0.2 mg/m^3 (322 mg/m^3 / 1,620).

In **Table 4.24** the MOE values for aniline concerning carcinogenicity are summarised. For several inhalation and dermal exposure scenarios **conclusion (iii)** is obtained as a consequence

of MOE values below 1,620. No scenario additionally gets in category (iii) because of combined inhalation and dermal exposure.

Table 4.24 Estimation of MOE values for cancer risks by aniline

		Inhalation			Dermal			Combined		
Starting point for MOE calculation		**322 mg/m³**			**3,220 mg/p/d**			**3,220 mg/p/d**		
Minimal MOE		**1,620**			**1,620**			**1,620**		
Critical exposure level		**0.2 mg/m³**			**2 mg/p/d**			**2 mg/p/d**		
		Exposure (mg/m³)	**MOE**	**Conclusion**	**Exposure (mg/p)**	**MOE**	**Conclusion**	**Exposure (mg/p)**	**MOE**	**Conclusion**
Production and further processing in the large-scale chemical industry [1]										
1a	Production, reduction of nitrobenzene by means of H_2	2.5	130		low	high	**iii** (low)	25	130	
1b		2.5	130		420	8	**iii**	445	7	
2a	Production, reduction of nitrobenzene by means of Fe	1.5	215		low	high	**iii** (low)	15	215	
2b		1.5	215	**iii**	420	8	**iii**	435	7	[2]
3a	Production by means of H_2 or by means of Fe	12	27		low	high	**iii** (low)	120	27	
3b		12	27		420	8	**iii**	540	6	
4a	Further processing to various products	2	160		low	high	**iii** (low)	20	160	
4b		2	160		420	8	**iii**	440	7	
Release of aniline as a decomposition product										
5	Vulcanisation of rubber plastics and rubber processing	0.8	400	**iii**	low	high [3]	**iii** (low)	8	400	[2]
6	Iron, steel and aluminium foundries	6.4	50	**iii**	low	high [3]		64	50	[2]
7	Different branches (e.g. plastics processing, electrical engineering)	0.1	3,200	**iii** (low)	low	high [3]		1	3,220	**iii** (low)

Table 4.24 continued overleaf

Table 4.24 continued Estimation of MOE values for cancer risks by aniline

			Inhalation			Dermal			Combined		
Starting point for MOE calculation			**322 mg/m³**			**3,220 mg/p/d**			**3,220 mg/p/d**		
Minimal MOE			**1,620**			**1,620**			**1,620**		
Critical exposure level			**0.2 mg/m³**			**2 mg/p/d**			**2 mg/p/d**		
			Exposure (mg/m³)	**MOE**	**Conclusion**	**Exposure (mg/p)**	**MOE**	**Conclusion**	**Exposure (mg/p)**	**MOE**	**Conclusion**
Use of products with residual aniline											
8a	Use of dyes with residual aniline (2%), used e.g. in the textile industry	liquid dyeing formulations	0.08	4,000	**iii** (low)	84	38	**iii**	84.8	38	2)
8b		powdery dyes (+ LEV)	0.02	16,000		17	190		17.2	190	
8c		powdery dyes (- LEV)	0.1	3,200		17	190		18	180	
9	Use of adhesives (0.3%) engineering, device and tool construction industries		0.08	4,000		0.6	5,370	**iii** (low)	1.4	2,300	**iii** (low)

1) In the chemical industry normally suitable gloves are worn (Scenarios 1a-4a), however it cannot be excluded that the use of unsuitable glove material provides only limited protection (Scenarios 1b-4b)
2) Conclusion (iii) already results from a single exposure component (inhalation or dermal), therefore it does not seem specific for combined exposure scenarios
3) According to a rough estimate in Table 4.6 exposure levels are supposed to lie < 1 mg/person/day, the respective MOS calculates to > 1,470

For carcinogenicity and repeated dose toxicity the same working areas are identified to be of concern and assigned to conclusion (iii) (production and further processing in the large-scale chemical industry, foundries with partial open systems, vulcanisation of rubber plastics and rubber processing and use of dyes with residual aniline). In the end it might not be important which property of aniline triggers the need for risk reduction measures as far as these measures will ensure that all essential risks in the critical areas will synchronously be mitigated. Air concentrations at the workplace should at least be controlled to a level below the critical exposure level of 0.2 mg/m^3. Special emphasis should be given to measures, which reduce dermal risks.

Risk characterisation for aniline as a whole is limited by the uncertainties concerning the mechanism of tumour formation and its relevance for humans. Since a genotoxic mechanism cannot be excluded concern is expressed for all exposure scenarios. However, for certain working scenarios conclusion (iii) (low) indicates that cancer risks are already very low and might not need immediate further action. It might be discussed as to whether the risk level of $1 \cdot 10^{-4}$, which is applied as decision mark between conclusion (iii) and (iii) (low), is precautious enough. If it is taken into account, however, that by the assumption of a genotoxic mechanism cancer risks of aniline probably are overestimated, the use of this value seems justified.

Conclusion (iii).

Toxicity for reproduction

Fertility impairment

Conclusive fertility studies are not available for aniline, but in a chronic toxicity feeding study (CIIT, 1982) with doses of 7, 22 and 72 mg/kg/day no significant effects were observed for male or female reproductive organs. At the highest dose severe chronic toxic effects and carcinogenicity has occurred in the study (compare **Table 4.23** and specific chapters). The results are interpreted as giving no indication for reproductive toxicity of aniline up to doses, which induce chronic toxic effects and carcinogenicity. For higher exposure levels no data on fertility are available.

There is no specific concern with respect to fertility up to dose levels, which induce chronic toxicity or carcinogenicity. For significant higher exposure levels fertility risks cannot be excluded on the basis of the available experimental data. With this qualification **conclusion (ii)** is proposed concerning fertility.

Developmental toxicity

In a developmental toxicity study in rats with oral doses of approximately 7, 22 and 72 mg aniline/kg/day maternal toxicity occurred at all dose levels. No indications for significant impairment of pre- or postnatal development were obtained. However, based on some indications for interference of aniline with the hematopoietic system of the conceptus and with postnatal viablility, a NOAEL of 21 mg aniline/kg/day concerning developmental toxicity was derived with a conservative approach (Section. 4.1.2.9). This animal dose will be used as a starting point for MOS calculation. The corresponding human dose is identified as 1,470 mg/person/day (21 mg/kg · 70 kg), the according air concentration at the workplace would be 147 mg/m^3 (1,470 mg/kg/day / 10 m^3).

Evaluation of the MOS values has to account for the following aspect: 1) assessment starts from a rat dose scaled per body weight, thus for species extrapolation a factor of 10 is introduced, 2) it seems not necessary to propose an additional uncertainty factor, because the NOAEL is derived with sufficient precaution. The minimal MOS for developmental toxicity therefore is 10. The according critical exposure level is 150 mg/person/day (1,470 mg/person/day / 10) or 15 mg/m^3 (147 mg/m^3 / 10).

Table 4.25 MOS values for developmental toxicity of aniline

			Inhalation			Dermal			Combined		
Starting point for MOS calculation			**147 mg/m³**			**1,470 mg/p/d**			**1,470 mg/p/d**		
Minimal MOS			**10**			**10**			**10**		
Critical exposure level			**15 mg/m³**			**150 mg/p/d**			**150 mg/p/d**		
			Exposure (mg/m³)	**MOS**	**Conclusion**	**Exposure (mg/p)**	**MOS**	**Conclusion**	**Exposure (mg/p)**	**MOS**	**Conclusion**
Production and further processing in the large-scale chemical industry [1]											
1a	Production, reduction of nitrobenzene by means of H_2		2.5	60	ii	low	high	ii	25	60	ii
1b			2.5	60		420	3.5	**iii**	445	3.3	[2]
2a	Production, reduction of nitrobenzene by means of Fe		1.5	100		low	high	ii	15	100	ii
2b			1.5	100		420	3.5	**iii**	435	3.4	[2]
3a	Production by means of H_2 or by means of Fe		12	12		low	high	ii	120	12.3	ii
3b			12	12		420	3.5	**iii**	540	2.7	[2]
4a	Further processing to various products		2	74		low	high	ii	20	74	ii
4b			2	74		420	3.5	**iii**	440	3.3	[2]
Release of aniline as a decomposition product											
5	Vulcanisation of rubber plastics and rubber processing		0.8	180	ii	low	high [3]	ii	8	180	ii
6	Iron, steel and aluminium foundries		6.4	23		low	high [3]		64	23	
7	Different branches (e.g. plastics processing, electrical engineering)		0.1	1,470		low	high [3]		1	1,470	
Use of products with residual aniline											
8a	Use of dyes with residual aniline (2%), used e.g. in the textile industry	liquid dyeing formulations	0.08	1,800	ii	84	18	ii	84.8	17	ii
8b		powdery dyes (+ LEV)	0.02	7,400		17	86		17.2	85	
8c		powdery dyes (- LEV)	0.1	1,470		17	86		18	80	
9	Use of adhesives (0.3%) engineering, device and tool construction industries		0.08	1,800		0.6	2,450		1.4	1,050	

1) In the large-scale chemical industry normally suitable gloves are worn (scenarios 1a-4a), however it cannot be excluded that the use of unsuitable glove material provides only limited protection (Scenarios 1b-4b)
2) Conclusion (iii) already results from a single exposure component (inhalation or dermal), therefore it does not seem specific for combined exposure scenarios
3) According to a rough estimate in Table 4.6 exposure levels are supposed to lie < 1 mg/person/day, the respective MOS calculates to > 1,470

As can be seen from **Table 4.25** dermal contact during production and further processing in the large-scale chemical industry in the case of unsuitable gloves lead to conclusion (iii). However, some aspects have additionally to be taken into account. Firstly, for aniline no severe effects on developmental toxicity have been described, the evaluation is based on a NOAEL which is derived with a conservative approach. Secondly, the dermal exposure in the scenarios of concern might to some extent be overestimated since the EASE model does not take into account if protection measures are applied. Thirdly, only exposure situations which already have been identified as high-risk scenarios under the aspect of chronic toxicity and carcinogenicity lead to MOS values below 10. With these arguments indication with conclusion (iii) might be questioned. On the other hand MOS values are clearly in the concern range and no uncertainty factor has been used for risk evaluation. Altogether the scenarios in question are judged to be borderline cases. Applying the precautionary principle (Delogu, 2000) concern will be expressed: **Conclusion (iii)**.

(1)-(4) Production and further processing in the large-scale chemical industry, unsuitable gloves

4.1.3.2.3 Summary of occupational risk assessment

As result of the occupational risk assessment for several workplaces with aniline exposure concern is raised and risk reduction measures have to be initiated. The most important toxicological endpoint is carcinogenicity in combination with genotoxicity. Although it is not yet proven, whether the carcinogenic properties of aniline are relevant for humans (classification as category III carcinogen), the according risks might be comparably high. For a summary of the most critical exposure scenarios in the order of risk see **Table 4.26** with respect to inhalation and **Table 4.27** with respect to dermal exposure. For matters of comprehension mutagenicity has not been included since conclusion (iii), associated with a high degree of uncertainty, applies for all scenarios.

Table 4.26 Ranking of the most critical inhalation exposure scenarios for aniline and associated health risks

Scenario [1]		Exposure level in mg/m³	Carcinogenicity	Repeated dose toxicity	toxicity	Developmental toxicity
			Critical exposure level in mg/m³			
			0.2	0.5	1.5	15
3a,b	Production by means of H_2 or by means of Fe	12	**iii**	**iii**	**iii**	ii
6	Iron, steel and aluminium foundries	6.4	**iii**	**iii**	**iii**	ii
1a,b	Production, reduction of nitrobenzene by means of H_2	2.5	**iii**	**iii**	**iii**	ii
4a,b	Further processing to various products	2	**iii**	**iii**	**iii**	ii

Table 4.26 continued overleaf

Table 4.26 continued Ranking of the most critical inhalation exposure scenarios for aniline and associated health risks [1)]

Scenario [2)]		Exposure level in mg/m³	Carcinogenicity	Repeated dose toxicity	toxicity	Developmental toxicity
			Critical exposure level in mg/m³			
			0.2	0.5	1.5	15
2a,b	Production, reduction of nitrobenzene by means of Fe	1.5	**iii**	**iii**	**iii**	ii
5	Vulcanisation of rubber plastics and rubber processing	0.8	**iii**	**iii**	ii	ii
other scenarios		≤ 0.1	iii (low)	ii	ii	ii

1) 1a-4a: suitable gloves, 1b–4b: unsuitable gloves

Table 4.27 Ranking of the most critical dermal exposure scenarios for aniline and associated health risks

Scenario [1)]		Exposure level in mg/p/d	Carcinogenicity	Repeated dose	Acute toxicity	Sensitisation	Developmental toxicity
			Critical exposure level in mg/p/d				
			2	5	15	n.d.[2)]	150
1b	Production, reduction of nitrobenzene by means of H_2	420	**iii**	**iii**	**iii**	**iii**	**iii**
2b	Production, reduction of nitrobenzene by means of Fe	420	**iii**	**iii**	**iii**	**iii**	**iii**
3b	Production by means of H_2 or by means of Fe	420	**iii**	**iii**	**iii**	**iii**	**iii**
4b	Further processing to various products	420	**iii**	**iii**	**iii**	**iii**	**iii**
8a	Use of dyes with residual aniline (2%), used e.g. in the textile industry, Liquid dyeing formulations	84	**iii**	**iii**	**iii**	**iii**	ii
8b,c	Use of dyes with residual aniline (2%), used e.g. in the textile industry, Powdery dyes	17	**iii**	**iii**	**iii**	**iii**	ii
other scenarios		≤ 1	iii (low)	ii	ii	ii	ii

1) 1a-4a: suitable gloves, 1b–4b: unsuitable gloves
2) For skin sensitisation a critical exposure level cannot be determined. However in several scenarios dermal exposure is expected to be low enough not leading to concern, either by technical reasons in combination with the use of personal protective equipment or because the aniline content of the formulation which leads to skin contact is below 1%.

For inhalation aniline exposures at the workplace on the background of cancer risks air concentrations of 0.2 mg/m³ should not be exceeded. By this measure risks from several other endpoints as repeated dose toxicity, acute toxicity or reproductive toxicity would similarly and effectively be mitigated too. Special emphasis has to be given to reduce dermal contact with aniline. Aniline easily penetrates human skin and risk assessment shows that the according risks might actually be higher than those from inhalation exposure. Even a significant lower dermal absorption as assumed would not sufficiently reduce the estimated risks. Therefore effective risk

reduction measures should be implemented and complied with at all working places. As a minimum standard it seems self-evident that suitable gloves should be provided.

In **Table 4.28** occupational exposure scenarios are listed in the order of scenario numbers to give an overview for all situations with concern. All toxicological endpoints are listed which at least in one case give reason for **Conclusion (iii)** (or iii concerning carcinogenicity). Under this aspect irritation, respiratory sensitisation and fertility are not included in **Table 4.28**. For aniline concern results either from inhalation or from dermal exposure. Combination of both exposure routes does not lead to identification of additional concern scenarios. Inhalation and dermal exposure scenarios are therefore addressed separately in **Table 4.28**, an extra column for combined exposure is not given.

Table 4.28 Summary of exposure scenarios with concern for aniline

Scenario		Acute toxicity		Sensitisation	Repeated dose toxicity		Mutagenicity	Carcino-genicity		Develop-mental toxicity	
		Inhalation	Dermal	Dermal	Inhalation	Dermal	Inhalation or dermal	Inhalation	Dermal	Inhalation	Dermal
Production and further processing in the large-scale chemical industry [1]											
1a	Production, reduction of nitrobenzene by means of H_2	**iii**	ii	ii	**iii**	ii	**iii**	**iii**	iii (low)	ii	ii
1b		**iii**	**iii**	**iii**	**iii**	**iii**	**iii**	**iii**	**iii**	ii	**iii**
2a	Production, reduction of nitrobenzene by means of Fe	**iii**	ii	ii	**iii**	ii	**iii**	**iii**	iii (low)	ii	ii
2b		**iii**	**iii**	**iii**	**iii**	**iii**	**iii**	**iii**	**iii**	ii	**iii**
3a	Production by means of H_2 or by means of Fe	**iii**	ii	ii	**iii**	ii	**iii**	**iii**	iii (low)	ii	ii
3b		**iii**	**iii**	**iii**	**iii**	**iii**	**iii**	**iii**	**iii**		**iii**
4a	Further processing to various products	**iii**	ii	ii	**iii**	ii	**iii**	**iii**	iii (low)	ii	ii
4b		**iii**	**iii**	**iii**	**iii**	**iii**	**iii**	**iii**	**iii**	ii	**iii**
Release of aniline as a decomposition product											
5	Vulcanisation of rubber plastics and rubber processing	ii	ii	ii	**iii**		**iii**	**iii**	iii (low)	ii	ii
6	Iron, steel and aluminium foundries	**iii**	ii	ii	**iii**		**iii**	**iii**	iii (low)	ii	ii
7	Different branches (e.g. plastics processing, electrical engineering)	ii	ii	ii	ii	ii	**iii**	iii (low)	iii (low)	ii	ii

Table 4.28 continued overleaf

Table 4.28 continued Summary of exposure scenarios with concern for aniline

Scenario			Acute toxicity		Sensitization	Repeated dose toxicity		Mutagenicity	Carcino-genicity		Develop-mental toxicity	
			Inhalation	Dermal	Dermal	Inhalation	Dermal	Inhalation or dermal	Inhalation	Dermal	Inhalation	Dermal
Use of products with residual aniline												
8a	Use of dyes with residual aniline (2%), used e.g. in the textile industry	liquid dyeing formulations	ii	**iii**	**iii**	ii	**iii**	**iii**	iii (low)	**iii**	ii	ii
8b		powdery dyes (+ LEV)	ii	**iii**	**iii**	ii	**iii**	**iii**	iii (low)	**iii**	ii	ii
8c		powdery dyes (- LEV)	ii	**iii**	**iii**	ii	**iii**	**iii**	iii (low)	**iii**	ii	ii
9	Use of adhesives (0.3%) engineering, device and tool construction industries		ii	ii	ii	ii	ii	**iii**	iii (low)	iii (low)	ii	ii

1) In the large-scale chemical industry normally suitable gloves are worn (Scenarios 1a-4a), however it cannot be excluded that the use of unsuitable glove material provides only limited protection (Scenarios 1b-4b)

4.1.3.3 Consumers

It is not known whether aniline is used as a component in consumer products. However, there is information from Spain, that aniline is a component of a product used for dying shoes. An internal exposure to aniline of $1.0 \cdot 10^{-4}$ mg/kg bw/d (adults) and of $4.3 \cdot 10^{-5}$ mg/kg bw/d (children) from wearing dyed leather shoes was estimated.

Acute toxicity

Acute intoxication of humans with aniline/aniline vapours is reported frequently. Average lethal inhalation dose for humans is reported to be 25 g/l air or 0.35-1.43 g/kg body weight. In experiments in rats and rabbits the acute toxicity of aniline is moderate, independent of the way of application (oral LD_{50}, rat: 442-930 mg/kg; dermal LD50, rabbit: 1,540 mg/kg; inhalation LC_{50}, rat: 1-3.3 mg/l/4h). Cats however, react much more sensitive, with a dermal LD_{50} of 254 mg/kg bw and death following oral application of as low as approximately 100 mg/kg. Aniline is absorbed through the skin and the lungs: **conclusion (ii).**

Irritation

Data on local irritancy to the skin and eyes of humans are not available. Aniline causes weak irritation to the skin but long lasting severe irritation with pannus formation to the eyes of rabbits: **conclusion (ii).**

Corrosivity

Human data on local corrosivity of aniline are not available. Aniline is not corrosive to the skin of rabbits; eye damage can be caused by small quantities entering the eye. According to the data

on local irritant properties of aniline, the substance is not to be classified as corrosive to skin: **conclusion (ii).**

Sensitisation

The contact sensitisation potential of aniline was demonstrated in guinea pigs. Animal data revealed a mild to moderate sensitisation rate. Sensitisation in humans has also been reported, often associated with para-group cross reactivity. Respiratory sensitisation has not been observed: **conclusion (ii).**

Repeated dose toxicity

The calculation of the dermal exposure of consumers due to shoes dyed with an aniline-containing product leads to an internal exposure of up to $1.0 \cdot 10^{-4}$ mg/kg bw/d.

A NOAEL has not been established; the LOAEL of systemic toxic effects (non neoplastic lesions) of 7 mg/kg bw/d was derived from the carcinogenicity study in rats (CIIT, 1982).

In the following text the database on repeated dose toxicity of aniline is considered to explain the conclusion about the appropriateness of the MOS for this endpoint.

Repeated dose oral studies in rats

Repeated oral administration of aniline to rats caused main toxic effects in the haematopoietic system with corresponding changes of the spleen, the bone marrow, the kidneys and the liver. Clinical symptoms were cyanosis, reduced body weight gain and food consumption and at high-doses premature deaths. Aniline treatment damaged erythrocytes resulting in a haemolytic anaemia. Formation of higher methaemoglobin levels and of Heinz bodies was observed. The damaged red blood cells were scavenged predominantly in the red pulp of the spleen followed by increased haemosiderin accumulation, sinusoidal congestion, higher organ weight and darkened appearance of the spleen. Prolonged exposure to aniline resulted in a continuous damage of erythrocytes. In response to the haemolytic effect reticulocyte counts were increased and erythropoietic activity was elevated in the bone marrow and at extramedullary sites (mainly in the spleen). After chronic administration also stromal hyperplasia and fibrosis, and chronic capsulitis with papillary projections occurred. Occasionally haemosiderosis of the kidneys and liver were observed.

The LOAEL (7 mg/kg bw/d in rats) representing haemotoxicity, haemolytic anaemia and the corresponding alterations as the most sensitive adverse effects after repeated oral application was derived from the 2-year CIIT study (1982) which was accepted as valid. This long-term study was considered to be the most appropriate one for risk assessment although the study did only include as clinical chemistry parameters alkaline phosphatase, blood urea nitrogen, and serum alanine aminotransferase. From all studies with chronic administration of aniline to rats no NOAEL could be derived.

Repeated dose oral studies in mice

The database of aniline related toxic effects on mice is less than that in rats, only two studies exist. The 8-week dose finding study (NCI, 1978) shows a NOAEL of 162 mg/kg bw/d, whereas in the 2-year study a LOAEL of 735 mg/kg bw/d could be derived (NCI, 1978).

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

- overall confidence in the database

The data taken into account for performing the risk characterisation have been evaluated with regard to their reliability, relevance and completeness according to section 3.2 of the TGD. The data were published in peer-reviewed journals or submitted to the Competent Authority in private reports being adequately detailed and in accordance with internationally recognised guidelines and to GLP.

The findings of all studies are not contradictory so that the judgment can be based on the database (cf. Sections 4.1.2.6 and 4.1.2.8).

There are no reasons to assume limited confidence.

- uncertainty arising from the variability in the experimental data

The studies cited above allow to conclude on the LOAEL of severe toxicity (non neoplastic effects and anaemia) from 14 studies in rats and mice. The LOAEL for non neoplastic effects has been derived from 9 oral studies in rats which resulted in LOAELs ranging from 7 mg/kg bw/d to 110 mg/kg bw/d. The LOAEL of 7 mg/kg bw/d from the CIIT study (1982) was considered to be the most appropriate value for risk assessment although in this study (2 years) only limited information on clinical chemistry (alkaline phosphatase, blood urea nitrogen, and serum alanine aminotransferase) has been obtained. The main findings of the other studies, which are not in full compliance to current test guidelines showed good consistency. The NOAEL of 23 mg/kg bw/d should not be considered because it was observed in a 4-day study in rats (Khan et al., 1997).

From the 2-year study in mice a LOAEL of 735 mg/kg bw/d was estimated.

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

- intra- and interspecies variation

Comparing the effect levels for non neoplastic lesions rats seem to be more sensitive than mice without any clear sex preference (LOAEL; rat: 7 mg/kg bw/d vs mice: 735 mg/kg bw/d).

Data on kinetics of the substance do not allow to calculate the intraspecies and interspecies variability by applying modern approaches. However, the available data give no hint on a particular high variability in kinetics. The variability of the data on the toxicodynamics has been described above and has been considered not to justify an increased MOS. For establishing the MOS, the LOAEL of the most sensitive animal study (rats) has been used.

- the nature and severity of the effect

The carcinogenic action of aniline in rats is proven. However, the data on carcinogenicity in humans are inadequate. No clear tumor response could be associated with aniline exposure to humans.

The effects described in rats as “low observed adverse effect” are haemotoxicity, haemolytic anaemia and the consequential alterations, these effects are considered to be severe health effects.

There are no reasons to assume that the non-carcinogenic effects shown in the animal experiments are limited to the species tested, thus being not of relevance for humans. Therefore there is concern, which has to be expressed in the magnitude of the MOS.

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

The estimated total body burden with an assumed absorption of 100% is compared with an oral LOAEL from a 2-year study.

There are no reasons to assume that special concern can be derived from this procedure.

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

Following the exposure scenario there is no reason to assume a special risk for elderly or children. There is concern on people suffering from special diseases like anaemia, which has to be expressed in the magnitude of the MOS.

- other factors

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

MOS for dermal exposure scenario

The calculation of the dermal exposure of consumers due to shoes dyed with an aniline-containing product leads to an internal exposure of up to $1.0 \cdot 10^{-4}$ mg/kg bw/d. The margin of safety between the

estimated exposure level of	0.0001 mg/kg bw/d

and the

oral LOAEL of	7 mg/kg bw/d

is judged to be sufficient, even if special considerations on intra- and interspecies variation, nature and severity of the effects and possible human populations at risk are taken into consideration and being aware that the exposure calculation is based on a worst-case model calculation: **conclusion (ii).**

Mutagenicity

Aniline is positive in mammalian cell cultures with respect to gene and chromosomal mutations. Stronger effects are induced in the presence of an exogenous metabolic activation system than in the absence. *In vivo*, aniline is an inducer of micronuclei in mouse and rat bone marrow cells. Whereas in mice positive effects occur only in high-doses in the toxic range, in rats a positive dose-related response can be seen in non-toxic doses. The *in vivo* mutagenicity of aniline is, furthermore, supported by mutagenicity data on structurally-related substances (4-aminophenol, azobenzene).

Aniline is classified as a category 3 mutagen and labelled “R 68, possible risks of irreversible effects”: **conclusion (iii).**

Carcinogenicity

There is clear evidence on carcinogenicity in rats, but not in mice. Occupational aniline exposure is not clearly associated with a tumor response in humans. *In vivo*, aniline is an inducer of micronuclei in rat and mouse bone marrow cells. DNA adduct formation was demonstrated in the target organ of carcinogenicity, the spleen. Aniline is considered to be a non-threshold carcinogen. Animal data and *in vivo* genotoxicity data give concern that aniline is carcinogenic to humans, too. Aniline is classified as a category 3 carcinogen and labeled with Xn, R 40: **conclusion (iii).**

Toxicity for reproduction

Fertility

Conclusive fertility studies are not available for aniline, but in a chronic toxicity feeding study (CIIT, 1982) with doses of 7, 22 and 72 mg/kg/day no significant effects were observed for male or female reproductive organs. At the highest dose severe chronic toxic effects and carcinogenicity have occurred in the study. The results concerning reproductive organs are interpreted as giving no indication for an impairment of fertility up to doses which induce toxic and tumorigenic effects (cf. Section 4.1.2.9). Therefore, fertility is not considered to be a relevant endpoint: **conclusion (ii).**

Developmental toxicity

The available data from animal studies did not give evidence for a specific embryotoxic, fetotoxic or teratogenic potential of aniline. The NOAEL/developmental toxicity of 30 mg aniline HCI, corresponding to 21 mg aniline/kg bw/d was obtained from the study of Price et al. (1985).

- MOS for the dermal exposure scenario

The calculation of the dermal exposure of consumers due to shoes dyed with an aniline-containing product leads to an internal exposure of $1.0 \cdot 10^{-4}$ mg/kg bw/d (adults) and of $4.3 \cdot 10^{-5}$ mg/kg bw/d (children). The margin of safety between the

estimated exposure level of	0.0001 mg/kg bw/d

and the

NOAEL of	21 mg/kg bw/d

is judged to be sufficient. Thus, the substance is of no concern in relation to dermal exposure from wearing dyed leather shoes: **conclusion (ii).**

4.1.3.4 Humans exposed via the environment

Indirect exposure via the environment is calculated using data for oral intake via food, drinking water, and air. Following the local scenario data (at a point source) an intake of a total daily dose of 0.74 mg/kg bw/d is calculated with the main contributions of the $DOSE_{stem}$ and $DOSE_{air}$ with fractions of 64% and 35%, respectively. Following the data for the regional scenario, the total daily dose is smaller ($4.4 \cdot 10^{-6}$ mg/kg bw/d) with a fraction of the $DOSE_{drw}$ of 84%. Due to the removal of aniline in the waterworks the total daily intake is reduced to $0.7 \cdot 10^{-6}$ mg/kg bw/d.

A daily intake of aniline via plants of 0.11 mg/kg bw/d was calculated.

Repeated dose toxicity

A NOAEL has not been established; the LOAEL of systemic toxic effects (non neoplastic lesions) of 7 mg/kg bw/d was derived from the carcinogenicity study in rats (CIIT, 1982).

- Local scenario

The total calculated internal dose after combined exposure is 0.74 mg/kg bw/d (local scenario). The margin of safety between the

estimated exposure level of	0.74 mg/kg bw/d
and the	
oral LOAEL of	7 mg/kg bw/d

is judged to be not sufficient: **conclusion (iii).**

- Regional scenario

The total calculated internal dose after combined exposure is $0.7 \cdot 10^{-6}$ mg/kg bw/d (regional scenario). The margin of safety between the

estimated exposure level of	$0.7 \cdot 10^{-6}$ mg/kg bw/d
and the	
oral LOAEL of	7 mg/kg bw/d

is judged to be sufficient, even if special considerations on intra- and interspecies variation, nature and severity of the effects and possible human populations at risk are taken into consideration and being aware that the exposure calculation is based on a worst-case model calculation: **conclusion (ii).**

Intake from plant protecting agents

The calculated intake from plants amounts to 0.11 mg/kg bw/d. The margin of safety between the

Calculated exposure	0.11 mg/kg bw/d
and the	
oral LOAEL of	7 mg/kg bw/d

is judged to be not sufficient, taking into account the nature and severity of the effects and possible human populations at risk and being aware that this exposure calculation is based on measured data: **conclusion (iii)**.

Mutagenicity

Aniline is positive in mammalian cell cultures with respect to gene and chromosomal mutations. Stronger effects are induced in the presence of an exogenous metabolic activation system than in the absence of such a system. *In vivo*, aniline is an inducer of micronuclei in mouse and rat bone

marrow cells. Whereas in mice positive effects occur only in high-doses in the toxic range, in rats a positive dose-related response can be seen in non-toxic doses.

Aniline is classified as a category 3 mutagen and labelled "R 68, possible risks of irreversible effects": **conclusion (iii)**.

Carcinogenicity

There is clear evidence on carcinogenicity in rats, but not in mice. Occupational aniline exposure is not clearly associated with a tumor response in humans. *In vivo*, aniline is an inducer of micronuclei in rat and mouse bone marrow cells. DNA adduct formation was demonstrated in the target organ of carcinogenicity, the spleen. Aniline is considered to be a non-threshold carcinogen. Animal data and *in vivo* genotoxicity data give concern that aniline is carcinogenic to humans, too: **conclusion (iii)**.

Toxicity for reproduction

Fertility

Conclusive fertility studies are not available for aniline, but in a chronic toxicity feeding study (CIIT, 1982) with doses of 7, 22 and 72 mg/kg/day no significant effects were observed for male or female reproductive organs. At the highest dose severe chronic toxic effects and carcinogenicity have occurred in the study. The results concerning reproductive organs are interpreted as giving no indication for an impairment of fertility up to doses which induce toxic and tumorigenic effects (cf. Section 4.1.2.9). As a consequence of carcinogenicity and chronic toxicity risk reduction measures have to be taken into account for aniline.

The NOAEL/developmental toxicity of 30 mg aniline HCl, corresponding to 21 mg aniline/kg bw/d was obtained from the study of Price et al. (1985): **conclusion (ii).**

Developmental toxicity

The available data from animal studies did not give evidence for a specific embryotoxic, fetotoxic or teratogenic potential of aniline.

- Local scenario

The total calculated internal dose after combined exposure is 0.74 mg/kg bw/d (local scenario). The margin of safety between the

exposure level of 0.74 mg/kg bw/d

and the

NOAEL of 21 mg/kg bw/d

is judged to be not sufficient. Thus, the substance is of concern in relation to indirect exposure via the environment: **conclusion (iii).**

- Regional scenario

The total calculated internal dose after combined exposure is $0.7 \cdot 10^{-6}$ mg/kg bw/d (regional scenario). The margin of safety between the

exposure level of $0.7 \cdot 10^{-6}$ mg/kg bw/d

and the

NOAEL of 21 mg/kg bw/d

is judged to be sufficient. Thus, the substance is of no concern in relation to indirect exposure via the environment: **conclusion (ii).**

- Intake from plant protecting agents

The calculated intake from plants amounts to 0.11 mg/kg bw/d. The margin of safety between the

calculated exposure 0.11 mg/kg bw/d

and the

NOAEL of 21 mg/kg bw/d

is judged to be sufficient taking into account the dose-response relationship, the nature and severity of the effects used in deriving the NOAEL, and even being aware that the exposure calculation is based on measured data: **conclusion (ii).**

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

In view of its chemical structure, aniline is not expected to have an oxidising potential. The substance is neither explosive nor flammable. Therefore with regard to the physico-chemical properties and with regard to the occupational (see Section 4.1.1.2) and consumer exposure (see Section 4.1.1.3) aniline is not expected to cause specific concern relevant to human health.

Conclusion (ii).

5 RESULTS

5.1 ENVIRONMENT

Aquatic compartment (incl. sediment)

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

This conclusion is reached because of the need for better information to adequately characterise the risks for the aquatic ecosystem as a consequence of exposure arising from rubber production sites.

The information and/or test requirements are:

- data about the formation of aniline from rubber chemicals, the releases into the wastewater and wastewater treatment processes which are representative for the European rubber industry.

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

This conclusion is reached because of:

- concerns for effects on the aquatic environmental spheres including sediment as a consequence of exposure arising from aniline production and further processing (4,4'-methylenedianiline and rubber chemicals) sites.

Atmosphere

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

This conclusion is reached because there is a need for better information to adequately characterise the risks to the atmosphere.

The information and/or test requirements are:

- data about releases into the atmosphere and the applied exhaust air purification techniques which are representative for the European rubber industry.

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

This conclusion is reached because of:

- concerns for effects on plants as a consequence of exposure via the air compartment arising from one aniline production site.

Terrestrial compartment

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

This conclusion is reached because there is a need for better information to adequately characterise the risks to agricultural soils from aniline as a degradation product of phenylurea and carbamate derivatives used as plant protection products.

The information and/or test requirements are:

- long term tests with plants, earthworms and micro-organisms.

However, since the risk to soil from the breakdown of plant protection agents is not covered by Regulation 793/93 it is proposed that this be considered within the frame of Council Directive 91/414/EEC.

5.2 HUMAN HEALTH

5.2.1 Human health (toxicity)

5.2.1.1 Workers

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

This conclusion is reached because of:

- concerns for acute toxicity as a consequence of:
  - inhalation exposure and/or dermal contact in case of unsuitable gloves arising from production and further processing in the large-scale chemical industry;
  - inhalation exposure arising from thermal degradation of plastics in iron, steel and aluminium foundries;
  - dermal exposure arising from the use of dyes containing residual aniline;
- concerns for skin sensitisation as a consequence of dermal exposure arising from production and further processing in the large-scale chemical industry (in case of unsuitable gloves), and the use of dyes with residual aniline;
- concerns for systemic toxic effects as a consequence of
  - inhalation exposure and/or dermal contact in case of unsuitable gloves arising from production and further processing in the large-scale chemical industry;
  - inhalation exposure arising from vulcanisation of rubber chemicals, and from thermal degradation of plastics in iron, steel and aluminium foundries;
  - dermal exposure arising from the use of dyes containing residual aniline;
- concerns for mutagenicity and carcinogenicity in all workplace scenarios, as the substance is identified as a non-threshold carcinogen. However, for the following specific working scenarios risks are already low:
  - release of aniline as a decomposition products in different industrial sectors (e.g. plastics processing, electrical engineering);
  - use of products with residual aniline (e.g. adhesives, engineering, device and tool construction industries);

This should be taken into account when considering the adequacy of existing controls and the feasibility and practicability of further specific risk reduction measures.

- concerns for developmental toxicity as a consequence of dermal exposure in case of unsuitable gloves arising from production and further processing in the large-scale chemical industry.

5.2.1.2 Consumers

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

This concusion is reached because of:

- concerns for mutagenicity and carcinogenicity as a consequence of exposure arising from use of products containing the substance, as aniline is identified as a non-threshold carcinogen.

5.2.1.3 Humans exposed via the environment

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

This conclusion is reached because of:

- concerns for systemic toxic effects, developmental toxicity, mutagenicity and carcinogenicity as a consequence of exposure arising from point sources.
- concerns for mutagenicity and carcinogenicity as a consequence of possible exposures at a regional level, as aniline is identified as a non-threshold carcinogen. However, exposures are already very low and 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 for risk reduction measures beyond those which are being applied already.

This conclusion is reached because:

- The risk assessment shows that risks are not expected. Risk reduction measures already being applied are considered sufficient.

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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
BOD	Biochemical Oxygen Demand
bw	body weight / *Bw*, *bw*
C	Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II 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
CEPE	European Committee for Paints and Inks
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 90 percent dissipation / degradation
E	Explosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II 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 II of Directive 67/548/EEC)
FAO	Food and Agriculture Organisation of the United Nations
FELS	Fish Early Life Stage
foc	Organic carbon factor (compartment depending)
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 II 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 II of Directive 67/548/EEC)
OC	Organic Carbon content
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 IV 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 II 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
ThOD	Theoritical Oxygen Demand
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
VOC	Volatile Organic Compound
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 II of Directive 67/548/EEC)
Xi	Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)

Appendix A Atmospheric deposition during production

(cf. Section 3.1.5).

I/C Model calculations for soil concentration

I	**Name of chemical**	**Aniline**		
	Partitioning between soil and pore water			
D	Density of air	RHO_air	1,3	kg_air/m^3_air
D	Density of water	RHO_water	1,000	kg_water/m^3_water
D	Density of the solids in soil	RHO_solid	2,500	kg_solid/m^3_solid
D	Volume fraction air in soil	Fair_soil	0,2	m^3_air/m^3_soil
D	Volume fraction water in soil	Fwater_soil	0,2	m^3_water/m^3_soil
D	Volume fraction solids in soil	Fsolids_soil	0,6	m^3_solids/m^3_soil
C	Bulk density of the soil	RHO_soil	1,700	kg_wet soil/m^3_soil
I	n-octanol/water partition coefficient	log Pow	0,9	-
D	Fraction organic carbon in soil	Foc_soil	0,02	kg_oc/kg_solid
D	Fraction organic matter in soil	Fom_soil	0,034	kg_om/kg_solid
S	Organic carbon-water partition coefficient	Koc	410	l/kg
C		=	0,41	m^3_water/kg_oc
C	Organic matter-water partition coefficient	Kom	0,241176471	m^3_water/kg_om
C	Solids-water partitioning coefficient in soil	Kp_soil	0,0082	m^3_water/kg_solid
C	Total soil-water partitioning coefficient	Ksoil_water	12,5	m^3_water/m^3_wet soil
	Partitioning between water and air			
I	Henry's law coefficient	Henry	0,106	Pa.m^3/mol
C	Air-water partirion coefficient	Kair_water	4,47354E-05	-
	Characteristics of soil and soil use			
D	Amount of sludge applied onto agricultural soil	APPL_agri	0,5	kg_dry sludge/m^2
D	Amount of sludge applied onto grassland	APPL_grass	0,1	kg_dry sludge/m^2
D	Depth of agricultural soil	DEPTHagri	0,2	m
D	Depth of grassland	DEPTHgrass	0,1	m
	Derivation of removal rate constants			
C	Pseudo first order rate constant for volatilisation			
C	from agricultural soil	kvolat_agri	2,74342E-05	d^{-1}
C	from grassland	kvolat_grass	5,48683E-05	d^{-1}
I	Pseudo first order rate constant for biodegradation	kbio_soil	1,98E-03	d^{-1}
C	Pseudo first order rate constant for leaching			
S	in agricultural soil	kleach_agri	0	d^{-1}
S	in grassland	kleach_grass	0	d^{-1}
C	First order rate constant for removal			
C	from agricultural soil	k_agri	2,01E-03	d^{-1}
C	from grassland	k_grass	2,03E-03	d^{-1}
	Concentration in soil through aerial deposition			
I	Annual average deposition flux	DEPtotal	3,80E-09	kg_chem/m2/d
C	aerial deposition flux per kg of soil			
	in agricultural soil	Dair_agri	1,11748E-11	kg_chem/kg_soil/d
	in grassland	Dair_grass	2,23495E-11	kg_chem/kg_soil/d

	Initial concentration after 10 a of aerial deposition			
C	in agricultural soil	Cdep_agri10(0)	5,56303E-09	kg_chem/m^3_soil
C	in grassland	Cdep_grass10(0)	1,09767E-08	kg_chem/m^3_soil
	Concentration in soil through sludge application			
I	Concentration in dry sewage sludge	Csludge	0,00E+00	kg_chem/kg_dry sludge
	Initial concentration on the first year of application			
C	in agricultural soil	Csludge_agri1(0)	0	kg_chem/kg_dry soil
C	in grassland	Csludge_grass1(0)	0	kg_chem/kg_dry soil
	Fraction of the substance at the end of the year			
C	in agricultural soil	Facc_agri	0,480603119	-
C	in grassland	Facc_grass	0,475814631	-
	Initial concentration after 10 a of sludge application			
C	in agricultural soil	Csludge_agri10(0)	0	kg_chem/kg_dry soil
C	in grassland	Csludge_grass10(0)	0	kg_chem/kg_dry soil
	Total concentration in soil			
	Initial total concentration after 10 a			
C	in agricultural soil	Csoil_agri10(0)	5,56303E-09	kg_chem/kg_dry soil
C	in grassland	Csoil_grass10(0)	1,09767E-08	kg_chem/kg_dry soil
D	averaging period for crops and grass (indirect exposure)	Texp_ind	180	d
D	averaging period for ecosystem	Texp_eco	30	d
	Total concentration in soil			
C	for terrestrial ecosystem	PEClocal_soil	5,56314E-09	kg_chem/kg_dry soil
		=	5,563137126	µg/kg dry soil
C	in agricultural soil for indirect exposure	PECagri_ind	5,56362E-09	kg_chem/kg_dry soil
		=	5,563617386	µg/kg dry soil
C	in grassland for indirect exposure	PECgrass_ind	1,09778E-08	kg_chem/kg_dry soil
		=	10,97780724	µg/kg dry soil
	Total concentration in soil porewater	**PEClocal**		
C	for terrestrial ecosystem	PEClocal_soil	7,56702E-07	kg_chem/m^3_water
C		=	0,756702362	µg/l
C	in agricultural soil for indirect exposure	PECagri_ind	7,56768E-07	kg_chem/m^3_water
C		=	0,756767688	µg/l
C	in grassland for indirect exposure	PECgrass_ind	1,49321E-06	kg_chem/m^3_water
C		=	1,493210122	µg/l

Appendix B Exposure from Fenuron and Siduron

Exposure from Fenuron

I/C Model calculations for soil concentration

I	**Name of chemical**	**Aniline**		
	Partitioning between soil and pore water			
D	Density of air	RHO_air	1,3	kg_air/m^3_air
D	Density of water	RHO_water	1,000	kg_water/m^3_water
D	Density of the solids in soil	RHO_solid	2,500	kg_solid/m^3_solid
D	Volume fraction air in soil	Fair_soil	0,2	m^3_air/m^3_soil
D	Volume fraction water in soil	Fwater_soil	0,2	m^3_water/m^3_soil
D	Volume fraction solids in soil	Fsolids_soil	0,6	m^3_solids/m3_soil
C	Bulk density of the soil	RHO_soil	1,700	kg_wet soil/m^3_soil
I	n-octanol/water partition coefficient	log Pow		-
D	Fraction organic carbon in soil	Foc_soil	0,02	kg_oc/kg_solid
D	Fraction organic matter in soil	Fom_soil	0,034	kg_om/kg_solid
I	Organic carbon-water partition coefficient	Koc	410	l/kg
C		=	0,41	m^3_water/kg_oc
C	Organic matter-water partition coefficient	Kom	0,241176471	m^3_water/kg_om
C	Solids-water partitioning coefficient in soil	Kp_soil	0,0082	m^3_water/kg_solid
C	Total soil-water partitioning coefficient	Ksoil_water	12,5	m^3_water/m^3_wet soil
	Partitioning between water and air			
I	Henry's law coefficient	Henry	0,106	Pa.m^3/mol
C	Air-water partirion coefficient	Kair_water	4,47354E-05	-
	Characteristics of soil and soil use			
D	Amount of sludge applied onto agricultural soil	APPL_agri	0,5	kg_dry sludge/m^2
D	Amount of sludge applied onto grassland	APPL_grass	0,1	kg_dry sludge/m^2
D	Depth of agricultural soil	DEPTHagri	0,2	m
D	Depth of grassland	DEPTHgrass	0,1	m
	Derivation of removal rate constants			
C	Pseudo first order rate constant for volatilisation			
I	from agricultural soil	kvolat_agri	0	d^{-1}
I	from grassland	kvolat_grass	0	d^{-1}
I	Pseudo first order rate constant for biodegradation	kbio_soil	1,98E-03	d^{-1}
C	Pseudo first order rate constant for leaching			
I	in agricultural soil	kleach_agri	0	d^{-1}
I	in grassland	kleach_grass	0	d^{-1}
C	First order rate constant for removal			
C	from agricultural soil	k_agri	1,98E-03	d^{-1}
C	from grassland	k_grass	1,98E-03	d^{-1}

Concentration in soil through aerial deposition

I	Annual average deposition flux	DEPtotal	0,00E+00	kg_chem/m^2/d
C	aerial deposition flux per kg of soil			
	in agricultural soil	Dair_agri	0	kg_chem/kg_soil/d
	in grassland	Dair_grass	0	kg_chem/kg_soil/d
	Initial concentration after 10 a of aerial deposition			
C	in agricultural soil	Cdep_agri10(0)	0	kg_chem/m^3_soil
C	in grassland	Cdep_grass10(0)	0	kg_chem/m^3_soil

Concentration in soil through sludge application

I	Concentration in dry sewage sludge	Csludge	0,00E+00	kg_chem/kg_dry sludge
	Initial concentration on the first year of application			
I	in agricultural soil	Csludge_agri1(0)	3,40E-07	kg_chem/kg_dry soil
C	in grassland	Csludge_grass1(0)	0	kg_chem/kg_dry soil
	Fraction of the substance at the end of the year			
C	in agricultural soil	Facc_agri	0,485439797	-
C	in grassland	Facc_grass	0,485439797	-
	Initial concentration after 10 a of sludge application			
C	in agricultural soil	Csludge_agri10(0)	6,60278E-07	kg_chem/kg_dry soil
C	in grassland	Csludge_grass10(0)	0	kg_chem/kg_dry soil

Total concentration in soil

	Initial total concentration after 10 a			
C	in agricultural soil	Csoil_agri10(0)	6,60E-07	kg_chem/kg_dry soil
C	in grassland	Csoil_grass10(0)	0	kg_chem/kg_dry soil
D	averaging period for crops and grass (indirect exposure)	Texp_ind	180	d
D	averaging period for ecosystem	Texp_eco	30	d

Total concentration in soil

C	for terrestrial ecosystem	PEClocal_soil	6,41051E-07	kg_chem/kg_dry soil
		=	641,0505951	µg/kg dry soil
C	in agricultural soil for indirect exposure	PECagri_ind	5,55433E-07	kg_chem/kg_dry soil
		=	555,4331855	µg/kg dry soil
C	in grassland for indirect exposure	PECgrass_ind	0	kg_chem/kg_dry soil
		=	0	µg/kg dry soil

Total concentration in soil porewater PEClocal

C	for terrestrial ecosystem	PEClocal_soil	8,71962E-05	kg_chem/m^3_water
C		=	87,19621478	µg/l
C	in agricultural soil for indirect exposure	PECagri_ind	7,55505E-05	kg_chem/m^3_water
C		=	75,55046624	µg/l
C	in grassland for indirect exposure	PECgrass_ind	0	kg_chem/m^3_water
C		=	0	µg/l

Exposure from Siduron

Partitioning between soil and pore water

D	Density of air	RHO_air	1,3	kg_air/m^3_air
D	Density of water	RHO_water	1,000	kg_water/m^3_water
D	Density of the solids in soil	RHO_solid	2,500	kg_solid/m^3_solid
D	Volume fraction air in soil	Fair_soil	0,2	m^3_air/m^3_soil
D	Volume fraction water in soil	Fwater_soil	0,2	m^3_water/m^3_soil
D	Volume fraction solids in soil	Fsolids_soil	0,6	m^3_solids/m^3_soil
C	Bulk density of the soil	RHO_soil	1,700	kg_wet soil/m^3_soil
I	n-octanol/water partition coefficient	log Pow		-
D	Fraction organic carbon in soil	Foc_soil	0,02	kg_oc/kg_solid
D	Fraction organic matter in soil	Fom_soil	0,034	kg_om/kg_solid
I	Organic carbon-water partition coefficient	Koc	410	l/kg
C		=	0,41	m^3_water/kg_oc
C	Organic matter-water partition coefficient	Kom	0,241176471	m^3_water/kg_om
C	Solids-water partitioning coefficient in soil	Kp_soil	0,0082	m^3_water/kg_solid
C	Total soil-water partitioning coefficient	Ksoil_water	12,5	m^3_water/m^3_wet soil

Partitioning between water and air

I	Henry's law coefficient	Henry	0,106	Pa.m^3/mol
C	Air-water partirion coefficient	Kair_water	4,47354E-05	-

Characteristics of soil and soil use

D	Amount of sludge applied onto agricultural soil	APPL_agri	0,5	kg_dry sludge/m^2
D	Amount of sludge applied onto grassland	APPL_grass	0,1	kg_dry sludge/m^2
D	Depth of agricultural soil	DEPTHagri	0,2	m
D	Depth of grassland	DEPTHgrass	0,1	m

Derivation of removal rate constants

C	Pseudo first order rate constant for volatilisation			
I	from agricultural soil	kvolat_agri	0	d^{-1}
I	from grassland	kvolat_grass	0	d^{-1}
I	Pseudo first order rate constant for biodegradation	kbio_soil	1,98E-03	d^{-1}
C	Pseudo first order rate constant for leaching			
I	in agricultural soil	kleach_agri	0	d^{-1}
I	in grassland	kleach_grass	0	d^{-1}
C	First order rate constant for removal			
C	from agricultural soil	k_agri	1,98E-03	d^{-1}
C	from grassland	k_grass	1,98E-03	d^{-1}

Concentration in soil through aerial deposition

I	Annual average deposition flux	DEPtotal	0,00E+00	kg_chem/m^2/d
C	aerial deposition flux per kg of soil			
	in agricultural soil	Dair_agri	0	kg_chem/kg_soil/d
	in grassland	Dair_grass	0	kg_chem/kg_soil/d
	Initial concentration after 10 a of aerial deposition			
C	in agricultural soil	Cdep_agri10(0)	0	kg_chem/m^3_soil
C	in grassland	Cdep_grass10(0)	0	kg_chem/m^3_soil

Concentration in soil through sludge application

I	Concentration in dry sewage sludge	Csludge	0,00E+00	kg_chem/kg_dry sludge
	Initial concentration on the first year of application			
I	in agricultural soil	Csludge_agri1(0)	6,40E-07	kg_chem/kg_dry soil
C	in grassland	Csludge_grass1(0)	1,28E-06	kg_chem/kg_dry soil
	Fraction of the substance at the end of the year			
C	in agricultural soil	Facc_agri	0,485439797	-
C	in grassland	Facc_grass	0,485439797	-
	Initial concentration after 10 a of sludge application			
C	in agricultural soil	Csludge_agri10(0)	1,24288E-06	kg_chem/kg_dry soil
C	in grassland	Csludge_grass10(0)	2,48575E-06	kg_chem/kg_dry soil
	Total concentration in soil			
	Initial total concentration after 10 a			
C	in agricultural soil	Csoil_agri10(0)	1,24E-06	kg_chem/kg_dry soil
C	in grassland	Csoil_grass10(0)	2,48575E-06	kg_chem/kg_dry soil
D	averaging period for crops and grass (indirect exposure)	Texp_ind	180	d
D	averaging period for ecosystem	Texp_eco	30	d
	Total concentration in soil			
C	for terrestrial ecosystem	PEClocal_soil	1,20668E-06	kg_chem/kg_dry soil
		=	1206,683473	µg/kg dry soil
C	in agricultural soil for indirect exposure	PECagri_ind	1,04552E-06	kg_chem/kg_dry soil
		=	1045,52129	µg/kg dry soil
C	in grassland for indirect exposure	PECgrass_ind	2,09104E-06	kg_chem/kg_dry soil
		=	2091,042581	µg/kg dry soil
	Total concentration in soil porewater	PEClocal		
C	for terrestrial ecosystem	PEClocal_soil	0,000164134	kg_chem/m^3_water
C		=	164,1340514	µg/l
C	in agricultural soil for indirect exposure	PECagri_ind	0,000142213	kg_chem/m^3_water
C		=	142,2126423	µg/l
C	in grassland for indirect exposure	PECgrass_ind	0,000284425	kg_chem/m^3_water
C		=	284,4252847	µg/l

Appendix C Regional and continental exposure – Humans exposed via the environment

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

Appendix D Reaction products of aniline derivatives with organic matter in soil and sediment

Aniline derivatives are known to react with the organic matter of soils and sediments. This property has consequences for several endpoints of the environmental risk assessment: distribution in the hydrosphere and soils, bioaccumulation, toxicity on soil and sediment organisms, and secondary poisoning.

For most of the aniline derivatives, only fragmentary experimental information is available. This appendix gives an overview about the available results from those substances being so far assessed, and a discussion how far this information can be used for the assessment of substances which were not tested.

Distribution behaviour in soils and sediments

Chemistry

Experiments with various ring-substituted anilines (aniline, toluidines, mono- and dichloroanilines, N-methylaniline), various humus extracts and model substances revealed that the anilines form covalent bonds with the organic fraction in soils and sediments. Reaction partners of the amino moiety were found to be aldehyde or keto groups as well as double bonds of quinoid systems which are typically for humic substances. The primary amines bind to humate in two phases. Initially, a reversible equilibrium is rapidly established, which is thought to represent the reaction with carbonyl groups to form imines. Subsequently, there is a slow (not readily reversible) reaction which is thought to represent 1,4-addition to quinone rings followed by tautomerization and oxidation to give an amino-substituted quinone.

Parallel experiments with 2- and 4-chloroaniline, and 4-methylaniline indicates that the ortho substituent inhibits the binding reaction, regardless of whether the substituent is electron withdrawing (Cl-) or donating (CH_3) (Parris, 1980). However, Fuchsbichler et al. (1978a) demonstrated that after 16 weeks of radiolabeled 2-chloroaniline incubation only a minor part of the radioactivity can be extracted from soil with organic solvents, while a higher fraction is not extractable. This result can be interpreted in that way that o-substituted anilines form covalent bonds as well as other derivatives, although with a lower reaction rate.

N-methylaniline cannot form imines, but reacts with p-benzochinone forming the same sort of product as the ring-substituted anilines (Parris, 1980).

Adsorption/Desorption Studies

Due to the formation of covalent bonds, the adsorption is much higher than it would be expected from the log Kow. In the following table, experimental distribution coefficients are compared with the calculated values using the equation proposed by the TGD:

$$\log Koc = 0.62 \log Kow + 0.85$$

Table D.1 Comparison of experimental distribution coefficients with calculated values

Substance	log Kow	Koc calc. [l/kg]	Koc exp. [l/kg]	Remarks	Reference
Aniline	0.9	26	130-410 310-910	sterile soil non- sterile soil, 120h	Pillai et al. (1982)
3,4-Dichloroaniline	2.7	330	1,900-10,400	2 soils, 1 sediment, after 53 h	Beyerle-Pfnür and Lay (1990)
4,4'-Methylenedianiline	1.59	69	7,041 10,729	aerobic, 7 d anaerobic, 7 d	III (1996)
2,4-Toluenediamine	0.14 (exp.)	8.6	9,763 4,454	aerobic, 7 d anaerobic, 7 d	III (1996)
2,6-Toluenediamine	0.34 (calc)	12	7,805 2,571	aerobic, 7 d anaerobic, 7 d	III (1996)

It should be kept in mind that the term "Koc" generally describes the distribution of a substance between the pore water and the organic matter where the substance is physically bound; if chemisorption occurs the use of this term is not quite correct.

Assessing the adsorption/desorption studies, attention has to be paid to:

- A sufficient reaction time has to be ensured until chemisorption is completed. e.g. for 3,4 dichloroaniline a low Koc of 190 l/kg was estimated after 2 h incubation, whereafter chemisorption is not completed (Briggs, 1981). Beyerle-Pfnür and Lay (1990) reached a plateau after 53 h, while in a study with 3 sediments the adsorption process was not finished after 28 d (Heim et al., 1994). From the available data a general rule for the incubation time needed to reach an equilibrium cannot be derived. Only those tests should considered to be valid which demonstrate an equilibrium state.
- If the ratio test substance to soil/sediment organics is too high, the sorption sites are saturated and the sorption constants are too low. E.g. Bishop et al. (1990) determined a Koc of 16 l/kg for aniline after incubation of 1 to 5 g soil (20% OC) with 40 ml of a 10 to 500 mg/l aniline solution. From the available tests a limit for the substance/soil or sediment concentration ratio cannot be estimated.

Analytics in soil/sediment

In field studies, the analytical determination of aniline derivatives in soils or sediments is not complete because of the formation of covalent bonds: with organic solvents only the physically adsorbed or in pore water dissolved fractions can be extracted, while by extraction with alkali the hydrolyzable fraction is removed additionally. The non-hydrolyzable fraction is inaccessible for analytical determination.

The most effective extraction method for 3,4-dichloroaniline, the Bleidner technique, is described by You and Bartha (1982). The field soil sample is treated with 12.5 N NaOH with simultaneous water steam distillation of removed 3,4-DCA, followed by extraction of the distillate with an organic solvent. The authors estimated a removal rate of 50%, 100 d after DCA application.

In laboratory experiments, there is the opportunity for a complete determination by using radiolabelled substance.

Degradation in soils and sediments

If a biodegradable aniline derivative is incubated into soil or sediment, degradation and chemisorption are competing. Generally degradation starts immediately, but the degradation rate decreases after chemisorption plays a part. The reaction product is assumed to degrade similar to the humic acid themselves. The following examples demonstrate this:

Süß et al. (1978) examined in a laboratory experiment the mineralization of ^{14}C-labelled aniline with four soil types in a concentration of 1 mg/kg over 10 weeks. In the different soils between 16.2 and 26.3% of the aniline was mineralized to $^{14}CO_2$ after 10 weeks. The degradation maximum was already reached after 1 week. After 2 weeks 50% of the totally formed $^{14}CO_2$ was found. Then the weekly degradation rates remained constant at ca. 1% until the end of the test. After extraction with water between 57.3 and 67.4% of the ^{14}C activity could be detected in the four soils.

The microbial degradation of 4,4'-methylenedianiline and 2,4- and 2,6-toluenediamne in soil was investigated under aerobic and anaerobic conditions using ^{14}C labeled MDA (International Isocyanate Institute, 1996). The results show, that biodegradation started immediately after mixing with the aerobic soil. With the binding of the amine to soil the degradation rate decreased later. The test indicates biodegradation of 2.9% after 3 days, 9.1% after 7 days and 11.6% after 56 days for MDA. For 2,4-TDA and 2,6-TDA biodegradation of 1.9% and 2.7% after 3 days, 4% and 6.2% after 7 days, 7.9% and 11.1% after 14 days, 10.8% and 14.2% after 28 days and 14.8% and 18.1% after 56 days, respectively, was found. The degradation rates indicate that biodegradation is decreased after MDA and TDA had formed covalent bounds with humic substances.Investigations on biodegradation of the soil bound 3,4-dichloroaniline show, that the degradability is dependent on the intensity of the attachment to the soil. Physically adsorbed plus chemically bound DCA is mineralized to 7.9% in 100 days, chemically bound to 4.3% in 100 days and non-hydrolyzable is mineralized to 1.5% only in 100 days. 3,4-DCA covalently bound to organic fraction is degraded almost similar to the humic acids themselves (Saxena and Batha, 1982).

The reaction products of all known aniline derivatives with humic matter have a very low degradation constant, with half-lives of one year or more. Together with the high sorption constants, high accumulation in soils and sediments is expected.

Bioavailability of the bound aniline derivatives in sediment

For aniline compounds, the experimentally derived bioaccumulation factors for fish are in the same range as those calculated with the log Kow.

Bioaccumulation studies with sediment organisms are available for 3,4-dichloroaniline. In the following table, BCF values from a single-species-test (conducted in water) are compared with BAFs (bioaccumulation factors) from a microcosm test containing sediment. All values are based on substance-specific measurements (Nagel, 1997):

Table D.2 Comparison between BCFs from a single-species-test and BAFs from a microcosm test containing sediment

Species	Single-species test BCF_{DCA}	Microcosm test BAF_{DCA}
Ceratophyllum demersum	82	210
Elodea canadensis	11	198
Daphnia magna	9	276
Asellus aquaticus	10	76
Planorbarius corneus	12	533
Tubifex tubifex	18	271
Lumbriculus variegatus	n.d.	572

Two uptake mechanisms for soil and sediment organisms are conceivable:

It is evident that the "free" aniline derivative dissolved in porewater is bioavailable.

In the above cited study, the bioaccumulation factors (BAF) are significantly higher than the bioconcentration factors (BCF) determined in the single species test without sediment. This is a strong indication that there is an additional uptake of the bound substance via the solid phase. Detailed examinations about the mechanism are not available, e.g. whether only the hydrolysable fraction or also the non- hydrolysable are taken up, or whether the aniline-humic acid adducts are stable in the organisms or metabolized under reformation of the anilines.

The bioavailability of the reaction product of aniline with humic acids in sediment was examined in a test with the benthic oligochaete *Lumbriculus variegatus* that was performed in parallel to a sediment toxicity test (Egeler and Nésa, 2002). The sediment used in the test was an artificial sediment consisting of 5% peat, 20% kaolinite clay and 75% quartz sand. The sediment had a mean organic matter content of 2 ± 0.5%. As food source for the test organisms urtica powder in a concentration of 0.5% of sediment dry weight was added. The test substance was added to the sediment as an aqueous solution. The test system was allowed to equilibrate for 48 hours before the worms were introduced. The oligochaetes were exposed to a nominal aniline concentration of 31.25 mg/kg dw for 28 days at 20 ± 2°C using a static system. Four replicate chambers, each containing 1-2 g wet weight of worms, were maintained. The worms were not fed throughout the test. Gentle aeration was used throughout the test, with the vessels being covered. The concentration of aniline in the pooled worm sample was 0.97 mg/kg worm tissue ww. The measured value was corrected for average recovery found in fortified worm tissue samples (73.3%). So a body residue of 1.32 mg/kg worm tissue ww was calculated. Body residue was normalised for the estimated dry weight and for the measured lipid content. **Table D.3** shows the obtained accumulation factors normalised for wet weight (AFww), dry weight (AFdw) and lipid/TOC (AFlipid) based on nominal and measured concentration:

Table D.3 Accumulation factors normalised for wet weight (AFww), dry weight (AFdw) and lipid/TOC (AFlipid)

	AF_{ww}	AF_{dw}	AF_{lipid}
Nominal	0.06	0.21	0.08
Measured	0.28	0.95	0.36

In all cases the concentrations found in the worms were below the concentration in the sediment. However, the values for the accumulation factor were not statistically evaluated and they have not been evaluated for steady state conditions. Although the study shows some limitations and can therefore not be used unrestrictedly for a quantitative assessment of the bioaccumulation of aniline for sediment organisms, the result from this study can at least give an indication that no bioaccumulation of aniline in the test organisms from sediment occurs.

Bioavailability of the bound aniline derivatives in soil

In laboratory tests, the bioavailability of 4-chloroaniline and 3,4-dichloroaniline for terrestrial plants was investigated. From a nutrient solution, the radiolabeled compounds were taken up by the roots. In tomatoes, oat, barley and wheat, 90-95% of the absorbed radioactivity remained in the roots, while in carrots both substances were found to be constantly distributed (Fuchsbichler et al., 1978b).

In a further test with two different soils, the uptake of covalently bound 4-chloroaniline and 3,4-dichloroaniline by oats was investigated. Three parallel tests were performed:

1) the test substance was preincubated 16 weeks before sowing
2) the test substance was preincubated 6 hours before sowing
3) the test substance was applied after sowing

After 3 and 6 weeks, the radioactivity taken up by the plants was measured. With 3,4-DCA, test 2 and 3 revealed similar results, while the uptake in test 1 was reduced by a factor of 2 to 4 compared with the others. A surprising result was found with 4-CA: the uptake was lower in the tests 2 and 3 compared to test 1. No conclusion can be drawn whether the compounds were taken up in the unreacted or in the bound form, as no substance-specific analytic was applied (Fuchsbichler et al., 1978c).

Toxicity on soil and sediment organisms

There are toxicity tests on soil and sediment organisms available, at which the test substance was added after the test organisms were inserted into the medium. Considering the fate of aniline derivatives (partial degradation, rapid formation of covalent bonds with organic matter) the practicability of these tests for the risk assessment appears to be questionable. In the test system, the organisms were initially (during a few hours or days) exposed to a high dosis of “free” aniline derivative, while later (when the equilibrium was reached) they were exposed to both free substance in the porewater (in a drastically reduced concentration) and the reaction product in the solid phase. Bioavailability and toxicity, however, are expected to be different in both cases.

In the environment, aniline compounds reach soils via metabolization from plant protecting agents or via deposition from the atmosphere. An equilibrium between the porewater and the solid phase (in the bound form) is formed leading to a long-termed accumulation. Sediments receive the compound from the polluted surface water, the equilibrium is continuously renewed. The above mentioned effect tests don’t reflect the environmental conditions.

To approximate these conditions, effect test should be performed at which the test substance is pre-incubated several weeks before the organisms are added. Pre-incubation guarantees that an equilibrium will be formed, like in the environment.

Soil

For the substance 3,4-dichloroaniline several tests with terrestrial organisms are available. These are described in the following:

Adult *Eisenia fetida* were exposed in an artificial soil to the test concentration of 1, 3.2, 10, 32, 100 and 320 mg/kg dw soil in two different variants (Bayer, 2000a). In the first variant (non-aged soil) the worms were exposed 2 h after application of the test substance, in the second variant the earthworms were introduced into the soil 5 weeks after application of 3,4-DCA (aged soil). 28 days after exposition of each test cohort the test concentration, the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offsprings was determined.

No effects on mortality and body weight of adults and the number of offspring was observed for the test concentrations of 1 to 100 mg/kg dw soil at both variants. The highest test concentration of 320 mg/kg provided significant effects on mortality and body weight of adults in the freshly contaminated soil; offsprings have also not been found at this concentration.

In aged soil some reduction in offspring numbers has been observed at 320 mg/kg dw soil only. These findings indicate that the bioavailability of the test substance decreased in the aged soil.

From these results a NOEC of 100 mg/kg dw based on nominal concentration can be deduced for mortality, body weight and offspring in freshly contaminated soil, and for offspring only in aged soil.

A comparison of this effect values with the LC50 for *Eisenia fetida* found in a short-term test with freshly contaminated soil (14d-LC50 = 130 mg/kg dw (van Gestel and van Dies, 1988) shows that preincubation of the soil with the test substance significantly reduce the toxicity.

In a test with soil microorganisms the influence of freshly applied and 5 weeks aged residues of 3,4-DCA on nitrogen mineralization was studied (Bayer, 2000b). A loamy sand soil with a organic carbon content of 0.7% was used. In the experiment with fresh residues, a lomy sand soil was treated with 0, 1, 3.2, 10, 32 and 100 mg a.i. 3,4-DCA/kg dw soil and immediately amended with lucerne-grass-green meal (5 g/kg dw soil). Soils were extracted 28 days later, and the quantities of NO3 in the extract were determined.

In the experiment with aged residues, the same quantities of 3,4-DCA were added to the loamy sand soil and allowed to age 5 weeks. After 5 weeks, the soil was mixed with Lucerne-grass-green meal to stimulate microbial metabolism. Soil was extracted 28 days later, and the quantities of NO3 in the extracts were determined. In the fresh-treated test, after 28 days, soil samples with 1, 3.2, 10 und 32 mg/kg dw soil contained more NO3 than in the untreated control. The quantities of NO3 increased as the concentration of 3,4-DCA increased. In soil treated with 100 mg/kg soil there was 91% less NO3 than in the control. The reason for the increasing nitrate concentration was not determined. However, the authors speculate that immediately after treatment, small quantities of the 3,4-DCA were available to–and were degraded by–the soil microflora. In addition, 3,4-DCA might have killed some microbial cells. In this case, these too would become available for degradation and mineralization to release NO3-N.

In the aged-residue test, after 5 weeks incubation, only the soil containing 100 mg/kg dw soil contained 40 % less NO3 than the control. 14 days after addition of the plant meel, differences between the sample treated with 100 mg/kg dw soil and the control (and the remaining samples) were strongly reduced, and after 28 days, differences between treated and control samples were no longer significant.

An exact ID50 could not be calculated. However, the data show that the ID50 for 3,4-DCA lies between 32 and 100 mg/kg dw soil.

From these studies a 28 d-NOEC of 32 mg/kg soil for inhibition of nitrification can be deduced for fresh treated soil. For the 5 week aged soil a 14 d-NOEC of 32 mg/kg and a 28 d-NOEC of 100 mg/kg can be deduced.

The studies show that aged residues of 3,4-DCA in soils , up to 100 mg/kg soil, do not have long-term influence on nitrogen mineralization in soil.

A study on the chronic toxicity of 3,4-DCA in soil to the two plant species *Avena sativa* und *Brassica rapa* (rapid cycling variant Rbr) was performed (ECT, 2001). The study was based on the ISO draft guideline ISO/TC 190/SC 4/WG 3 N 58–Soil Quality–Chronic Toxicity in Higher Plants (February 2000), except inclusion of a storage period of 14 days of the soils after application of 3,4-DCA. The organic carbon content of the soil was 2.17 ± 0.05%. The seeds were sown in a standard soil amended with 3,4-DCA at concentration of 0, 31.25, 62.5, 125, 250, 500 and 1,000 mg/kg 14 d prior to the sowing. After 35 days the shoot length, biomass and seed pod (silique) production of *Brassica rapa* and after 51 days the shoot length, biomass and flower production of *Avena sativa* were determined. In addition the seedling emergence after day 3, 4, 5 and 8 and the growth at day 14 were recorded.

Concentration of 31.25 and 125 mg/kg promoted the growth of the plant. At a concentration of 500 and 1,000 mg/kg no plant of the two species developed. The EC50 for the emergence of *Avena sativa* was 514 mg/kg and for *Brassica rapa* 304 mg/kg 3,4-DCA. The lowest concentration at which less than 75% of the seedlings emerged was 500 mg/kg for both species. Thus the NOAEC for the emergence was 250 mg/kg.

The EC50 values for the shoot length, shoot dry weight, number of flowers, dry weight of flowers and total dry weight of *Avena sativa* at day 51 ranged from 202 to 242 mg/kg with the number of flowers being the most sensitive endpoint.

Statistical analysis showed a significant reduction ($P < 0.05$) between the control and treatment levels of and above 250 mg/kg 3,4-DCA in the shoot length, shoot dry weight, number of flowers, dry weight of flowers and total dry weight of *Avena sativa* at day 51. Therefore, the NOAEC for *A. sativa* derived from this study was 125 mg/kg 3,4-DCA.

The EC50 values for the shoot length, shoot dry weight, number of seed pods, dry weight of seed pods and total dry weight of *Brassica rapa* at day 35 ranged from 251 to 264 mg/kg with the shoot length and the number of seed pods being the most sensitive endpoints.

Statistical analysis showed a significant reduction ($P < 0.05$) between the control and treatment levels of and above 500 mg/kg 3,4-DCA in the shoot dry weight, seed pot number, dry weight of seed pods and total dry weight of *Brassica rapa* at day 35. It also showed a significant reduction in the shoot length, between the control and treatment levels of and above 250 mg/kg 3,4-DCA. Therefore, the NOAEC for *B. rapa* derived from this study was 125 mg/kg.

Again, a comparison of the results from this chronic study with the EC50 for *Lactuca sativa* obtained in a short-term test with freshly contaminated soil (14d-EC50 = 10 mg/kg dw, Hulzebos et al., 1993) shows a significant reduction of toxicity due to the preincuabtion of the soil with 3,4-dichloroaniline.

Sediment

For the substance 3,4-dichloroaniline sediment tests with the benthic species *Lumbriculus variegatus* and *Chironomus riparius* were performed by Oetken et al. (2000).

First the acute toxicity of 3,4-dichloroaniline to both species was determined in a test without sediment. *Lumbriculus variegatus* was exposed in multi-well plates for 4 days to 3,4-dichloroaniline concentrations in the range of 3.125 to 50 mg/l. Lethal endpoints were paralysis as well as failure of circulation of hemolymphe. To determine sublethal effects, morphallaxis, convulsive motion and increased defecation were reported. Another toxicological endpoint was the incidence of deformations. For the test with *Chironomus riparius* first instar larvae were exposed in multi-well plates for 48 hours to 3,4-dichloroaniline concentrations from 2.5 to 40 mg/l. Lethality of the organisms was determined by immobility and/or lack of reaction to touching. No sublethal effects were determined.

For *Lumbriculus variegatus* a 96-hour LC50 of 25.2 mg/l was found, while the 48-hour LC50 for *Chironomus riparius* was 9.2 mg/l.

The sediment tests were conducted over a period of 28 days. An artificial sediment with a grain size of 100-2,000 µm was used. As carbon sources 1% pulverized leaves of each stinging-nettle (*Urtica* spec.) and alder (*Alnus glutinosa*) were used. The organic carbon content of the sediment was about 1.8%. Due to these carbon sources it was not necessary to feed the animals during the tests.

For each species two assays were performed with sediment preincubated with 3,4-dichloroaniline either for 2 days or for 14 days. It is assumed that after 14 days an equilibrium is reached.

For the sediment test with *Lumbriculus variegatus* worms of the same physiological and developmental status were exposed to the sediment spiked with 3,4-dichloroaniline in the range of 1 to 625 mg/kg dw for 28 days. At the end of the test the endpoints survival, deformations and morphallaxis were monitored.

First instar larvae of *Chironomus riparius* were exposed to the sediment spiked with 3,4-dichloroaniline in the range of 0.064 to 40 mg/kg dw. As endpoints the following effects were monitored at the end of the test: total emergence, rate of emergence, gender ratio and eggs per clutch.

The results of the sediment tests with both species are summarised in **Table D.4**:

Table D.4 Results of sediment tests with *L. variegates* and *C. riparius*

	48h water	28d-sediment bioassay			
Species, endpoint	**L(E)C50 [mg/l]**	**NOEC [mg/kg dw]**		**LOEC [mg/kg dw]**	
		2 days	**14 days**	**2 days**	**14 days**
L. variegatus	25.2				
Number of worms					
Total					
Large		25	5	125	25
Small		25	5	125	25
Biomass		25 [1)]	25	125 [1)]	125
Deformations		n.d.	5	n.d.	25
		1[1)]	1[1)]	5[1)]	5[1)]
C. riparius	9.2				
Emergence		40	40	>40	> 40
Rate of Emergence		8	< 0.064	40	0.064
Gender ratio		40	40	>40	>40
Eggs per clutch		40	< 0.064	> 40	0.064

1 effect was obvious, but not statistically significant to the solvent control

The lowest NOEC was found for *Chironomus riparius* for the endpoint rate of emergence and eggs per clutch. Even at the lowest tested concentration of 0.064 mg/kg dw the emergence of the larvae was statistically significant earlier (> 10 < 20 %) than in the solvent control. According to the TGD a NOEC for rate of emergence of 0.032 mg/kg can be calculated as LOEC/2.

For *Lumbriculus variegatus* a NOEC of 5 mg/kg dw was found for the endpoints total number of worms, number of large worms and biomass.

The effects observed in both sediment tests are not caused by the concentration of the substance in the waterphase, as these concentrations were far below the concentration at which effects were found in short-term tests. At the LOEC of 25 mg/kg dw for *Lumbriculus* the concentrations of 3,4-dichloroaniline in the overlying water and the porewater were 0.033 mg/l and 0.53 mg/l, respectively. at day 28, while the 96-hour LC50 for *Lumbriculus* from the water only study was 25 mg/l. At the LOEC of 0.064 mg/kg dw for *Chironomus* no 3,4-dichloroaniline was detectable in both overlaying water and porewater. The results of the tests with Lumbriculus and Chironomus show that the preincubation of the sediment with the test substance for 14 days does not reduce the toxicity. Therefore, it can be concluded that the reaction products of 3,4-dichloroaniline with humic substances are bioavailable and toxic for sediment-dwelling organisms.

The results of the test with *Chironomus riparius* (LOEC of 0.064 mg/kg) by Oetken et al. (2000) are questioned because for the endpoints rate of emergence as well as for eggs per clutch no statistically significant dose related response was found and therefore the test was repeated. The new study (Bayer AG, 2001) was intended to be performed in two parts to investigate the influence of the aging period of the spiked sediment onto the development of chironomids. Unfortunately unfavourable oxygen concentrations in several test vessels induced a high mortality in some replicates of all test concentration of the study were the larvae were added to the test vessels 2 days after spiking. Therefore only results for the 14 days aged sediment are available. The test was carried out with an artificial sediment which was prepared 2 days before spiking. It consist of 75% fine quartz, 2.0% dried, finely ground peat, as a food source 1% stinging-nettle (*Urtica* spec.) and 1% leaves of alder, 20% kaolin and around 1% calcium carbonate to adjust the pH value to 7 + 0.5. The test substance was added as a mixture of

unlabeled and labelled test substance. The initial nominal test concentration was chosen as follows: 10, 32, 56, 100, 180, 320 and 1,000 mg/kg dw.

The endpoints determined in the study were emergence rate, development rate (pooled sex), development rate (male) and developments rate (female). The results (nominal concentration) of the sediment test are summarised in **Table D.5**:

Table D.5 Results of sediment tests

	EC_{10} [mg/kg dw]	EC_{15} [mg/kg dw]	EC_{50} [mg/kg dw]
Emergence rate (pooled sex)	219	223	239
Development rate (pooled sex)	129	165	>180
Development rate (male)	122	>180	>180
Development rate (female)	104	154	>180

No emergence was found at 320 and 1,000 mg/kg dw.

The findings of all test concentration indicate that the major part of radioactivity (60-77%) was found in the spiked sediment, that the proportion of activity in the test water rose with the increasing test concentration (1.5–19%) and that after about two weeks after spiking an equilibrium between sediment and water was established. The portion of radioactivity in the pore water was generally low, however, a similar trend was visible: At the lowest concentration (10 mg/kg dw) the proportion was below 0.3%, while at the highest test concentration (1,000 mg/kg dw) the radioactivity in pore water exceeded 1.3%. The portion of extractable activity from the sediment in the HCL-eluate rose with increasing test concentration as well: at 10 mg/kg dw, about 1.4% of the radioactivity were found in the HCL-eluate, at 100 mg(kd dw 2.1% and at 1,000 mg/kg dw 7.8%.

Also for aniline prolonged sediment tests with *Chironomus riparius* and *Lumbriculus variegatus* were performed (Egeler et al., 2002, Egeler and Nésa, 2002). The sediment used in the tests was an artificial sediment consisting of 5% peat, 20% kaolinite clay and 75% quartz sand. The sediment had a mean organic matter content of 2 ± 0.5 %. As food source for the test organisms urtica powder in a concentration of 0.5% of sediment dry weight was added. The test substance was added to the sediment as an aqueous solution. The test system was allowed to equilibrate for 48 hours before the test organisms were introduced. The endpoints determined in the studies were emergence ratio, developmental rate and total number of adults emerged for *Chironomus riparius* and survival, reproduction and biomass for *Lumbriculus variegatus*. Analysis of the concentration of the test substance in sediment, pore water and overlying water was carried out for the control and three test concentrations at days –2, 0, and 28 of the test. An overall mean recovery rate of 30% was found in the test with *Chironomus riparius* and of 37.2% in the test with *Lumbriculus variegatus*. The difference between nominal and measured aniline concentrations can be both attributed to biodegradation and formation of non-hydrolyzable binding to sediment components that is inaccessible for analytical determination. To consider the decrease of the aniline concentration by biodegradation, as a rough approach the overall mean recovery is used to correct the nominal aniline concentrations. As this recovery also covers formation of non-hydrolyzable binding of aniline to the sediment it represents a worst-case approach. Ammonium concentration was measured twice during the test period. The measurements were performed with overlying water removed from replicate of control and one of each concentration level. The measurements show an increase in ammonium content related

with increasing aniline concentration As ammonia in its unionized form is toxic to most aquatic organisms, it cannot be excluded that the increased ammonium concentration has an influence on the biological results and that the observed results are not directly related to the toxicity of aniline. However, since the increased ammonium concentration was observed only in treated vessels, it may have occurred as a secondary effect of the presence of aniline in the sediment-water system. In **Tables D.6** and **D.7** the results of the studies are presented:

Result from test with *Chironomus riparius*:

Table D.6 Result from test with *Chironomus riparius*

	mg/kg sediment (dw)			
	Emergence ratio		Developmental rate	
	Nominal	Corrected for mean recovery of 30%	Nominal	Corrected for mean recovery of 30%
NOEC	125	37.5	250	75
LOEC	250	75	500	150
EC10	113.2	34	245.3	73.6

Results from test with *Lumbriculus variegatus* for endpoint survival:

Table D.7 Results from test with *Lumbriculus variegatus*

	mg/kg sediment (dw)	
	Nominal	Corrected for mean recovery of 37.2%
NOEC	125	46.5
LOEC	250	93.1
EC10	34.5	15.3

For the endpoints biomass and reproduction there were clear effects from the lowest concentration level on. Therefore, no dose-response relationship could be calculated and no NOEC/LOEC estimation could be made. It can only be stated that the NOEC for reproduction and growth is < 31.25 mg/kg dw (nominal) or < 11.6 mg/kg dw (measured).

For MDA a long-term sediment test with *Lumbriculus variegatus* was performed (Egeler, 2002). The test concentrations were 60, 30, 7.5, and 3.74 mg/kg dw. The peat content of sediment amount to 5% of the sediment dry weight. The food source consists in finely ground leaves of stinging nettle (*Urtica* sp.) spiked to sediment once at the start of the test.

Due to binding behaviour of MDA to sediment, MDA was preincubated with the sediment 16 day before addition of test organisms. After 28 days the endpoints reproduction, biomass reduction and survival were examined. In **Table D.8** the results for each endpoint are summarised:

Table D.8 Results for survival, biomass reduction and reproduction

Endpoint	Parameter	NOEC (mg/kg dw)	LOEC (mg/kg dw)	EC50 (mg/kgdw)
Survival	surviving worms	≥ 60	> 60	n.d.
Biomass	Dry weight	< 3.75	3.75	42.6
Reproduction	Total number of worms	3.75	7.5	59.5
	Number of regenerated worms	3.75	7.5	14.1

The available sediment studies give a strong indication that the reaction products of aniline derivatives with humic acids are bioavailable. Therefore, there is in general a need to perform sediment and soil toxicity tests for a proper assessment of the risk of these substances to the environment.

Accumulation via the food chain

Aniline derivatives do not accumulate to a large extent in fish exposed via the water phase. Therefore, the exposure route water–fish–fish eating bird or mammal is likely to be not relevant.

However, the reaction products of aniline compounds with humic acids accumulate in soils and sediments and are assumed to be bioavailable. Therefore, a biomagnification via the terrestrial and benthic food chain cannot be excluded.

As there is up to now no guidance how to calculate biomagnification of chemical substances via several food chain members (e.g. sediment-dwelling worm–worm-eating fish–fish-eating bird or mammal), only a simplified food chain model (sediment- or soil-dwelling worm–worm-eating bird or mammal) can be used. For the substance 3,4-dichloroaniline a bioaccumulation factor for the sediment-dwelling worm *Lumbriculus variegatus* was measured by Nagel. With this factor a 3,4-dichloroaniline concentration in these worms could be calculated that was used as PEC_{oral} for the assessment of secondary poisoning. This PEC_{oral} was compared to the $PNEC_{oral}$ that was derived from mammalian laboratory tests according to the TGD.

In a sediment bioaccumulation study with aniline using the benthic oligochaete *Lumbriculus variegatus* as test organism, BAF < 1 were found, indicating that the bioaccumulation of aniline for sediment organisms is low (Egeler and Nésa, 2002).

As the bioaccumulation potential of the different aniline compounds cannot be compared, a bioaccumulation study with sediment-dwelling organisms in sediment should be conducted for a proper assessment of the endpoint secondary poisoning via the benthic food chain.

For the assessment of secondary poisoning via the terrestrial food chain, a bioaccumulation study with soil-dwelling organisms should be conducted in analogy to the sediment compartment.

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Appendix E Hepatic metabolism of aniline

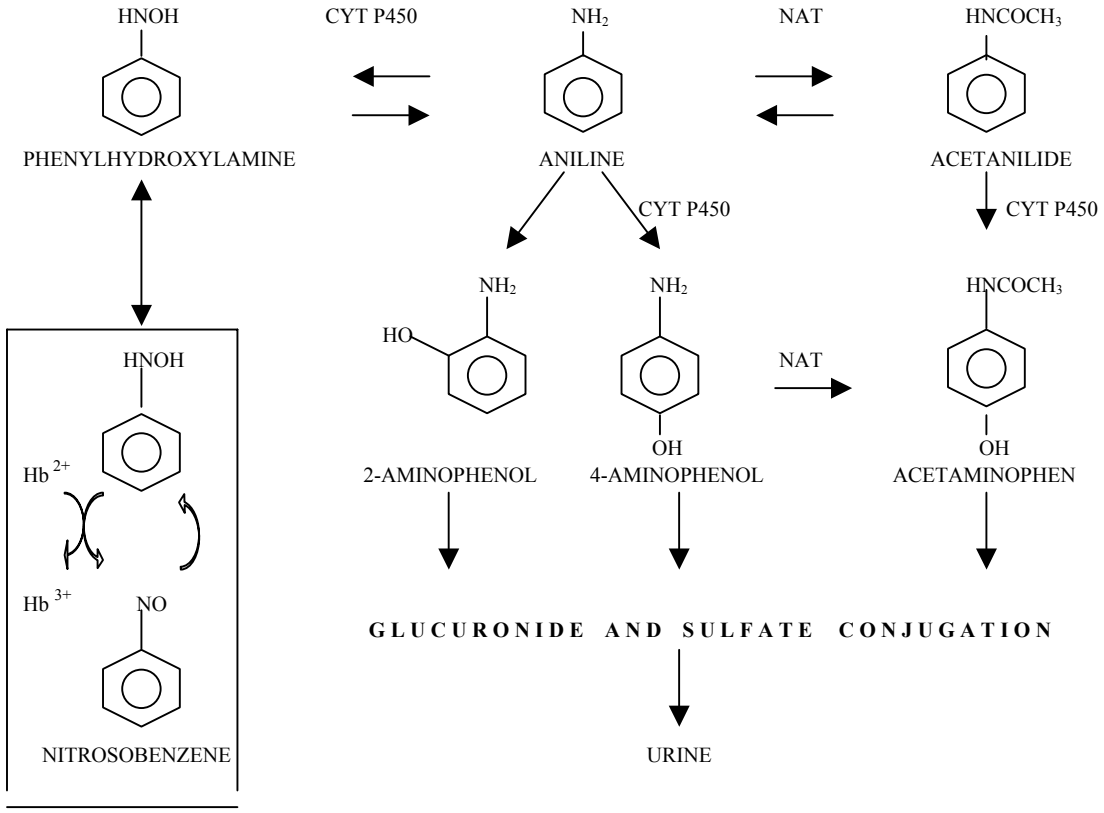
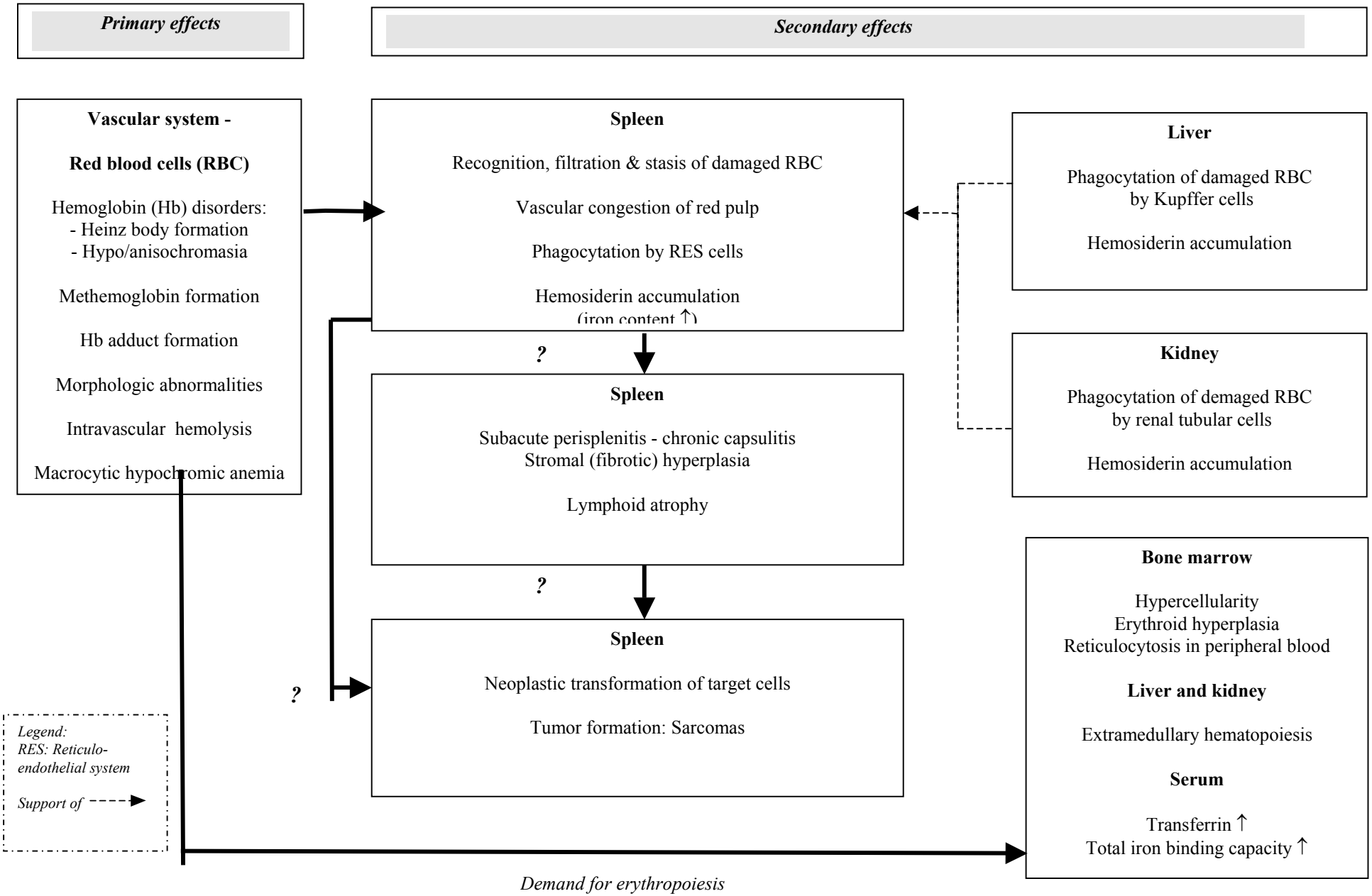


Fig. 1 Hepatic metabolism of aniline (from Harrison and Jollow, 1987)

P450, cytochrome P450 ; NAT, N-acetyltransferase ;
Hb^{2+}, haemoglobin; Hb^{3+}, methaemoglobin

Appendix F Schematic representation of main toxic effects related to aniline erythrotoxicity



European Commission

EUR 21092 EN **European Union Risk Assessment Report aniline, Volume 50**

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Environment and quality of life series

The report provides the comprehensive risk assessment of the substance aniline. It has been prepared by Germany in the frame of Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances, following the principles for assessment of the risks to humans 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 aniline concludes that there is at present concern for workers, consumers and for humans exposed via the environment, risk reduction measures have to be initiated.

The environmental risk assessment for aniline concludes that there is a need for limiting the risks for the aquatic and air compartments. There is also a need for better information to adequately characterise the risks for the aquatic and terrestrial ecosystems, and the atmosphere.

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