European Chemicals Bureau

Institute for Health and Consumer Protection

European Chemicals Bureau

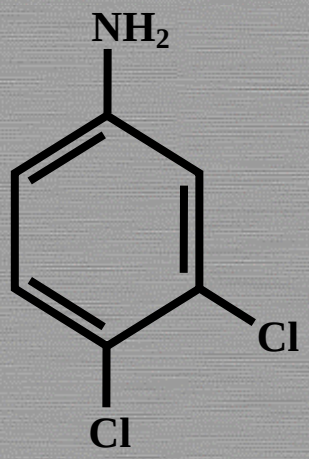
Existing Substances

European Union Risk Assessment Report

CAS: 95-76-1

EINECS: 202-448-4

3,4-dichloroaniline (3,4-DCA)



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3rd Priority List

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The mission of the IHCP is to provide scientific support to the development and implementation of EU polices related to health and consumer protection. The IHCP carries out research to improve the understanding of potential health risks posed by chemical, physical and biological agents from various sources to which consumers are exposed.

The Toxicology and Chemical Substances Unit (TCS), commonly known as the European Chemicals Bureau (ECB), provides scientific and technical input and know-how to the conception, development, implementation and monitoring of EU policies on dangerous chemicals including the co-ordination of EU Risk Assessments. The aim of the legislative activity of the ECB is to ensure a high level of protection for workers, consumers and the environment against dangerous chemicals and to ensure the efficient functioning of the internal market on chemicals under the current Community legislation. It plays a major role in the implementation of REACH through development of technical guidance for industry and new chemicals agency and tools for chemical dossier registration (IUCLID5). The TCS Unit ensures the development of methodologies and software tools to support a systematic and harmonised assessment of chemicals addressed in a number of European directives and regulation on chemicals. The research and support activities of the TCS are executed in close co-operation with the relevant authorities of the EU MS, Commission services (such as DG Environment and DG Enterprise), the chemical industry, the OECD and other international organisations.

European Commission
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3,4-DICHLOROANILINE

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RISK ASSESSMENT

3,4-DICHLOROANILINE

CAS No: 95-76-1

EINECS No: 202-448-4

RISK ASSESSMENT

Final Report, 2006

Germany

The risk assessment of 3,4-dichloroaniline has been prepared by Germany on behalf of the European Union.

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Date of Last Literature Search:	**2000**
Review of report by MS Technical Experts finalised:	**2001**
Final report:	**2006**

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 Health and Environmental Risks (SCHER) which gives its opinion to the European Commission on the quality of the risk assessment.
If a Risk Assessment Report concludes that measures to reduce the risks of exposure to the substances are needed, beyond any measures which may already be in place, the next step in the process is for the "Rapporteur" to develop a proposal for a strategy to limit those risks.
The Risk Assessment Report is also presented to the Organisation for Economic Co-operation and Development as a contribution to the Chapter 19, Agenda 21 goals for evaluating chemicals, agreed at the United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992 and confirmed in the Johannesburg Declaration on Sustainable Development at the World Summit on Sustainable Development, held in Johannesburg, South Africa in 2002.
This Risk Assessment improves our knowledge about the risks to human health and the environment from exposure to chemicals. We hope you will agree that the results of this in-depth study and intensive co-operation will make a worthwhile contribution to the Community objective of reducing the overall risks from exposure to chemicals.

Roland Schenkel
Director General
DG Joint Research Centre

Mogens Peter Carl
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:	95-76-1
EINECS No:	202-448-4
IUPAC Name:	3,4-dichlorophenylamine
Synonyms:	3,4-dichloroaniline

Overall results of risk assessment

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account. [4]

Conclusion (i) There is a need for further information and/or testing. [5]

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

– the non-agricultural use of diuron on sealed areas as total herbicide,

which is expected to cause a risk to the aquatic environment.

An environmental pollution of 3,4-dichloroaniline from the use of diuron as antifouling agent and as algicide in the construction sector has to be expected. These releases could not been taken into account in the risk characterisation, as neither sufficient exposure relevant information nor an appropriate exposure model are available. Diuron is more toxic than 3,4-DCA and probably occurs in higher concentrations, thus the 3,4-DCA exposure from these applications should be covered by a diuron assessment. It is recommended to perform an assessment for diuron in the frame of the Biocides Directive 98/8/EC.

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

For the releases of 3,4-DCA from the non-agricultural use of diuron on sealed areas as a total herbicide the PEC/PNEC ratio for sediment is above 1. The data basis can be improved by performing a long term test with a third sediment organism representing a further exposure pathway (*Hyalella azteca*). However, this requirement for further testing was awaiting the outcome of the risk reduction strategy for the aquatic compartment. Because the measures recommended are expected to sufficiently reduce concentrations in the aquatic compartment, the test is now no longer deemed necessary.

[4] Commission Recommendation 2006/283/EC of 13th April 2006 on risk reduction measures for various substances including 3,4-DCA, OJ L 104/46.

[5] Commission Communication 2006/C 90/04 of 13th April 2006 on the results of the risk evaluation and the risk reduction strategies for various substances including 3,4-DCA (90/07).

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.

Based on the available information the exposure of workers to 3,4-dichloroaniline is generally low with the exception of occasional dermal contact during cleaning, maintenance and repair work. On that background for skin sensitisation concern has to be raised.

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

For the other toxicological endpoints the risk orientated conclusions result in no concern with the consequence that risk reduction measures are of low priority. Although the hazard assessment revealed significant toxicological properties for 3,4-dichloroaniline, exposure levels reported at the workplace are below the concern range.

Consumers

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

Humans exposed via the environment

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

Human Health (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

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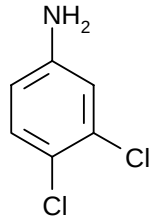
1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS No: 95-76-1
EINECS No: 202-448-4
IUPAC Name: 3,4-dichlorophenylamine
Synonyms: 3,4-dichloroaniline, 3,4-dichlorobenzeneamine, 3,4-DCA
Empirical formula: $C_6 H_5 Cl_2 N$
Molecular weight: 162 g/mol

Structural formula:



1.2 PURITY/IMPURITIES, ADDITIVES

Purity > 95%, typical concentration 99%

Impurities Cyclohexylamine (< 2%),
Chlorobenzene (1%),
1,2-dichlorobenzene (0.2%),
Aniline (< 1%),
2-chloroaniline (0.1%),
3,4-dichloronitrobenzene (< 0.7%),
4-chloroaniline (< 0.1%),
3-chloroaniline (< 0.1%),
2-chloro-4-aminotoluene (0.2%),
2,5-dichloroaniline (0.1%),
2,3-dichloroaniline (0.6%),
water (< 0.1%),
3,3',4,4'-tetrachloroazobenzene (< 0.01%),
3,3',4,4'-tetrachloroazooxybenzene (approximately 15 ppm) and
Morpholine (< 0.4%).

1.3 PHYSICO-CHEMICAL PROPERTIES

Table 1.1 Physico-chemical properties

Parameter	Value	Reference
Physical state	solid at 20°C	
Melting point	72°C	Lide and David (1991)
Boiling point	272°C at 1013 hPa	Lide and David (1991)
Density	1.57 g/cm^3 at 20°C	Ghetti et al. (1985)
Vapour pressure	0.184 Pa at 20°C	Bayer AG (1989a)
Surface tension	71.8 mN/m at 19.8°C (0.54 g/l solution in water)	Bayer AG (1995)
Water solubility	580 mg/l at 20°C	Bayer AG (1987b)
Partition coefficient (log P_{ow})	2.7 (Shake Flask - method)	Eadsforth (1986)
Flash point	not determined, solid	
Auto flammability	no auto flammability up to the melting point at 72°C	derived from the properties of the substance
Flammability	not highly flammable	derived from the properties of the substance Tests A.10, A.12 and A.13 not conducted
Explosive properties	no explosive properties	no test because of structural reasons
Oxidising properties	no oxidising properties	no test because of structural reasons

1.4 CLASSIFICATION

Classification according to Annex I, 29th ATP[6]

Toxic	R 23/24/25	Toxic by inhalation, in contact with skin and if swallowed
Dangerous for environment	R50/53	Very toxic to aquatic organisms, may cause long- the term adverse effects in the aquatic environment
Concentration limits:	none	
Irritant	R 41	Risk of serious damage to eyes
Sensitising	R 43	May cause sensitisation by skin contact

[6] The classification of the substance is established by Commission Directive 2004/73/EC of 29 April 2004 (29th ATP) adapting to technical progress for the 29th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances, OJ. L 152 of 30/04/2004.

2 GENERAL INFORMATION ON EXPOSURE

2.1 PRODUCTION AND IMPORT

In Western Europe, 12,000 tonnes 3,4-DCA was produced in 1991 (BUA, 1994). Presently there are two production sites in the European Union:

- Bayer AG, Leverkusen (GER)
- Tolochimi, Toulouse (F).

In the period 1996-1998, their total production volume was 13,500-15,500 tonnes/annum, from this 3,750-4,600 tonnes/annum were exported.

+3,4-DCA is exclusively used as an intermediate in the chemical industry for the synthesis of 3,4-dichlorophenylisocyanate, the herbicide propanil and an azo dye for polyester fabrics (UC = 33, IC = 3). Actually, there are no direct uses of 3,4-DCA without chemical transformation (BUA, 1994).

3,4-dichlorophenylisocyanate is sold and further used for the production of phenylurea herbicides (diuron, linuron) and the bactericide trichlorocarbanilide by further sites.

2.2 USE PATTERN

Table 2.1 The quantitative use pattern is (all figures related to 3,4-dichloroaniline [tonne/annum]):

Product	2nd Generation Products	Processing Volume	Export	Use in EU
3,4-Dichlorophenyl-isocyanate	Diuron Linuron	9,200-10,200	4,900-5,300	4,300-4,900
3,4-Dichlorophenyl-isocyanate	Trichlorocarbanilide	780	714	66*
Propanil	-	confidential (1 site)		
5-Amino-2,3-dimethylbenzenesulph-ethanol-amide	Dyes	100	?	?

* Recent information from TCC producers in the EU indicate that the total amount of TCC used in the EU is only 30 tonnes/annum.

In the Danish Product Register (June 1996), 2 products (not specified) with a 3,4-DCA content of < 0.1% are recorded with a total quantity of < 1 tonnes/annum. The substance is recorded neither in the Swedish (1992) nor in the Norwegian Product Register (1995).

3,4-DCA is a biodegradation product of several phenylcarbamates, phenylurea and acylanilide herbicides (You and Bartha, 1982).

3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 General discussion

3.1.1.1 Release into the environment

3.1.1.1.1 Releases during production of 3,4-DCA

3,4-DCA is produced by catalytic hydrogenation of 3,4-dichloronitrobenzene (synonym: 1,2-dichloro-4-nitrobenzene). During production, releases occur via waste water into the hydrosphere. According to the data of both producers, the total yearly emissions into the hydrosphere are maximum 1,700 kg/annum.

Emissions into the atmosphere are < 37 kg/annum (sum of both sites).

3,4-DCA is also formed in industrial waste water treatment plants by microbial transformation of 3,4-dichloro-nitrobenzene (BUA, 1990). This compound can be released into the waste water during its production and processing thus leading to an emission of 3,4-DCA. According to the IUCLID database the announced producers of the nitro compound are identical with the DCA-producers, so this emission source is covered by the present assessment.

3.1.1.1.2 Releases during processing of 3,4-DCA to 3,4-dichlorophenylisocyanate

The major part of the 3,4-DCA is processed to 3,4-dichlorophenylisocyanate by reaction with phosgene. One producer states that the equipment is cleaned with water, and 3,4-DCA may be formed by hydrolysis of the isocyanate. The environmental emissions of this site are estimated by measurements in the sewage, so this emission source is covered by the present assessment. At the second 3,4-DCA processing site, all residues are incinerated.

3.1.1.1.3 Releases during processing of 3,4-DCA to Propanil

Propanil is produced by one European site. While no emissions into the atmosphere occur, the emissions into surface waters are estimated to 4.9 kg/annum.

3.1.1.1.4 Releases during production of Diuron and Linuron

3,4-dichlorophenylisocyanate is marketed and processed to both plant protection agents partially at other than the DCA production sites. Releases of 3,4-DCA into the waste water are expected due to hydrolysis of the isocyanate and due to biodegradation of the plant protection agents in treatment plants.

In Europe, diuron is produced at two sites. At one site, no environmental releases occur, as the equipment is cleaned only with organic solvent, and all residues are incinerated. The second site

is identical with one of the 3,4-dichlorophenylisocyanate producers cited above, and the releases are covered by the respective scenario.

Linuron is produced at two sites. At one site, no emissions into the atmosphere and the waste water occur. At the second site, 1.2 kg/annum 3,4-DCA is emitted via waste water into the receiving surface water.

3.1.1.1.5 Releases during use of plant protecting agents and biocides

3,4-DCA is formed by biotransformation from certain crop protecting agents produced from 3,4-DCA. Additionally 3,4-DCA is released as it is an impurity of these agents, the total amount emitted via this pathway was estimated to be 310 kg/annum in Germany (BUA, 1994). The major part is released in agricultural soils. When these agents are released into the hydrosphere, unknown amounts of 3,4-DCA will be formed as well (see Section 3.1.2.4).

DCA forming plant protection products and biocides come under the Council Directive 91/414/EEC (due to the use as herbicide) respectively under the Biocide Directive 98/8/EU (due to the uses as antifouling agent and algicide). 3,4-DCA is formed by metabolisation from these agents leading to an environmental exposure which adds to the emissions from DCA production, processing etc. In the present report the release sources of 3,4-DCA from the use of herbicides and biocides are as far as possible assessed according to the principles of the *Technical Guidance Documents*.

The following agents containing a 3,4-dichlorophenyl moiety are of interest:

Linuron

3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea (CAS-No. 330-55-2)

Linuron is applied to field beans, soya beans, potatoes, asparagus, peas, carrots, leeks, celery, parsley, gladiolus, ornamentals, artichokes, fennel, parsnips, herbs and spices, onions, garlic, cereals, maize, sorghum, cotton, flax, sunflowers, sugar cane, bananas, cassava, coffee, tea, rice, peanuts, shrubs, established vines and other crops (BBA, 1990; The Pesticide Manual, 1994).

In the following table, the available information on linuron application is presented:

Table 3.1 Linuron application

Country	Volume	Remarks
Germany	n.a.	Field beans, peas, ornamentals, gladiolus, and vine-branches, 1 - 2.9 kg/ha once a year
UK	92.2 tonnes/annum (1994/97)	Arable crops, outdoor bulbs and flowers, grassland and fodder crops, glasshouse crops, hardy nursery stock, soft fruit and vegetables, 0.10 - 1.31 kg/ha
Finland	4.2 tonnes (1997)	Beans, leek, potatoes, fruit trees, berries and ornamentals, 0.675-1.350 kg/ha
Netherlands	26 tonnes (1993) 40 tonnes (1995)	Plant protection agent
Denmark	4.1 tonnes (1994) 11.3 tonnes (1995) 10.6 tonnes (1996) 9.6 tonnes (1997)	Plant protection agent
Sweden	4.3 tonnes (1995)	Last approval 1995

Presently, the application of linuron is object of the review programme in the frame of Council Directive 91/414/EEC. As the maximum application rate, 0.95 kg/ha is intended.

Diuron

3-(3,4-dichlorophenyl)-1,1-dimethylurea (CAS-No. 330-54-1)

Diuron is applied

- in the agricultural sector: to selective control of germinating grass and broad-leaved weeds in many crops, including asparagus, tree fruit, bush fruit, citrus fruit, vines, olives, pineapples, bananas, sugar cane, cotton, peppermint, alfalfa, forage legumes, cereals, maize, sorghum, perennial grass-seed crops, and ornamentals (BBA, 1990; The Pesticide Manual, 1994);
- in the non-agricultural sector to total control of weeds and mosses on non-crop areas like garden paths, yards, and railway tracks;
- as antifouling agent on ships. In Europe, 25 – 42 tonnes diuron were used in 1998. The main use is in international shipping, and to a minor extent in recreational boating. The diuron content in antifouling paints is 2-4% (Bayer AG, 1999), according to CEPE (1999) the maximum level is 13%. Because there is no appropriate model available, in the present report a quantitative risk assessment for 3,4-DCA metabolised from diuron cannot be performed. It is recommended to assess this use in the frame of the Biocide Directive 98/8/EU;
- in the construction sector as algicide in façade paints and plasters. The European use amount is 110-170 tonnes/annum, the content in paints is 0.2-0.5% and in plasters 0.05-0.3%. Paints are renewed at the earliest after 5 years and plasters after 10 years (Bayer AG, 1999). At this use, diuron is enclosed into a matrix. A fraction is expected to be leached with rainwater, but the releases cannot be quantified. Because of lack of data and an appropriate model, in the present report a quantitative risk assessment cannot be performed. It is recommended to assess this use in the frame of the Biocide Directive 98/8/EU.

In the following table, the available information on diuron application is presented:

Table 3.2 Diuron application

Country	Volume	Remarks
Germany	?	Agric.: fruit and vine growing, 2.7 to 4.1 kg/ha once a year, application on sealed areas prohibited since 1997
UK	9.2 tonnes/annum (1994/97) ?	Outdoor bulbs and flowers, hardy nursery stock, hops, orchards, soft fruit and vegetables, 0.15 to 2.79 kg/ha antifouling agent, for amateur and professional use
Netherlands	112 tonnes (1993) 108 tonnes (1995)	Plant protection agent
Denmark	23.4 tonnes (1994) 31.4 tonnes (1995) 7.4 tonnes (1996) 7.2 tonnes (1997) 2.6 tonnes (1997)	Plant protection agent Plant protection agent Plant protection agent Plant protection agent Antifouling agent, on approximately 50,000 small ships, last year of approval 1997
Sweden	Small use, volume confidential	Antifouling agent

Table 3.2 continued overleaf

Table 3.2 continued Diuron application

Country	Volume	Remarks
Finland	0.8 tonnes/annum 40 kg/annum	Antifouling agent for recreational boating Wood preservatives
Belgium		Not allowed as antifouling Not authorised as pesticide

Propanil

N-(3,4-dichlorophenyl)-propanamide (CAS-No. 709-98-8)

Propanil is used as a contact herbicide in rice (2.5-5.0 kg/ha) and also in wheat cultures (The Pesticide Manual, 1994). At present, no propanil containing agents are permitted in Germany. As well, propanil is not used in UK and Finland. In Sweden, in 1994 propanil was approved the last time.

In France, the application of propanil on rice fields is homologated with a rate of 3.6-3.7 kg/ha. According to an industry information, the propanil application rate is 2.5 to 5 kg/ha (Rhône-Poulenc, 1999).

Neburon

1-butyl-3-(3,4-dichlorophenyl)-1-methylurea (CAS-No. 555-37-3)

This substance is used for pre-emergence control of annual broad-leaved weeds and grasses in beans, peas, alfalfa, garlic, cereals, beet, strawberries, ornamentals, and in forestry (The Pesticide Manual, 1994). At present, no neburon containing agents are permitted in Germany. As well, neburon is not used in UK, Sweden and Finland. Presently, there is no neburon production in Europe.

The following compounds, though at one time marketed or widely reported, are believed to be currently of little commercial interest (The Pesticide Manual, 1994):

Methazole:	2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione (CAS-No. 20354-26-1)
Phenobenzuron:	1-benzoyl-1-(3,4-dichlorophenyl)-3,3-dimethylurea (CAS-No 3134-12-1)
Chloranocryl:	N-(3,4-dichlorophenyl)-2-methyl-2-propenamide (CAS-No. 2164-09-2)
Cypromid:	N-(3,4-dichlorophenyl)-cyclopropanecarboxamide (CAS-No. 2759-71-9)
Swep:	methyl (3,4-dichlorophenyl) carbamate (CAS-No. 1918-18-9)
Chloraniformethan:	N-[2,2,2-trichloro-1-(3,4-dichloroanilino)-ethyl]formamide (CAS-No. 20856-57-9)
Benzoylprop[ethyl]:	N-benzoyl-N-(3,4-dichlorophenyl)-DL-alanine [ethyl ester] (CAS-No. 22212-55-1)

3.1.1.1.6 Releases during production and use of Trichlorocarbanilide [TCC; 3-(4-chlorophenyl)-1-(3,4-dichlorophenyl)-urea]

TCC is produced either from 3,4-DCA and 4-chlorophenyl isocyanate, or from 4-chloroaniline and 3,4-dichlorophenyl isocyanate. It is used as a deodorant and soap bactericide in household products.

3,4-DCA is a metabolite of TCC. Therefore, environmental releases of 3,4-DCA are expected from production and use of TCC.

Gledhill (1975) examined the biodegradation of TCC in a continuous-flow activated sludge system. The TCC influent concentration was 200 µg/l, the concentration of MLSS was 4,000 mg/l and the mean retention time was 10 hours. TCC was ^{14}C labeled either in the p-chloroaniline ring or in the dichloroaniline ring. For the study in which the ^{14}C-DCA-TCC was used, 30% of the total radioactivity was found in the effluent, 26% was found as $^{14}CO_2$ and 35% in the activated sludge. Thus, elimination in a municipal sewage treatment plant of 70% can be assumed for TCC and its metabolites. A further analysis of the effluent by thin-layer chromatography showed that about 17% of the total radioactivity in the effluent was identified as 3,4-DCA. As the recovery of the radioactivity in the effluent by the employed method (thin-layer chromatography) was only about 40%, it is assumed for the assessment that about 40% (17% of 40% recovery) conversion of TCC to 3,4-DCA occurs.

Currently there are 3 TCC production sites in Europe. Releases into the environment are expected when TCC is emitted into a treatment plant, where the urea moiety can be biologically cleaved under formation of 3,4-DCA. One site is identical with DCA producer A, and the emissions during TCC production are covered by the respective scenario. Both further sites state that there are no releases into the environment.

TCC is used in household articles. After their use it is completely emitted into the household sewage. The European TCC use volume is 30 tonnes/annum. Assuming removal in sewage treatment plants of 70% and conversion of TCC to 3,4-DCA of 40% as found by Gledhill, gives 3.6 tonnes/annum TCC that are converted to 3,4-DCA (30 tonnes/annum · 30% · 40%). As TCC consists of both a DCA-ring and a p-chloroaniline ring, 3.6 tonnes of TCC result in the formation of 1.84 tonnes 3,4-DCA that are released due to this source.

3.1.1.2 Degradation

3.1.1.2.1 Hydrosphere

Biodegradation

As different test results confirm, 3,4-DCA has to be regarded as not readily biodegradable:

A modified MITI-I-Test (OECD 301 C) with activated sludge (10 components) indicated 0% degradation after 14 days (CITI, 1992). The Closed Bottle Test (OECD 301 D) showed after 28 days 0% degradation (Bayer AG, 1987a; Janicke and Hilge, 1980). Therefore, 3,4-DCA is regarded as not readily biodegradable in surface waters.

A Coupled Units Test (OECD 303 A) with activated sludge indicates < 5% degradation after 29 days, a prolongation of the duration time has no influence on the degradation process (Janicke

and Hilge, 1980). Therefore, 3,4-DCA is regarded as not biodegradable in municipal waste water treatment plants (WWTPs).

However, in a Closed-Bottle-Test (OECD 301 D) performed with adapted industrial sludge 82% of 3,4-DCA were degraded after 28 days (Bayer, 1987a). The application of this test result to environmental conditions and to municipal WWTPs is not possible.

A degradation test with Rhine-water showed primary degradation between 45% after 30 days at a substance concentration of 0.01 mg/l and > 95% within 50 days at a concentration of 1 mg/l (Bayer AG, 1992). With water from the North Sea as inoculum no biodegradation could be detected (Kuiper and Hanstveit, 1984).

As no standard tests on inherent biodegradability are available, 3,4-DCA is regarded as not readily biodegradable. In surface water and under environmental conditions no mineralisation is to be expected.

Measurements in WWTP influent and effluent of a production site revealed that the 3,4-DCA concentration in the effluent is sometimes higher than in the influent (BUA, 1994). This phenomenon is explained by assuming the formation of the substance from 3,4-dichloronitrobenzene by microbial reduction and no significant elimination of the 3,4-DCA.

In the following assessment it is assumed that no significant biodegradation occurs in WWTPs. According to the SIMPLETREAT model, with a log Pow of 2.7 and a log H of -1.5, a WWTP elimination of 6% (due to adsorption) is predicted, so 94% are emitted into the receiving surface water.

Sediment

Biodegradation tests under anaerobic conditions with fresh-water-sediment and pond-sediment indicate primary degradation through reductive dechlorination to monochloroaniline (3- and 4-chloroaniline) only. The dechlorination started after 20 days of incubation in unacclimated sediments. Approximately 90% of the 3,4-DCA was dechlorinated within the next 40 days. After 60 days 44% of the 3,4-DCA was present as 3-chloroaniline and 33% as 4-chloroaniline. Further degradation of the monochloroanilines was not observed in these studies. The monochloroanilines, however, appeared to be resistant to further dechlorination (Struijs and Rogers, 1989). No information on mineralisation is available.

Tests on biodegradation in aerobic sediments are not available. Thus, in the following exposure assessment, for the aerobic sediment layer the same degradation half-life of 1,000 days as for soils is used.

Abiotic degradation

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

The UV-spectrum (λ_{max} at 298 nm; log ε_{max} = 3.22; Reber et al., 1979) indicates that direct photolysis in the hydrosphere may occur. In several tests, both direct and indirect photolysis was demonstrated.

The quantum yield of the direct phototransformation in water was determined; from this a half-life of 0.4 hours (yearly average value concerning the water surface at 40-50° latitude) was calculated (Lemaire et al., 1985). A test with natural sunlight (40° latitude, summer) resulted in half-lives of 6 ± 3.6 hours at the water surface (Yager and Yue, 1988).

In model ponds, half-lives in the range of 4.1 to 6.3 days were determined, the main route for loss of 3,4-DCA was predicted to be direct phototransformation. The ponds' depth was 1 m, the test was provided in spring at the 52 degree of latitude. There is no information about the concentration of suspended matter (Wolff and Crossland, 1985).

In view of the stability to hydrolysis and the poor biodegradability, photolysis should be the major degradation pathway for 3,4-DCA in the hydrosphere. A half-life of 6 days appears to be a realistic value for the degradation under environmental conditions. However, this value was determined for a water depth of 1 m, while according the TGD the mean water depth is 3 m. As photolysis only takes place in the upper water layer, a half-life of 18 days is used in the exposure models.

There are several investigations dealing with the reaction products of 3,4-DCA photolysis. By irradiation with a sunlight lamp ($\lambda > 290$ nm) of a 0.8 mg/l solution in distilled water, the main product was found to be 2-chloro-5-aminophenol (lower limit of conversion 78 ± 5%) which degraded three times more rapidly than DCA in a separate photolysis experiment. A minor amount (2%) of 3-chloroaniline was also produced (Miller et al., 1979). Similar results were found in artificial and natural sea water (Miille and Crosby, 1983).

Further investigations regarding the formation of 3,3',4,4'-tetrachloroazobenzene are cited in Section 3.1.4.4.

3.1.1.2.2 Geosphere

The degradation (mineralisation) of radiolabelled 3,4-DCA in soil was investigated in a laboratory experiment with four different soil types and a concentration of 1 ppm over 16 weeks. Between 3.9 and 11.9% of the 3,4-DCA was mineralised in the different soils (Süß et al., 1978). On the basis of this results DT50 values for the mineralisation between 470 and 1,500 days can be extrapolated for 3,4-DCA.

Other experiments confirm these mineralisation rates, moreover they indicate, and that the rate of mineralisation decreases with increasing concentration of 3, 4-DCA (Lee and Fournier, 1978). Investigations on biodegradation of the soil bound 3,4-DCA show, that the degradability is dependent on the intensity of the attachment to the soil. Physically adsorbed plus chemically bound DCA is mineralised to 7.9% in 100 days, chemically bound to 4.3% in 100 days and non-hydrolysable is mineralised to 1.5% only in 100 days. That means 3,4-DCA covalently bound to organic fraction is degraded almost similar to the humic acids themselves (Saxena and Batha, 1982).

For the further assessment of the geosphere a half-life of 1,000 days is assumed.

3.1.1.2.3 Atmosphere

According to the method established by Atkinson, a half-life of 9 hours for photochemical-oxidative degradation with OH-radicals ($C_{OH} = 5 \cdot 10^5$ molec/cm^3) in the atmosphere is calculated.

3.1.1.2.4 Summary

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

Table 3.3 Degradation rates

$kdeg_{water}$	0.039 d^{-1}	i.e. photolysis, $t_{1/2}$ = 18 d
$kbio_{sed}$	$6.9 \cdot 10^{-4}$ d^{-1}	aerobic layer
$kbio_{soil}$	$6.9 \cdot 10^{-4}$ d^{-1}	
$kdeg_{air}$	1.8 d^{-1}	

3.1.1.3 Distribution

With a vapour pressure of 0.184 Pa and a water solubility of 580 mg/l, a Henry's law constant of 0.05 $Pa \cdot m^3 \cdot mol^{-1}$ is calculated. Based on this value, only a low volatilisation from the hydrosphere is to be expected.

According to the TGD equation (log Koc = 0.62 log Kow + 0.85), a Koc value of 334 l/kg is calculated from the log Pow of 2.7.

Experiments with radiolabelled 3,4-DCA revealed that the substance forms covalent bonds with the organic fraction in soils and sediments. The chemical attachment to humic substances occurs by at least two mechanisms, in a hydrolysable and in a non-hydrolysable manner. The bonding processes were almost completed within 1 - 2 days. The chemisorbed fraction is dependent on the 3,4-DCA concentration and on the nature of soil. Typical values at use-relevant concentrations are in the range above 90% (Hsu and Bartha, 1974; Parris, 1980). Therefore, the analytical detection of 3,4-DCA in soils is always incomplete: with organic solvents only the physically adsorbed or in pore water dissolved fractions can be extracted, while with alkaline extraction the hydrolysable fraction is removed.

There are experimentally determined values for soil partition coefficients available. In 4 loam soils with an organic content of 1.09 - 4.25%, after 2 hours incubation (where after chemisorption is not completed) an average value for K_{om} of 112 (corresponding to a Koc of 190 $l \cdot kg^{-1}$) was estimated (Briggs, 1981).

In a further experiment with two soils and one sediment, Koc-values from 1,900-10,400 $l \cdot kg^{-1}$ were determined after 53 hours incubation when chemisorption was completed (Beyerle-Pfnür and Lay, 1990). 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.

In a laboratory experiment, the distribution of ^{14}C-labelled 3,4-DCA in the sediment/water system was examined. 3 sediments collected from a creek, a pond and a drainage ditch of a fruit-growing plantation were tested. Radioactivity left in the water phase was 21.8% (after 8 days) in the creek sediment, 10.4% (90 days) in the pond sediment and 1% (90 days) in the ditch sediment. The adsorption process was not finished after 28 d as the radioactivity decreased until day 90. 3,4-DCA was converted predominantly to non-extractable residues, presumably without preceding microbial transformation because no significant CO_2 amounts (maximum 3.8%) could be detected (Heim et al., 1994).

Because of the strong adsorption onto the organic fraction, 3,4-DCA is considered to be immobile in soils. Under outdoor conditions, the substance could not be detected in the leaching water of a soil incubated with 3,4-DCA (Viswanathan et al., 1978).

Because of the poor degradation of 3,4-DCA and its metabolites 3- and 4-chloroaniline and their binding properties onto humic substances, a high accumulation of the formed complexes in soil and in sediment is expected.

The substance was measured in a municipal waste water infiltration system. The plants' beds are composed of stratified sands. While a waste water concentration of 6.11 µg/l was detected, only 0.2 µg/l were found in a well 90 m away from the plant. An elimination of 97% is calculated from these values (Bedient et al., 1983).

The available studies demonstrate a strong chemisorption onto humic acids. This is supported by studies on biodegradation in soils where mineralisation dropped to a very low rate after the covalent bounds were formed (see Section 3.1.1.2). Based on the investigation of Beyerle-Pfnür and Lay (1990), a Koc of 10,000 $l \cdot kg^{-1}$ is chosen for modelling. With this figure, the following distribution constants are calculated in accordance to the TGD models:

Table 3.4 Distribution constants for 3,4-DCA

Kp_{soil}	200 $l \cdot kg^{-1}$	$K_{soil\text{-}water}$	300 $m^3 \cdot m^{-3}$
Kp_{susp}	1,000 $l \cdot kg^{-1}$	$K_{susp\text{-}water}$	251 $m^3 \cdot m^{-3}$
Kp_{sed}	500 $l \cdot kg^{-1}$	$K_{sed\text{-}water}$	251 $m^3 \cdot m^{-3}$

With a concentration of 15 mg suspended matter per litre river water, 1.5% of the 3,4-DCA is particle-bound.

3.1.1.4 Accumulation

The measured log Pow value of 2.7 indicates no high potential for bioaccumulation. This corresponds with measured BCF obtained for fish e.g. for carp between 4 and 14 l/kg (CITI, 1992), for *Brachydanio rerio* between 30 and 38 l/kg (CT_{50} < 48 hours) (Hertl et al., 1993; Ensenbach and Nagel, 1991; Kalsch et al., 1991) and for *Oncorhynchus mykiss* of 45 l/kg (Crossland, 1990). In the following exposure assessment, a BCF value of 45 l/kg is used.

In single species tests with different invertebrates and submerse macrophytes bioconcentration factors on the basis of ^{14}C-activity amounted to 113 (*Ceratophyllum demersum*), 79 (*Elodea canadensis*), 29 (*Daphnia magna*), 28 (*Asellus aquaticus*), 15 (*Planorbarius corneus*), 35 (*Tubifex tubifex*), 30 (*Limnodrilus hoffemeisteri*), and 800 l/kg (*Lumbriculus variegatus*). For the oligochaete *Lumbriculus variegatus* the BCF of 800 l/kg was much higher than those found in other invertebrates. As the BCF values are based on radioactivity measurements, the distribution of both DCA and transformation products being formed in the test system or in the organisms is represented (Nagel, 1997).

Applying HPLC-analysis the amount of parent substance (3,4-DCA) of the total radioactivity in the holding water as well as in the organism-extracts was measured and from this the following bioconcentration factors for 3,4-DCA were determined: 82 (*Ceratophyllum demersum*), 11 (*Elodea canadensis*), 9 (*Daphnia magna*), 10 (*Asellus aquaticus*), 12 (*Planorbarius corneus*), 18 (*Tubifex tubifex*), and 16 l/kg (*Limnodrilus hoffemeisteri*). The values were lower than those BCF based on ^{14}C-activity (Nagel, 1997).

In a laboratory microcosm (40 l) providing conditions resembling a natural environment, bioaccumulation was studied (Nagel, 1997). The stability of the test system was checked in 4 replicate microcosms over the time course of more than 5 months.

For the invertebrates and macrophytes of the microcosm the following ^{14}C-activity based bioaccumulation factors (BAFs) were found: 113 (*C. demersum*), 139 (*E. canadensis*), 78 (*D. magna*), 106 (*A. aquaticus*), 73 (*P. corneus*), 158 (*T. tubifex*), and 566 l/kg (*L. variegatus*). The BAFs mentioned are mean values of 3 measurements (after 94 hours, 121 hours and 144 hours test duration). The BAF for *C. demersum* corresponds to the BCF. For *L. variegatus* the BAF value of 566 l/kg for 3,4-DCA was lower than the BCF of 800 l/kg. The other BAFs were higher than the corresponding BCFs. For the accumulation in sediment an average sorption factor (SF) of 4 could be determined (Nagel, 1997).

After differentiation of ^{14}C- activity in water, sediment and organisms of the microcosm the following BAFs based on the parent substance (3,4-DCA) could be calculated: 210 (*C. demersum)*, 198 (*E. canadensis*), 276 (*D. magna*), 76 (*A. aquaticus*), 533 (*P. corneus*), 271 (*T. tubifex*) and 572 l/kg (*L. variegatus*) (Nagel, 1997).

Hence the calculated bioconcentration factors for 3,4-DCA substance were between 2.6 times (for *C. demersum*) and 44.4 times (for *P. corneus*) higher than the corresponding bioconcentration factors for 3,4-DCA obtained in the single species tests. The parent compound related sorption factor for the sediment amounted to 31 (Nagel, 1997).

The partitioning of total radioactivity and parent substance among the organisms and compartments (water and sediment) was measured. Approximately 71% of the parent substance was bound to sediment particles. 5.5% of 3,4-DCA could be detected in *Ceratophyllum demersum*, this species accounting for only 0.14% of the total mass of the system (approximately 35%). 2.4% of the radioactivity was bound to suspended matter in the water (Nagel, 1997).

Summarising the results, it can be concluded, that for several aquatic invertebrates and macrophytes correlations were established between log K_{OW} and log BCF for the test substances. These calculations revealed basically a linear relationship between log K_{OW} and log BCF for invertebrates and macrophytes. However the goodness of fit and the characteristic parameters of the regression line depend in this case on the chosen species. Therefore a direct extrapolation of BCFs from fish to other aquatic invertebrates or macrophytes and the extrapolation of data derived from single species tests to the complex situation in laboratory microcosm is currently not possible (Nagel, 1997).

The results indicate a high bioaccumulation for *L. variegatus, C. demersum, E. canadensis, T. tubifex* and *P. corneus* with BAF, and BCF > 100 to 800 l/kg. Therefore a biomagnification via sediment dwelling organism - fish and/or birds cannot be excluded.

In addition, the results of Nagel (1997) for the epi- and endobenthic organism *Asellus aquaticus* and *Tubifex tubifex* of the microcosm study in comparison to the single species show clearly, that the bioaccumulation factors (BAF) are significantly higher than the bioconcentration factors (BCF) determined in the single species test without sediment. This is a very strong indication, that the 3,4-DCA bound onto sediment is bioavailable.

An additional evidence of bioavailability of bound 3,4-DCA for Tubificides was supplied by the results of Egeler et al. (1997). It could be shown that ^{14}C-DCA is accumulated in *Tubifex tubifex* from loaded sediment. For 3,4-DCA the highest BAF of the three investigated substances (3,4-DCA, Lindan, HCB) could be obtained, although 3,4-DCA is the substance with the lowest log Pow-value.

The available studies demonstrate a relatively low bioaccumulation due to the uptake via water. A higher accumulation occurs when sediment solids are taken up, and when food is pre-loaded with the test substance. For the assessment of secondary poisoning the BAF of 570 l/kg obtained

for *Lumbriculus variegatus* is chosen, because it is the highest accumulation factor derived using substance-specific analytics.

3.1.2 Aquatic compartment

3.1.2.1 PEClocal during production and processing/Generic approach

In the *Technical Guidance Document*, a generic exposure scenario for the release of intermediates during production and processing into surface water is proposed. A total release factor of 1% into the sewage, a sewage flow of 2,000 m^3/day, a WWTP elimination of 6% and subsequent release during 300 days per year into a river with a flow of 60 m^3/s are assumed.

- production quantity 10,000 tonnes/annum
- release into sewage 100 tonnes/annum
- production period 300 days/annum
- release into sewage 333 kg/day
- elimination in stp 6%
- Ceffl = 160 mg/l
- Clocal = 60 µg/l
- PECregional = 0.0042 µg/l (see Section 3.1.6)
- PEClocal = 60 µg/l

3.1.2.2 PEClocal during production and processing/site-specific approach

Company A

The production and processing volumes were submitted for the years 1998. The substance is produced at one site and processed in two sites in close vicinity.

At the first site, 3,4-DCA is processed to diuron via 3,4-dichlorophenylisocyanate. The substance was measured in the effluent (248 samples) with only one positive detection. Based on the detection limit, sewage and river flow, the exposure is calculated to

Clocal = < 0.010 µg/l

At the second site where DCA is produced, the substance is measured weekly in the WWTP effluent (weekly combined samples). The average and a 90%ile value were submitted (data for 1998). With the sewage and river flow, the exposure is calculated based on the 90%ile value:

Clocal = 0.057 µg/l

As both sites are emitting into the same river, both Clocal figures are added to the PECregional:

PEClocal = 0.07 µg/l

In 1986, a study on the discharges of 3,4-DCA was performed by the EU, and recommendations for the reduction of emissions via waste water into the hydrosphere were made. At that time, the

annual emission from company A had been quantified to 1.5 tonnes (Donnez, 1986). According to the information provided by this company, a similar amount is stated as the current emission. An improvement of the Rhine exposure in the last decade cannot be constated.

Company B

In 1994, 3,4-DCA production and processing was ceased by this company. However, the company is still a producer of linuron.

Company C

At this site, 3,4-DCA is not produced but processed to the isocyanate derivative. The processing volume was not submitted. The phosgenation is a water-free process, the equipment is cleaned with the reaction solvent which is incinerated. Releases into the hydrosphere are not expected.

Company D

At this site, 3,4-DCA is produced, and the total production is sold. The following parameters were submitted (to keep confidentiality the figures are not shown):

- production volumes for the years 1994-1997
- production period
- emission into the waste water, no treatment plant!
- sewage and river (low) flow

From these parameters, the exposure is calculated to

- Clocal = 22 µg/l
- PEClocal = 22 µg/l

3.1.2.3 Releases during manufacture of plant protection agents

Releases of 3,4-DCA occur at one diuron and at one linuron production site. The diuron producer is identical with the via 3,4-dichlorophenylisocyanate producer (company A), thus the emissions are covered by the scenario above.

From the linuron producer, the daily emission, flow of waste water and receiving river are known. The resulting concentrations are

- Ceffluent = 0.33 µg/l
- Clocal = $6.5 \cdot 10^{-4}$ µg/l
- PEClocal = 0.0049 µg/l

The emission of the propanil producer was estimated to 4.9 kg/annum. The production period is known, for the sewage and river flow site-specific parameters are used:

- Ceffluent = 2.9 µg/l
- Clocal = 0.12 µg/l
- PEClocal = 0.12 µg/l

3.1.2.4 Releases from the use of plant protecting agents

Diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea]

Diuron is used as a total and semi-total herbicide in the private and industrial sector for weeds control on garden pathes, yards etc. (ARW, 1995). In Section 3.1.1.1, further agricultural uses are specified. As the substance is applied on sealed areas, a significant part is discharged via runoff into the hydrosphere.

Diuron is measured periodically in German rivers and in the Rhine at Lobith (NL). The measured concentrations are cited in Appendix A.

In order to find out the major sources of diuron in the hydrosphere, the substance was measured in two small German creeks at different locations. Diuron concentrations up to 1.78 µg/l were detected. The highest concentrations were found after a thundery rain near a railway embankment and a settlement and industrial area (AWWR, 1995). These results prove that the origin of the diuron detected in the hydrosphere is the private and industrial use and not agricultural (AWWR, 1995).

There is a number of tests available dealing with the formation of 3,4-DCA during diuron degradation:

The biodegradation in aerobic systems has been investigated in water, sediment and soil, and the anaerobic degradation in sediment (Attaway et al., 1982). From these results different degradation pathways for aerobic and anaerobic degradation can be proposed. As it has been demonstrated that under aerobic conditions a sequential N-demethylation and the cleavage of urea bonding occurs under subsequent formation of N-(3,4-dichlorophenyl)-N'-methylurea, N-3,4-dichlorophenylurea and 3,4-DCA. The mechanism of anaerobic degradation proceeds via reductive dechlorination. During the proposed anaerobic pathway 3,4-DCA is not formed.

In a test with a water/sediment system, the anaerobic degradation pathway investigated by Attaway was confirmed: 3,4-DCA is not formed from diuron as dechlorination is the first metabolisation step (Hausmann, 1992).

In a further test with a water/sediment system diuron degraded with a half-life of 33 days. During the test time of 30 days, no 3,4-DCA could be detected. At the end of the test, diuron (52%), N-(3-chlorophenyl)-N',N'-dimethylurea (25%) and N-(3,4-dichlorophenyl)-N'-methylurea (8.4%) were found indicating that both aerobic and anaerobic biodegradation took place. 3,4-DCA could not be detected (Hausmann and Kraut, 1992), probably because of the short test time. We interprete this test as follows: The formation of N-(3,4-dichlorophenyl)-N'-methylurea indicates that the aerobic degradation pathway proposed by Attaway occurs, although in competition with the anaerobic pathway. 3,4-DCA was not formed because of the short test duration, but its formation has to be expected after a longer reaction time.

A laboratory test showed that under environmental conditions hydrolysis of diuron will not occur as half-lives of several years were determined (Bayer, 1982).

In a laboratory test on aqueous photolysis of diuron (10 ppm solution), 3,4-DCA was not detected at 0.006 ppm. The half-life under natural sunlight was calculated to 43 d (latitude 30°-50°; 12 hours sunlight; related to the water surface). In natural waters a longer half-life has to be expected because of dullness and self-absorption of the water (Hawkins et al., 1988).

Comparing the reaction constants of photolysis, hydrolysis and biodegradation, the last is expected to be the major degradation mechanism in the environment. Diuron released into

surface waters should adsorb onto the upper (aerobic) sediment layer, where biodegradation occurs (as well as in the water phase), and the formation of 3,4-DCA has to be expected. The DCA formation half-life is in the range of several months.

When 3,4-DCA comes into contact with sediments, covalent bounds to the organic fraction are rapidly formed (see Section 3.1.1.3), and these complexes are known to be relatively stable against biodegradation (see Section 3.1.1.2). If 3,4-DCA is formed in sediments from diuron, a strong accumulation of its complexes with humic substances would have to be expected.

Linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea]

Lachmann (1988) studied the metabolism of ^{14}C-labelled linuron over a period of 92 days. In two water/hydrosoil-systems 6-7% and 15- 21% of the applied radioactivity remained in the sandy loam soil and the silty loam soil, respectively. Non-extractable residues made up to 4% and 14% of the respective soil. Methanol and ethyl acetate were used for extraction of soil. Neither methanol nor ethyl acetate is suitable for extraction of bound 3,4-DCA. The amount of every single metabolite was very low (< 1%). However, the sediment of the flask that had yielded an exceptionally high mineralisation rate of linuron (53% in one of the flasks containing silt loam soil) contained 4-10% 3,4-DCA. Keeping in mind the unsuitable extraction method used, it is clear that the quantity of 3,4-DCA detected in this flask represents only a small part of the whole 3,4-DCA formed. The following metabolites were found in the soils mentioned above: N-(3,4-dichlorophenyl)-N'-methoxy-N'-methyl-urea, N-(3,4-dichlorophe-nyl)-N'-methyl-urea, N-(3,4-dichlorophenyl)-N'-methoxy-urea, N-(3,4-dichlorophenyl)-urea, 3,4-dichloroaniline and 3,4-dichlorophenyl-hydroxamic acid. The author proposes the degradation scheme according to Attaway (see above).

A test on hydrolysis of linuron in aqueous solution showed that this degradation mechanism is of minor importance. Reaction half-lives of 1,220 days, 1,460 days, and 1,080 days were determined at pH 5, 7, and 9, respectively. The product of hydrolysis was 3,4-DCA both in acid and basic medium (Hoechst AG, 1982).

Linuron is measured periodically in the Rhine and its tributaries. In the last years, all measured concentrations were below the detection limits of 0.02 and 0.05 µg/l, respectively (LWA, 1992, 1993; RIWA, 1992-1994; IAWR, 1994). Because of the lower concentrations (compared with diuron), this possible 3,4-DCA source would be of minor importance for the hydrosphere.

Neburon (1-butyl-3-(3,4-dichlorophenyl)-1-methylurea)

Because of the structural relationship with diuron and linuron (one methyl or respectively one methoxy group is replaced by butyl) the same degradation mechanism forming 3,4-DCA can be assumed. As no present use of neburon in Europe is known, no exposure is expected.

Propanil (N-(3,4-dichlorophenyl)-propanamide)

3,4-DCA is also a metabolite of the herbicide propanil. Formation occurs by hydrolysis, by microorganisms in soil and in rice field water, and by enzymes in plants (Pothuluri et al., 1991). There are monitoring data for soil available, but not for the water phase. Thus emissions into the hydrosphere cannot be quantified.

3.1.2.5 Releases during use of diuron as antifouling

Diuron is used as an active ingredient in antifouling agents on ships and as algicide in façade paints and plasters. From these uses, unknown amounts of diuron are released into the environment, where it is degraded to 3,4-DCA.

There is no specific information about concentration levels in the environment, and no model is available. Therefore, in the present report a quantitative risk assessment cannot be performed. Diuron as the mother substance is much more toxic than 3,4-DCA, and its environmental concentration levels are higher, thus a risk assessment for diuron is necessary. Both uses come under the Biocides Directive 98/8/EC.

3.1.2.6 Releases during production and use of trichlorocarbanilide (TCC)

As mentioned in Section 3.1.1.1, one TCC producer is identical with a DCA producer, and its emissions are covered by the respective exposure scenario. The two further TCC producers have no emissions.

In Section 3.1.1.1, the DCA releases during the use of TCC as a bactericide are estimated to 1.84 tonnes/annum. According to the TGD, 0.184 tonnes are emitted in the European standard region. With a fraction of the local main source of 0.002, 0.368 kg DCA/annum (1 g/day) are released by a municipal treatment plant.

- Ceffluent = 0.5 µg/l
- Clocal = 0.05 µg/l
- PEClocal = 0.054 µg/l

3.1.2.7 Monitoring

3,4-DCA is measured periodically in German and Dutch rivers (LUA NRW, 1999; RIWA, 1996-1998). The measured concentrations [µg/l] are given below:

Table 3.5 Measured concentration in rivers

River (Site)	1995	1996	1997
Donau (Ulm)		< 0.05 n=13	
Elde (Dömitz)	< 0.05 n=12		
Erft (Neuss)	< 0.1 n=13	< 0.1-0.27 90%ile 0.26 n=11	< 0.1-0.42 90%ile 0.36 n=12
Havel (Krughorn)		< 0.05 n=9	

Table 3.5 continued overleaf

Table 3.5 continued Measured concentration in rivers

River (Site)	1995	1996	1997
Ijsselmeer (Andijk)		median < 0.05 max. 0.08	
Lippe (Wesel)	< 0.1-0.19 90%ile 0.17 n=12	< 0.1 n=5	< 0.1-0.12 90%ile 0.11 n=11)
Main (Bischofsheim)	< 0.05 n=52	< 0.05 n=52	< 0.05-0.10 90%ile 0.05 n=53
Meusse (Eijsden)	median 0.08 max. 0.31	median < 0.05 max. 0.56	
Meusse (Keizersveer)	median 0.06 max. 0.21	median < 0.05 max. 0.68	
Neckar 4 sites	< 0.05 n=24	< 0.05 n=51	< 0.05 n=52
Rhein (Mannheim)	< 0.05 n=12	< 0.05 n=13	< 0.05 n=13
Rhein (Mainz)	< 0.05-0.19 90%ile < 0.05 n=26	<0.05-0.05 90%ile < 0.05 n=27	< 0.05 n=26
Rhein (Koblenz)	< 0.05-0.08 90%ile <0.05 n=26	< 0.05 n=11	< 0.05 n=12
Rhein (Bad Honnef)	< 0.1-0.15 90%ile <0.1 n=26	< 0.1-0.29 90%ile 0.22 n=12	< 0.1-0.22 90%ile 0.17 n=12
Rhein (Kleve-Bimmen)	< 0.1-0.60 90%ile <0.17 n=22	< 0.1-0.26 ∅ <0.1 n=10	< 0.1 n=12
Rhein (Lobith)	< 0.05-0.16 90%ile 0.10 n=13	< 0.05-0.27 90%ile 0.15 n=14	< 0.05-0.08 90%ile 0.07 n=12
Ruhr (Duisburg)	< 0.1 n=12	< 0.1-0.27 ∅ <0.1 n=7	< 0.1-0.32 90%ile 0.29 n=11
Sieg (Bergheim)	< 0.1-0.32 90%ile 0.21 n=13	< 0.1-0.31 ∅ <0.1 n=10	< 0.1-0.60 90%ile 0.46 n=12
Spree (Spandau)		< 0.05 n=10	
Teltowkanal (Kohlhasenbrück)		< 0.05 n=9	
Wupper (Leverkusen)	< 0.1-0.11 90%ile <0.1 n=13	< 0.1-0.27 ∅ <0.1 n=8	< 0.1-0.30 90%ile 0.26 n=12

For the maximum concentration measured at Meusse (Kreizersveer) of 0.68 µg/l, a sediment concentration of 0.15 mg/kg could be calculated

Apparently at several sampling sites the measured concentrations are above the PNEC of 0.2 µg/l. For the interpretation of the positive detections, the following life-cycle steps have to be considered:

- 3,4-DCA production and processing to 3,4-dichlorophenylisocyanate. The detections in the Rhine at Kleve-Bimmen and Lobith are clearly related to one producer.
- Releases during the production of plant protecting agents. These sites are not located at the monitored rivers.
- Releases during the application of plant protecting agents. Concentrations above the PNEC (0.2 µg/l) are often detected in rivers where no industrial sources are located. In the same rivers, diuron is frequently detected. Diuron is degraded to 3,4-DCA (see Section 3.1.2.4).
- Diuron is considered as the more important source, rather than linuron. Both substances have a very similar structure (methyl moiety instead of methoxy), and thus similar physico-chemical properties. Diuron was often detected in surface waters, while linuron was never found. Because of the similar properties, the different environmental occurrence is caused by the different use. Both substances are applied directly onto agricultural soils, where the runoff rate is relatively small. However, the application of diuron as total herbicide on sealed areas is of high relevance for releases into the hydrosphere because of the relative high runoff rate, and is expected to be the main source of the detected diuron and DCA concentrations.
- It is unlikely that the use of diuron as antifouling is the source of the detected 3,4-DCA, as the latter is detected in rivers without shipping. As well, the use as algicide in the construction sector is unlikely the source, as diuron is enclosed in a matrix and is not expected to lead to high releases into surface waters.

As a result of routine control monitoring in UK, 3,4-DCA was measured at two sites. While at the first site 5 measurements were below the detection limit of 0.5 µg/l, the concentrations at the second site were < 0.5 to 6.2 µg/l (∅ 3.6 µg/l, n = 6) in 1996. In 1997, the concentrations at the second site were < 0.5 to 3.8 µg/l (∅ 2.1 µg/l, n = 6). The pollution source is a former 3,4-DCA production site which has now been demolished, but contaminated groundwater remained.

3.1.2.8 Sediments

Because of the binding properties of 3,4-DCA onto organic matter, a high accumulation of the substance in sediments is expected.

With a Kp_{susp} of 1,000 l/kg, the following $PEClocal_{sed}$ are calculated:

Table 3.6 Calculated $PEClocal_{sed}$ values

Site	$PEClocal_{water}$ [µg/l]	$PEClocal_{sed}$ [mg/kg ww]
DCA production and processing, generic	60	13
DCA producer A	0.07	0.015
DCA producer D	22	4.8
Production of Linuron	0.0049	0.001
Production of Propanil	0.12	0.026
Use of trichlorocarbanilide	0.054	0.012

In stream and estuary sediments near pineapple and sugarcane fields in Hawaii, where among other weed control chemicals diuron is extensively applied, 3,4-DCA was detected in concentrations up to 950 µg/kg dw. As the substance was extracted with an organic solvent, only the physically adsorbed 3,4-DCA was detected. It has to be assumed that in fact the concentrations of bound 3,4-DCA were even much higher (see Section 3.1.1.3). The corresponding concentrations in water were not measured (Green et al., 1977).

Because the binding properties of 3- and 4-chloroaniline in soils and sediments are similar to 3,4-DCA, accumulation of these metabolites in sediments is expected as well.

3.1.2.9 3- and 4-Monochloroaniline

As demonstrated in a laboratory experiment (see Section 3.1.1.2), 3- and 4-monochloroaniline are formed under anaerobic conditions in sediments. The dechlorination started after 20 days of incubation in unacclimated sediments. Approximately 90% of the 3,4-DCA was dechlorinated within the next 40 days. After 60 days 44% of the 3,4-DCA was present as 3-chloroaniline and 33% as 4-chloroaniline (Struijs and Rogers, 1989). Photolysis of 3,4-DCA is of minor importance for the formation of monochloroanilines: 3-chloroaniline was formed as transformation product with a yield of only 2-5% (Miller, 1980; Miille and Crosby, 1983).

As the monochloroanilines are subsequent products from 3,4-DCA, these compounds have to be considered in the environmental risk assessment. Detailed descriptions of both monochloroanilines are given in BUA (1991 and 1995). Here only the most important properties are mentioned:

- Biodegradation tests of 3-chloroaniline showed that the substance is not readily biodegradable in water. The mineralisation was below 10% (Bayer AG, 1979-1984).
- Primary biodegradation was detected in tests with water from a pond (Oconee-river) with a half-life of 4.8 months (at bacteria concentration of 10^8/l). As metabolite, 4-chlorobrenzkatechine was identified (Paris and Wolfe, 1987).
- The aerobic degradation (mineralisation) of radiolabelled 3-chloroaniline in soil was investigated in a laboratory experiment with 8 different soil types over 16 weeks with a concentration of 1 mg/kg ww. Between 8.9 to 22.7% of the 3-chloroaniline was mineralised (Fuchsbichler et al., 1978a).
- Results of degradation tests with 3-chloroaniline under anaerobic conditions in sediment (Struijs and Rogers, 1989) and soil (Pettigrew et al., 1985) indicate that there is neither mineralisation nor primary biodegradation.

- Results of biodegradation tests with 4-chloroaniline indicate that the substance is inherently biodegradable with a high fraction of adsorption (e.g. 52% in 3 hours) included in the elimination process (BUA, 1995).
- The aerobic degradation of 4-chloroaniline in soil was investigated with two different soil types over 16 weeks at a concentration of 1 mg/kg ww. The results show mineralisation of 8.4 - 9%. A prolongation of the test over 24 weeks resulted in a mineralisation of 14.4% (Fuchsbichler, 1977). In other tests with 4 different soil types a mineralisation rate of 12.3-17.2% was obtained (Süß et al., 1978).
- Under anaerobic conditions, neither mineralisation (Bollag and Russel, 1976, Shelton and Tiedje, 1981) nor primary degradation was detected after 16 weeks (Struijs and Rogers, 1989).

These investigations indicate that the biodegradation of 3- and 4-monochloroaniline is as poor as of 3,4-DCA. Covalent bounds with the organic matter in soils and sediments were formed, analogously to 3,4-DCA. Therefore, a secondary environmental exposure of these substances has to be expected. Both monochloroanilines were detected in the environment. As these substances are used in much lower quantities than 3,4-DCA, the environmental occurrence of the monochloroanilines may be origin from the DCA-derivatives (BUA 1991, 1995).

3.1.3 Atmosphere

Because no significant releases of 3,4-DCA into the atmosphere during production and processing are expected, a risk assessment for this compartment is not necessary.

3.1.4 Terrestrial compartment

No direct releases into soil were identified except of small amounts of 3,4-DCA contained as impurity in plant protection agents which are its subsequent products. However, additional 3,4-DCA is originated from this agents by microbial degradation.

3.1.4.1 Releases from the use of plant protection agents

Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea)

During aerobic biodegradation of diuron in water and sediment, 3,4-DCA is formed as a metabolite (Attaway et al., 1982; cited in Section 3.1.2.4). Generally, it can be assumed that the biodegradation pathway under aerobical conditions in soil is the same. As the degradation rate in soil, a DT_{50} depending on soil type and humidity of 90-180 days is cited (The Pesticide Manual, 1994).

There are two investigations available which examined the biodegradation of diuron and the formation of 3,4-DCA in soil:

In the study of Elder (1978), two silty clay loam soils were fortified with [Phenyl-(U)-14C] diuron equivalent to an application rate of approximately 0.75 kg a.i./ha. The study was conducted under aerobic conditions. The degradation half-life of diuron was 4.6 to 5.6 months. As a nonpolar metabolite, monomethyldiuron was detected after extraction with benzene-acetone-methanol-water. This extraction method is not applicable for 3,4-DCA as the

substance can only partially be extracted (40-50%) from fortified soil samples. With acid hydrolysis, some 3,4-DCA was released, but could not be quantified. The author proposes the degradation pathway investigated by Attaway: diuron was demethylated twice, and subsequently cleaved to 3,4-DCA which probably bound to soil organic matter and was therefore not detectable in soil extracts.

In a study on metabolism of ^{14}C-diuron in soil under aerobic conditions, a half-life for diuron of 372 days was calculated. Thin-layer chromatography of soil extracts showed that N-(3,4-dichlorophenyl)-N'-methylurea (22%) and N-3,4-dichlorophenylurea (< 1%) were formed. Thus, the authors assume the same metabolisation pathway as proposed by Attaway. 3,4-DCA extractable with organic solvents could not be found. However, the authors assume that 3,4-DCA is an intermediate with a short half-life (Hawkins et al., 1990).

The cited investigations confirm that in soils the aerobic degradation pathway of diuron proposed by Attaway occurs. According to the TGD, a period of 10 years subsequent application of diuron has to be considered. After this period, we expect that diuron is completely metabolised with 3,4-DCA as the product.

In Section 3.1.1.3, the formation of covalent bounds between 3,4-DCA and humic substances was elaborated. When 3,4-DCA is formed from diuron, it will react rapidly with the organic matter, therefore the "free" 3,4-DCA only occurs as an intermediate. Its humic acid complexes are relatively stable against further degradation, thus the complexes will accumulate in agricultural soils.

Linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea)

Under aerobic conditions, 3,4-DCA is also a degradation product of the urea herbicide linuron. The degradation pathway is the same as for diuron.

Hassink (1996) investigated leaching properties and distribution of ^{14}C-labelled linuron according to BBA part IV 4-3 over a period of 4 years. Linuron was applicated to soil columns. The main objective of this study was to test the leaching of linuron from soil, so the main work focused on analysis of the water having passed through the soil column. 64-68% of applied radioactivity was determined in soil. For extraction of metabolites in a first step acetone was used. Metabolites could not be detected this way. The adsorption of 3,4-DCA to soil is well known, as it is known that acetone is not an appropriate solvent to desorb it. The soil was extracted a second time using NaOH. 37-38% of radioactivity were extracted by this way. It was concluded that this part had been bound to humin and fulvo acids. The non-extractable residues of 23-25% were bound to the humin fraction.

Dulka (1980) studied degradation of ^{14}C-labelled linuron in soil. Again the solvents used for extraction were not suitable for extracting 3,4-DCA. Consequently no 3,4-DCA was detected. Detected metabolites were 3-(3,4-dichlorophenyl)-1-methoxy-urea, 3-(3,4-dichlorophenyl)-1-methyl-urea, 1-(3,4-dichlorophenyl)-urea as well as some polar materials (not further specified). Because of unsuitable solvent systems no direct prove of the formation of 3,4-DCA was possible. In spite of this the formation of 3,4-DCA can be concluded by the fact that the same metabolites known from the degradation pathway of diuron were detected for linuron, too. Thus 3,4-DCA will be formed by aerobic degradation of linuron, too. A study on degradation of linuron in a water/sediment-system (see Section 3.1.1.2) supports this conclusion.

The test results cited above can be summarised as follows: under aerobic conditions, 3,4-DCA is formed from diuron and linuron by the pathway proposed by Attaway (subsequent dealkylation

and further hydrolysis of the urea). The released 3,4-DCA reacts quickly with the organic fraction of soils and sediments.

The analytical detection of the bound substance (respectively its metabolites) is only possible if the sample is extracted with alkali and not with organic solvents.

Propanil (N-(3,4-dichlorophenyl)-propanamide)

3,4-DCA is also a metabolite of the herbicide propanil. Formation occurs by hydrolysis, by microorganisms in soil and in rice field water, and by enzymes in plants (Pothuluri et al., 1991). After the application of 6.8 kg propanil/ha (which is an excessive application, the authors state an amount of 3.4 kg/ha to be normal), 3,4-DCA was measured in the top soil layer after the field was flooded. Concentrations of 29 mg/kg after 3 days, 8 mg/kg after 7 and 6 mg/kg after 14 days were detected, after this times only traces of propanil were present (Deuel et al., 1977).

3.1.4.2 Monitoring

In soil samples from field plots, which had been treated for 12 consecutive years with 2.24 and 4.48 kg diuron/ha as well as samples treated with a single application of 2.24 kg linuron/ha, no 3,4-DCA was measured with a detection limit of 0.1 mg/kg soil one year, in the case of linuron 2 months after the last application. The incubation of 500 mg/kg of diuron and linuron (which would be an unrealistic high application) yielded in approximately 1 mg 3,4-DCA/kg or less after 31 days (Belasco and Pease, 1969). In orchard soil treated for 7 years with 4.5 kg diuron/ha, a 3,4-DCA concentration of 21 µg/kg was detected 5 months after the last application (Khan et al., 1976).

For the interpretation of the two investigations it has to be considered that the soil samples were treated with organic solvents which can only extract “free” 3,4-DCA. The results show that at most 3,4-DCA occurs in traces. This confirms that 3,4-DCA forms complexes with humic substances as described in Section 3.1.1.3. These complexes can only be detected when the samples are hydrolysed under drastically alkaline conditions, as it was done in the following investigation:

In a field soil treated with 1.76 kg diuron/ha over the past 10 consecutive years, the 3,4-DCA residue was measured 99 days after the last application. The soil sample was extracted with acetone, when diuron and its non-bound metabolites were removed. The remaining diuron content was estimated to 0.005 mg/kg (Using the model below, an initial concentration of 0.59 mg/kg is estimated, both values would correspond to a degradation half-life of 14 days. In the degradation tests cited above, half-lives between 3 and 12 months were determined. The cause of the significant shorter half-life in the monitoring study is not known, a possibility could be an adapted biodegradation during 10 years of application). The residue of the acetone extraction was further treated with alkali. Using the Bleidner distillation technique (which releases the hydrolyzable bound fraction, that is about one half of the total bound 3,4-DCA), a concentration of 1 mg DCA/kg was measured. Considering the non-hydrolyzable fraction, the authors estimated a total 3,4-DCA concentration of about 2 mg/kg soil (You and Bartha, 1982). This investigation is the most valid from all field measurements, as the best available analysis method was used.

3,4-DCA was measured in a monitoring program in Bavarian agricultural soils. The measurements were performed according to the Specht method, where the samples are hydrolysed. The concentrations (not reported whether related to dry or wet weight) were above

10 µg/kg in 33 out of 352 samples (Lepschy and Müller, 1991). It is unknown how frequently the soils were treated with 3,4-DCA derivatives, also the amount is unknown. By the analytical method, an exact determination of the concentration is not possible as a part of the detected substance may have its origin in the herbicides and the 3,4-DCA-containing metabolites which are released by hydrolysis during the sample preparation. On the other hand, 3,4-DCA covalently bound to humic acids may only partially be detected. The recovery rate is not known.

3.1.4.3 Calculation of $PEClocal_{soil}$

As in the Technical Guidance Documents no suitable exposure model for plant protecting agents is proposed, the sewage sludge model is used. It is assumed, that 4.1 kg diuron/ha, (which is the highest amount applied in agriculture permitted in Germany, BBA 1995) are applied once a year. On a worst case approach, we assume that it is completely metabolised to 3,4-DCA (2.9 kg/ha). As the TGD scenario proposes a period of ten years, and the degradation half-life of diuron is in the order of several months, this assumption is not unrealistic. Furthermore, it is assumed that 3,4-DCA is distributed in a 20 cm soil layer, completely bound onto organic matter and the complexes are degraded with a half-life of 1,000 days.

The initial concentration of 3,4-DCA (after single application of diuron) is

$$C_{soil\ 1} = \frac{APPL_{DCA}}{DEPTH_{soil} \cdot RHO_{soil}} = 0.97 \text{ mg/kg dw}$$

with

$APPL_{DCA}$	=	application rate (290 $mg \cdot m^{-2}$)
$DEPTH_{soil}$	=	mixing depth in soil (0.2 m)
RHO_{soil}	=	density of dry soil (1,500 $kg \cdot m^{-3}$)
$C_{soil\ 1}$	=	initial concentration in soil in first year

Furthermore, the accumulation during a 10 years period has to be considered. The calculation is presented in Appendix B. The 3,4-DCA concentrations due to diuron application are :

Table 3.7 3,4-DCA concentration due to diuron application

	Soil [mg/kg dw]	Porewater [µg/l]	Endpoint
PEClocal_soil	3.9	22	For terrestrial ecosystem
PECagri_ind	3.7	21	In agric. soil for indirect exposure
PECgrass_ind	0	0	In grassland for indirect exposure

It has to be noted that the amounts and concentrations are calculated as 3,4-DCA equivalents, while in soils the substance always occurs as reaction product with humic substances.

There is a good agreement between the calculated PEC and the reported concentration of 2 mg/kg (based on measurements, with a application of 1.76 kg diuron/ha; You and Bartha, 1982). With an application of 1.76 kg diuron/ha and the above model, a concentration of 1.7 mg/kg dw is calculated.

When diuron is used as a total herbicide in non-agricultural areas, the application amounts are up 5.6 kg diuron/ha, and with this figure a PEClocal_soil of 5.3 mg/kg dw is calculated.

Presently, for linuron application rates of maximum 2.9 kg/ha (corresponding to 1.9 kg DCA/ha) are permitted. With the same model calculated above for diuron, a PEClocal_soil of 2.6 mg/kg dw is calculated. According to the proposal of the EU review programme in the frame of Councel Directive 91/414/EEC, the maximum proposed application rate is 0.95 kg/ha, which leads to a PEClocal_soil of 0.85 mg/kg.

For propanil, application rates of 2.5 to 5 kg/ha are reported (see Section 3.1.1.1). In a study, 6 mg 3,4-DCA/kg soil were measured 14 days after the application of 6.8 kg propanil/ha (Pothuluri et al., 1991). Converted to an application of 5 kg/ha, a PEClocal_soil of 4.4 mg/kg dw is calculated.

3.1.4.4 3,3‘,4,4‘-tetrachloroazobenzene (TCAB), and 3,3‘,4,4‘-tetrachloroazoxybenzene (TCAOB)

Due to transformation of propanil and other plant protection agents, 3,4-DCA is released to soil, where conversion to 3,3’,4,4’-tetrachloroazobenzene (TCAB), 3,3’,4,4’-tetrachloroazoxy-benzene (TCAOB) and other azobenzenes formed by microbial peroxidases occur. These compounds are approximately isosteric to 2,3,7,8-tetrachlorodibenzodioxine (TCDD) and toxic by mechanisms similar to those of TCDD (Pothuluri, 1991).

Both TCAB and TCAOB are impurities in propanil (no concentrations reported; Pothuluri, 1991). These impurities origin from incomplete hydrogenation of 3,4-dichloronitrobenzene during the synthesis of 3,4-DCA. In the IUCLID, TCAB contents of ≤ 0.1% and < 0.01% respectively in 3,4-DCA are stated by two producers. Assuming that these TCAB concentrations are unchanged during the subsequent processing to the plant protecting agents and their application, a TCAB concentration of 1 µg/kg soil (with a Csoil1 of 0.97 mg/kg for 3,4-DCA) after a single application is expected.

TCAB and TCAOB can be formed by biodegradation of 3,4-DCA. The reaction was examined in laboratory tests with NO_3^- reducing bacteria and fungi cultures in the presence of peroxidases (a review is presented in BUA, 1994). Not all studies are cited here, as some of them used reaction conditions which are not relevant for the environment. The most important studies examined the formation in soils in concentrations occuring during regular application of the plant protecting agents:

In a silty clay loam soil spiked with ^{14}C-radiolabelled 3,4-DCA (1-1,000 mg/kg), TCAB was detected with conversion rates in the range of 0.2% to 13.9%. TCAB formation strongly increases with increasing DCA amounts applied. With the lowest 3,4-DCA concentrations of 1 and 10 mg/kg, TCAB yields of 0.2% and 1% (i.e. 2 and 100 µg/kg, respectively) related to the applied radioactivity were found (Kearny and Plimmer, 1972).

In studies on the extent to which TCAB formation from 3,4-DCA is depending on temperature, DCA concentration and incubation time, it was found that the percentage of transformation reached the following maxima: temperature 25°C, 3,4-DCA concentration 500 mg/kg, incubation time 7 and 21 days. At the lowest test concentration of 30 mg 3,4-DCA/kg, a conversion of 0.03% (i.e. 8 µg/kg) on day 7 was found, while the substance disappeared after 21 days. The authors assume that TCAB is degraded abiotically (Sprott and Corke, 1971).

On the other hand, in some studies neither TCAB nor TCAOB were detected: In Keyport silt loam soil treated with 19.2 mg diuron/kg soil, both compounds could not be found with a detection limit of 5 µg/kg one year after diuron application (Hawkins et al., 1990; see Section 3.1.4.1). In a diuron degradation test in a water/sediment system, TCAB and TCAOB were not found with a detection limit of 10 µg/kg (Hausmann, 1992; see Section 3.1.2.4). Also during diuron photolysis, both compounds were not found with a detection limit of 2 µg/l (Hawkins et al., 1988; see Section 3.1.2.4).

Analysis of a Keyport-type field soil which had been treated with up to 4.48 kg diuron/ha for 12 consecutive years as well as with 2.24 kg linuron/ha, TCAB could not be detected with a detection limit of 100 µg/kg. TCAB was measured in a laboratory experiment (105 mg/kg after 14 days and 143 mg/kg after 31 days) when the soil was treated with an unrealistic high propanil application of 500 mg/kg (Belasco and Pease, 1969; see Section 3.1.4.2).

TCAB was found in beans (*Phaseolus vulgaris L.*) and carrots (*Dacus carota*) grown in soil under greenhouse/laboratory conditions (Worobey, 1984, 1988), and in barley plants (*Hordeum vulgare L.*) grown outdoor on 3,4-DCA treated soil (Viswanathan et al., 1978). The residues of azobenzene in barley grains were well below 5 µg/kg, while for the other plants the concentrations are not reported. TCAB found in rice grain was expected to origin from soil-bound 3,4-DCA (Still et al., 1980).

Different experimental results are reported on the formation of 3,3',4,4'-tetrachloroazobenzene (TCAB) from photolysis of 3,4-DCA. An overview is given in the table:

Table 3.8 Formation of TCAB

[3,4-DCA]	Medium	TCAB formation	Reference
1 and 10 mg/l	Distilled water	< 0.3 µg/l	Miller et al., 1980
1 mg/l	Rice paddy water	1.6 - 2.6 µg/l	Miller et al., 1980
10 mg/l	Rice paddy water	23 - 74 µg/l	Miller et al., 1980
20 and 50 mg/l	Dist. and sea water	traces	Miille and Crosby, 1983
100 mg/l	Distilled water	detected (concentr. not reported)	Moilanen and Crosby, 1972
200 mg/l - 1 g/l	Distilled water	yield 7%	Mansour et al., 1975

In a monitoring study in soils of five rice growing states in the USA, TCAB (0.01-0.05 mg/kg) was detected in 6% of all samples analysed. Whereas, TCAB was detected in 12.5% of the Arkansas soil samples alone. For the last 20 years, on the average, between 3.36 and 6.72 kg/ha of propanil was annually applied (EPA, 1972). Further studies performed between 1966 and 1968 showed detectable levels of TCAB (0.01 - 0.06 mg/kg) in 77% of all soil samples analysed after three consecutive years of propanil application (3.4 and 5.6 kg/ha) (Smith, 1974). TCAOB was not measured. In these publications there is no information whether the detected TCAB origins from propanil impurities or is biologically formed in soil.

The available studies reveal that the formation of TCAB and TCAOB is more related to the application of propanil, rather than to diuron and linuron. For an initial approach, for TCAB a PEC of 60 µg/kg can be determined from the monitoring study.

3.1.5 Secondary poisoning

A biomagnification via food chain may not occur via the route water-fish.

Due to the high bioaccumulation and bioconcentration factors for sediment dwelling organisms biomagnification may occur for the route sediment - sediment dwelling organisms - worm-eating fish or bird.

For an exposure scenario for the sediment food chain (sediment - worm - bird or mammal) it is assumed that 50% of the diet comes from the surrounding of a point source, thus the mean value between PEClocal and PECregional (0.0042 µg/l) is used. For the different scenarios, the mean sediment concentrations - shown in **t**able 3.9 - are calculated, which are considered for exposure via the food chain. The $PECs_{oral,\ worm}$ are calculated from the mean aquatic concentration and a BAF for *Lumbriculus variegatus* of 570 l/kg obtained by Nagel, 1997 (see Section 3.1.1.4).

Table 3.9 Mean sediment concentration

Site	$PEClocal_{aqua}$ [µg/l]	Mean PECaqua [µg/l]	$PEC_{oral,\ worm}$ [mg/kg]
DCA production and processing, generic	60	30	17
DCA producer A	0.07	0.037	0.02
DCA producer D	22	11	6.3
Production of Linuron	0.0049	0.0046	0.0026
Production of Propanil	0.12	0.062	0.035
Use of trichlorocarbanilide	0.054	0.029	0.016

3.1.6 Regional concentrations

For the estimation of the total European releases, emissions from the following life-cycle steps are considered:

Table 3.10 Emission from life-cycle steps

Life-cycle step	Water	Air	Soil
Production of 3,4-DCA	1,700 kg/annum	37 kg/annum	0
Processing of DCA to Propanil	4.9 kg/annum	0	0
Use of TCC	1,840 kg/annum	0	0
Production of Linuron	1.2 kg/annum	0	0
Total	3,546 kg/annum	37 kg/annum	0

3,4-DCA formed in soils from the agricultural use of plant protection agents is chemically bound to the organic matter, this reaction product is not considered here. As well, the DCA releases from the uses of diuron as total herbicide on sealed areas and as antifouling agent on ships is not considered, because of missing data.

In Section 3.1.2.7 it is elaborated that the source of the pollution in most rivers is caused by the non-agricultural use of diuron as total herbicide. In the calculation, these releases could not be considered, as there is no information about several parameters like runoff rate, total residence time of diuron in soil before runoff, in water and sediments etc.

Table 3.11 Input for the EUSES model

	Water	Air	Soil
Continental	3,191 kg/annum	33.3 kg/annum	0
Regional	355 kg/annum	3.7 kg/annum	0

The results of the EUSES calculation (see Appendix C) are:

Table 3.12 Rebional and continental PECs

Compartment	Continental concentration	Regional concentration
Hydrosphere	$5.7 \cdot 10^{-4}$ µg/l	0.0042 µg/l
Sediment	$2.1 \cdot 10^{-4}$ mg/kg ww	$1.5 \cdot 10^{-3}$ mg/kg ww
Atmosphere	$3.5 \cdot 10^{-8}$ µg/m^3	$1.9 \cdot 10^{-7}$ µg/m^3
Agric. soil	$1.3 \cdot 10^{-8}$ mg/kg ww	$7 \cdot 10^{-8}$ mg/kg ww
Agr. soil, porewater	$7.5 \cdot 10^{-8}$ µg/l	$4 \cdot 10^{-7}$ µg/l
Industr. soil	$4.9 \cdot 10^{-8}$ mg/kg ww	$2.6 \cdot 10^{-7}$ mg/kg ww
Nat. Soil	$4.9 \cdot 10^{-8}$ mg/kg ww	$2.6 \cdot 10^{-7}$ mg/kg ww

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

3.2.1 Aquatic compartment

For 3,4-DCA further results from acute and long-term tests are available, but only those valid tests are listed below which are relevant for the risk assessment.

3.2.1.1.1 Vertebrates

Oncorhynchus mykiss (Hodson, 1985)
96-hour LC_{50} = 1.94 mg/l

Pimephales promelas
96-hour LC_{50} = 6.99 mg/l
28-day NOEC = 0.0051 mg/l

(effect: growth and weight - egg-fry, ELS, flow-through, lake water, effective concentration) (Call et al., 1987)

Brachydanio rerio (zebrafish) (Becker et al., 1990)
96-hour LC_{50} = 8.5 mg/l
42-day NOEC = 0.02 mg/l[a,b)]
42-day LOEC = 0.2 mg/l[a,b)]

(Effect: survival in F I generation, ELS, flow through, effective concentration) (a) Nagel 1988, b) Schäfers and Nagel, 1991)

42-day NOEC	=	0.002	mg/l[b)]
42-day LOEC	=	0.02	mg/l[a)]

(Effect: survival in F II generation, ELS, flow through, effective concentration) (a) Nagel 1988, b) Schäfers and Nagel, 1991)

42-day NOEC	=	0.02	mg/l

(Effect: reproduction and growth in F I generation, flow through, effective concentration) (Schäfers and Nagel, 1991)

In a complete life cycle tests with zebrafish it was demonstrated that the reproduction of adult fish was not influenced by an exposure up to 200 µg/l (F_0). Though growth was affected, a NOEC of 60 µg/l could be obtained for growth of the adults. For the F_I-generation a NOEC of 20µg/l was found for both reproduction and growth (Nagel, 1988; Schäfers and Nagel, 1991).

The toxicity of 3,4-DCA was further investigated in early-life stage tests (ELS) with zebrafish over 48 days in eight laboratories (Nagel et al., 1991). A LOEC of 200 µg/l and a NOEC of 20 µg/l could be obtained in the F_I-generation. Significant effects were reduction of survival rate and malformation. In zebrafish larvae of the F_{II}-generation a LOEC of 20 µg/l and a NOEC of 2 µg/l was found.

The ELS study was conducted with two parallel groups at each concentration (control, 2, 20 and 200 µg/l DCA) using the flow-through method. The spawn of unexposed parent fish were collected in glass dishes with stainless steel wire mesh covers. Green glass trees served as sprawning substrate. About 1 hour after the light had been switched on, the sprawn was removed and counted, and groups of about 150-250 ova were exposed in glass cylinders. The fertilisation rate was determined after 24 hours, the unfertilised ova removed, and the continued with groups of 100 ova. The larvae were transferred into glass aquaria after 15 days (Nagel et al., 1991).

The studies with zebrafish shown, that survival of the larvae (F_{II}-generation) with a NOEC of 2µg/l is the most sensitive parameter for r-strategists like zebrafish (Schäfers and Nagel, 1991).

Poecilia reticulata (guppy)

96-hour LC_{50}	=	8.7	mg/l
28-day LC_{50}	=	5.5	mg/l

(Static, Adema and Vink, 1981)

42-day NOEC	=	0.002	mg/l
42-dayLOEC	=	0.02	mg/l

(Effect:growth in F I generation)

42-day LOEC	=	0.002	mg/l

(Effect: reproduction in F I, flow through, effective concentration) (Schäfers and Nagel, 1991)

A complete life cycle test was also performed with the guppy. It was demonstrated that in contrast to survival, reproduction (as mean cumulative sum of surviving offspring) in the F_I-generation is influenced more severely than guppies exposed as adults (F_0). While in F_0 significant effects was only found at 200 µg/l (LOEC) since the second litter is within the

exposure period, in F_I it is evident even at 2 µg/l (LOEC). A NOEC for reproduction in F_0 of 20 µg/l could be obtained (Schäfer and Nagel, 1991).

Corresponding results to reproduction were shown by the female body length and weights. The body weights of female after the last litter within the test period were lowered significantly at 200 µg/l in F_0 (NOEC = 20 µg/l) and at 20 µg/l (NOEC = 2 µg/l) in F_I, respectively. No effect was observed on body weights of the males (Schäfer and Nagel, 1991). Additionally the authors could find significant effects at 2 µg/l body weight of juveniles in F_{II} for guppy. The survival of newborn guppies was not affected at 200 µg/l in F_I and F_{II} (NOEC = 200µg/l).

The life cycle test with guppy was initiated with a pre-exposure phase to gain information about the reproduction of each pair. One non-reproductive pair was replaced. After each female had one litter, high and low reproducing pairs were apportioned equally to each concentration and the exposure was started. Offspring were removed daily. Eggs, stillborn larvae and surviving newborn larvae were differentiated. The length of newborn larvae was measured for one (F_0) or two (F_I) litters. Length and body weight of the adults were measured at the end of the test. For each concentration, 30 newborn larvae of the third litters of the F_0-(one start) and F_I-generation (two parallels) were randomly chosen and reared in the test vessel until the age of 42 days was reached. Survival rate and body weight were determined after 16 and 42 days in order to compare these endpoints with early life stage (ELS) test data. After the end of the "early life stage" period (42 days) for the F_I-guppies, the pairs of the F_0-generation were replaced by juveniles of the F_I-generation. These pairs were exposed for the time required to produce five F_{II}-generation litters. The test was performed under flow-through conditions. The 3,4-DCA concentration was determined weekly (Schäfers and Nagel, 1991).

In comparison with zebrafish, the guppy is more sensitive to 3,4-DCA, possible because of the higher, and thus more affectable growth rate of adult females due to its reproductive strategy (k-strategist). At a concentration which didn't influence the zebrafish life cycle (2 µg/l), reproduction of the guppy is reduced by 35%. Zebrafish react more drastically, at 200 µg/l zebrafish populations will be eliminated, while in guppies there is only a reduction of offspring of 40% (Schäfers and Nagel, 1991).

It could be shown by Allner (1997) that 3,4-DCA is rapidly taken up by fish and metabolised into 3,4-dichloroacetanilide. Back-metabolisation to 3,4-DCA was observed.

3.2.1.1.2 Invertebrates

Daphnia

Results from short-term tests

Daphnia magna	48-hour LC_{50}	=	0.23	mg/l
	96-hour LC_{50}	=	0.16	mg/l

(Effect: mortality, 1 mm larvae, effective concentration, (Adema and Vink, 1981)

Daphnia longispina	48-hour EC_{50}	=	0.44	mg/l

(Effect: immobilisation, effective concentration, (Crossland and Hillaby, 1985)

Artemia salina	48-hour LC_{50}	=	9.0	mg/l
	96-hour LC_{50}	=	5.5	mg/l

(effect: mortality, larvae: 3 days old, 1 mm, enriched seawater, effective concentration) (Adema and Vink, 1981)

Results from long-term toxicity tests

Daphnia magna	21-day NOEC	=	0.005	mg/l
	21-day LOEC	=	0.01	mg/l

(Effect: reproduction, semi-static (renewing once a week), effective concentration (UBA, 1994)

	21-day NOEC	=	0.01	mg/l
	21-day LOEC	=	0.02	mg/l

(Effect: reproduction, semi-static (renewing 3 times per week), effective concentration) (WaBoLu, 1994)

	21-day NOEC	=	0.0065	mg/l

(Effect: mortality and reproduction, effective concentration) (Adema and Vink, 1981)

	14-day NOEC	=	0.025	mg/l[c)]
	14-day NOEC	=	0.005	mg/l[d)]
	14-day NOEC	=	0.0025	mg/l[e)]

(Effect: c) first batch of eggs in the brood pouch, d) aborted eggs, e) offspring; semistatic: renewal after 48 h; nominal concentration; Diamantino et al., 1997)

	14-day NOEC	=	> 0.050	mg/l[c)]
	14-day NOEC	=	0.005	mg/l[d)]
	14-day NOEC	=	0.005	mg/l[e)]

(Effect: c) first batch of eggs in the brood pouch, d) aborted eggs, e) offspring; flow-though; nominal concentration; closed system; Diamantino et al., 1997)

The slightly lower sensitivity within the flow-through than under semistatic conditions is in the opinion of the authors probably a result of the higher food concentration available within the flow-through test design. The concentration of the stock solution was analysed at days 9 and 14 and it was not significantly different from the initial concentration. In experimental conditions similar to those of the present study, no significant loss of concentration could be detected (Diamantino et al., 1997).

It could be shown in different investigations that 3,4-DCA influences the reproduction of daphnids, especially on embryogenese proceeding in the brood chamber. The inhibition of reproduction can be manifested in an extended juvenile development, in reduced daphnids survival and reduced numbers of live young produced, in reduced total egg production and in a disturbed embryonal development.

Artemia salina	28-day NOEC	=	0.032	mg/l)

(Effect: reproduction and mortality, larva: 3d, 1 mm, enriched seawater, effective concentration) (Adema and Vink, 1981)

Gastropoda (Molluscs):

Lymnaea stagnalis	16-day NOEC	=	0.13	mg/l

(Effect: mophology and hatching of eggs, effective concentration) (Adema and Vink, 1981)

Plants

Phaeodactylum tricornutum	72-hour EC_{50}	=	1.1	mg/l

(Effect: growth inhibition, effective concentration (analysed at the end of test, > 10%) (Kusk and Nyholm, 1992)

	96-hour EC_{50}	=	0.45	mg/l

(Effect: growth inhibition, enriched seawater) (Adema and Vink, 1981)

Scenedesmus pannonicus	96-hour EC_{50}	=	4.8	mg/l
	96-hour NOEC	=	1.0	mg/l

(Effect: growth inhibition) (Adema et al., 1982)

Multispecies test

In a research project aiming at the development of laboratory multispecies toxicity tests reflecting the predator/prey (Hydra/Daphnia) interaction and species competition within the same trophic level (Gammarus/Asellus) a subchronic impact of 3,4-DCA could be determined only at concentration levels above (mostly > 100 µg/l) the NOEC‘s resulting from the earlier presented tests with long-term exposure of single species. In addition, part of the effect values obtained were not dose-related and in certain aspects (as for the impact of development over time difficult to interprete) (EU, 1995).

In outdoor experiments within the stream and pool communities no population and ecosystem level responses could be detected at 3,4-DCA treatment levels of up to 0.0075 mg/l (streams) and 0.0017 mg/l (pools). At 0.037 mg/l the abundance of a few invertebrate taxa was reduced.

In summary, the results obtained in these outdoor studies and in supplementary experiments conducted with the selected members of the mentioned communities confirmed the high long-term sensitivity of early life stages in fish and that of certain invertebrates (especially crustacea, were examplified by low effects values regarding behavioural parameters and reproduction (EU, 1995).

3.2.1.1.3 Endocrine effects

3,4-DCA is known to have endocrine effects on fish. It could be shown (Allner, 1997) that 3,4-DCA causes lowered androgen synthesis at tested concentrations of 200 and 400 µg/l in breeding male Sticklebacks (*Gasterosteus aculeatus*). The changes in androgen metabolism are accompanied by changes in the secondary sex characters at concentration of 100, 200 und 400 µg/l. The splendid colour typical of breeding males become regressive and courtship behavior occurs no longer.

In an investigation of mechanism for Lydig cell tumorigenesis by linuron in rats it was found (Cook et al, 1993) that linuron and four of its metabolites, among them 3,4-DCA, compete *in*

vitro for binding to the androgen receptor from ventral prostate cytosol of rats. The IC_{50} value for displacement of [^{3}H]testosterone from the androgen receptor for 3,4-DCA and for Linuron are 110,000 nM and 64,000 nM. As positive control DHT (Dihydrotestosterone) with a IC_{50} of 1.4 nM was used.

These data, in connection with the observation that linuron decreases accessory sex organ weights, suggest that linuron is an androgen receptor antagonist. This conclusion is supported by hormonal changes, specifically the increased serum LH (luteinising hormone) levels. The author's conclusion is, that all data support the hypothesis linuron produces Leydig cell tumors via an antiandrogenic mechanism where sustained hypersecretion of LH appears to be responsible for the development of Leydig cell hyperplasia and adenomas.

For 3,4-DCA only the *in vitro* binding studies to the androgen receptor are available.

Determination of $PNEC_{aqua}$

In comparison to other chemicals, the effect data of 3,4-DCA show a large acute to chronic ratio (ACR). This is observed for daphnids, polychets and fish. The most sensitive organism from standard tests is *Daphnia magna* (14-day NOEC = 2.5 µg/l). Non-standard tests which examined more sensitive life stages are revealed: *Ophryotrocha diadema* (38-day NOEC = 3.2 µg/l, see sediment), the fish-species *Brachydanio rerio* (42-day NOEC = 2 µg/l, survival in FII generation) and *Poecilia reticula* (42-day NOEC = 2 µg/l, growth in F1 generation). As there are significant effects at 2 µg/l on body weights of juveniles in FII and on reproduction in FI, an ACR for long-term effects in guppy of > 4,000 is obtained, corresponding to a value of 1,200 for the fathead minnow. The available experimental results reveal that 3,4-DCA is highly toxic to reproduction.

For the calculation of the PNEC the lowest NOEC of 2 µg/l obtained from tests with two different fish species is used. This value is supported by the other low aquatic effect concentrations mentioned above.

According to the EU *Technical Guidance Document*, the assessment factor is set at 10 for the aquatic compartment, as data from long-term tests on 3 trophic levels are available. The PNEC is calculated as follows:

$$PNEC_{aqua} = 2\ \mu g/l / 10 = 0.2\ \mu g/l$$

3.2.1.1.4 Microorganisms:

Photobacterium phosphoreum 30-min EC_{50} = 0.65 mg/l

(Effect: inhibition, static, nominal concentration) (Ribo and Kaiser, 1984)

Pseudomonas putida 18-hour TGK = 19.0 mg/l

(Effect: celldensity, static, nominal concentration) (Janicke and Hilge, 1980)

16-18-hour TGK = 23 mg/l

(Effect: growth inhibition, nominal concentration) (Bayer AG, 1979)

Activated sludge	3-hour EC_{50}	=	44	mg/l

(Effect: O_2-consumption) (Hoechst AG, 1990)

Protozoa:

Tetrahymena pyriformis	24-hour IC_{50}	=	9.0	mg/l

(effect: cell density, static) (Yoshioka et al., 1985)

Determination of $PNEC_{WWTP}$

For the determination of $PNEC_{WWTP}$ different tests with microorganisms are available. Although the test with *Photobacterium phoshoreum* (IC_{50} = 0.5 mg/l) cannot be used for the determination of the PNEC according to TGD.

The lowest effect data for organisms important for the function of WWTPs are as follows:

Pseudomonas putida	TGK = NOEC = 19 mg/l, AF = 1 PNEC = 19.0 mg/l
Activated sludge	EC_{50} = 44 mg/l, AF = 100 PNEC = 0.44 mg/l
Tetrahymena pyriformis	EC_{50} = 9 mg/l, AF = 10 PNEC = 0.9 mg/l

For the assessment of WWTPs the PNEC derived from activated sludge is used.

$$PNEC_{WWTP} = 0.44 \text{ mg/l}$$

3.2.1.1.5 Sediment

Oligochaeta (benthic):

Pristina longiseta	96-hour LC_{50}	=	2.5	mg/l

(Static, nominal concentration) (Schmitz and Nagel, 1995)

Tubifex tubifex	24-hour LC_{50}	=	11	mg/l
	48-hour LC_{50}	=	11	mg/l

(Validity could not be checked, without sediment, no more information available, Yoshioka et al., 1986)

Polychaeta:

Ophryotrocha diadema	24-hour LC_{50}	=	25.0	mg/l
(marine)	7-day LC_{50}	=	2.8	mg/l
	38-day NOEC	=	0.0032	mg/l

(Effect: reproduction, enriched seawater without sediment, effective concentration) (Adema and Vink, 1981)

3.2.1.1.6 Results from short-term toxicity tests with sediment:

Chironomiden (epibentic):

Chironomus riparius	10-day LC_{50}	=	> 0.45 < 0.55 g/kg
	10-day LC_{100}	=	0.55 g/kg
	10-day LOEC	=	0.25 g/kg *
	10-day LOEC	=	0.35 g/kg **

(Effect: dry weight* and length**, spiked sediment, analytical measurement of interstitial and overlaying water, Naylor et al. 1997)

The spiking system and test sediment used resulted in approximately 10% of the DCA in all concentrations being detectable in the water phase.

The test has been used as a model system to investigate how test design can influence test outcome. Three sizes of larvae were used (early 1st, late 1st or early 2nd) and both survival and the two sub-lethal endpoints dry weight and length were investigated. Each of the tests contained both a control treatment with acetone and one without (10 replicates of each).

When length was used as an endpoint, starting instar did not effect the test sensitivity as all tests generated a LOEC of 0.35 g/kg. Dry weight measurements generated a LOEC of 0.25 g/kg for early 1st and early 2nd instar and a LOEC of 0.35 g/kg for late 1st instar.

The survival of larvae after 10 days in all 3 treatments was reduced as DCA concentration increased and there was no survival in the top concentration of 0.55 g/kg. The greatest difference between levels of survival in the 3 treatments is found at the 0.45 g/kg concentration, where treatment A (1 larva per test and 10 mg of food) has 80% survival, treatment C (1 larva per vessel and 1 mg of food) has 40% and value for the replicates in B (5 larva per test vessel and 50 mg of food) range between 0 and 100% with a mean of 56.4%. A precise determination of the LC_{50} is not possible. The concentration of 0.45 mg/kg can be considered only as an approximation and is therefore used as LC_{50}.

Results from long-term toxicity tests with sediment:

For the substance 3,4-dichloroaniline long-term 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 LC_{50} of 25.2 mg/l was found, while the 48-hour LC_{50} for *Chironomus riparius* was 9.2 mg/l.

The long-term sediment tests were conducted over a period of 28 days. Artificial sediment with a grain size of 100-2,000 µm was used. As carbon sources 1% pulverised 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 pre-incubated with 3,4-dichloroaniline either for 2 days or for 14 days. It is assumed that after 14 days 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 (nominal concentration) of the sediment tests with both species are summarised in the following table:

Table 3.13 Summary of the results

	48-hour water	28-day sediment bioassay			
Species, Endpoint	**L(E)C_{50} [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		25	5	125	25
Large		25	5	125	25
Small		25[1)]	25	125[1)]	125
Biomass		n.d.	5	n.d.	25
Deformations		1[1)]	1[1)]	5[1)]	5[1)]
C. riparius					
Mortality	9.2	not tested	not tested	not tested	not tested
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 LOEC was found for *Chironomus riparius* for the endpoints rate of emergence (EmT_{50}) as well as for 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% effect) than in the solvent control (Oetken et al., 2000).

In the following the ecological relevance of the endpoint rate of emergence will be discussed. To minimise the risk of emerging at unfavorable environmental conditions (rain, wind, low atmospheric humidity) the *C. riparius* midges emerge over a longer time span. In the above mentioned experiment with 14 days aged sediment, the main emergence period (from 20 up to 80% emerged organisms) in the control ranges from 13.1 to 18.3 days. The main emergence

period lasted 5.2 days and the EmT_{50}-value was 15.7 days. In contrast the main emergence period of the midges in the treatment 0.064 mg/kg dw was between 13.1 and 14.4 days after insertion in the test. The main emergence period lasted 1.3 days only and the EmT_{50}-value was 13.8 days. The emergence of the residual treatments was also temporary abbreviated. The consequence for a *C. riparius* population exposed to 3,4-DCA contaminated sediment are evident. If emergence takes place during an abbreviated period of 1.3 days a whole generation can become distinct by unfavorable environmental conditions. Hence, the population of *C. riparius* will be at risk (Oetken et al., 2000).

Additionally, in the present study a correlation between the number of eggs per clutch and the EmT_{50} was observed. This means the midges emerge earlier and the number of clutch will be smaller. This reduction in the number of eggs per clutch of 3,4-DCA treatments could be a consequence of insufficient food variety (detritus and bacteria) that inserted larvae of the parental-generation have had due to potential bactericidal effects of 3,4-DCA. The decreased quantity of food could also result in an earlier emergence. To extrapolate the results to the field, it is presumed that the disturbance of reproduction strategy as well as a reduction in the number of eggs per clutch will influence the success of survival of a population exposed to 3,4-DCA contaminated sediment (Oetken et al., 2000).

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 will be repeated. The effects observed in both sediment tests are not caused by the concentration of the substance in the water phase, 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 concentration of 3,4-dichloroaniline in the overlying water and the porewater were 0.0433 mg/l and 0.53 mg/l, respectively at day 28, while the 96-hour LC_{50} for *Lumbriculus* from the water only study was 25 mg/l. At the LOEC of 0.064 mg/l dw for *Chironomus* no 3,4-DCA was detectable in both overlaying water and porewater. The results of the tests with *Lumbriculus* and *Chironomus* show that the pre-incubation 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 prolonged sediment study with the midge Chironomus riparius (Oetken et al., 2000) was repeated due to the above mentioned problems. 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 artificial sediment which was prepared 2 days before spiking. It consist of 75% fine quartz sand (84% of the sand had a particle size of 0.06 – 0.2 mm), 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 were chosen as follows: 10, 32, 56, 100, 180, 320 and 1,000 mg/kg dw. The range was selected in order to define the EC_{15}.

For biological evaluation, three replicates per study were prepared for each test concentration. For analytical purposes (radioactice measurement) additional parallel replicates were prepared. The larvae were not fed throughout the test. Gentle aeration was used throughout the test.

Although test vessels were covered with clear plastic plates, some parts of the water evaporated and were compensated by deionised water once per week.

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 the following table:

Table 3.14 Summary of the results

	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 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%.

Determination of $PNEC_{sediment}$

A number of short-term tests with benthic invertebrates are available, but in these tests the organisms were exposed to 3,4-DCA in water and not to contaminated sediment. Only one acute 10 days growth test on *Chironomus riparius* with sediments spiked with 3,4-DCA is available. The test investigated survival and the sub-lethal endpoints length and dry weight. Due to the short time of test duration, the data refer only to a restricted period of larval development, the stage of emergence is not reached and more over no reproduction is investigated. As it is known that 3,4-DCA influences the reproduction of different species and that the acute to chronic ration for 3,4-DCA is very high, the test cannot be used as a long-term test. Additionally two long-term tests with *Chironomus riparius* and one with *Lumbriculus variegatus* with sediments spiked with 3,4-DCA are available. The lowest endpoint available for *Chironomus riparius* with a LOEC of 0.064 mg/kg dw was questioned therefore the test was repeated. From the repeated test an EC_{10} of 104 mg/kg dw for developmental rate (female) was obtained. For *Lumbriculus variegatus* a NOEC of 5 mg/kg dw was found for the total number of worms. This can be seen as the endpoint relevant for the assessment. The assessment factor for two valid long-term tests is set to 50 according TGD and therefore a PNECsediment of 0.04 mg/kg ww (0.1 mg/kg dw) is calculated.

With the equilibrium partitioning method (EPM), a PNECsediment of 0.039 mg/kg ww is calculated from the PNECaqua of 0.2 µg/l.

3.2.2 Atmosphere

Because there are no fumigation tests available, an effects assessment for this compartment can not be performed.

3.2.3 Terrestrial compartment

3.2.3.1.1 Microorganisms

The effect of 3,4-DCA on growth and respiration of different soil organisms was tested. In addition, the influence on mycelium growth on different phytopathogenic fungi was examined. In concentrations of 32 and 49 mg/l 3,4-DCA influenced the growth of microorganisms. For *Bacillus subtilis* a growth inhibition of 80% at 32 mg/l could be found. 40 - 60% growth inhibition could be obtained for *Nocardia sp*., *Pseudomonas fluorescens* (anaerobic conditions) *Rhizopus japonicus* and *Ustilago maydis* at a concentration of 49 mg/l. 3,4-DCA reduced considerably (52%) the rate of anaerobic nitrate-respiration in *Pseudomonas fluorescencs* at a concentration of 49 mg/l (Huber et al., 1980).

A radiorespirometric technique was used to examine the effect of 100 ppm diuron and 3,4-DCA on respiration of fresh sandy loam soil. *P. putida* was inoculated to the sterilised soil following 18 hours incubation at 25ºC. The results show that diuron had no inhibitory effect on P. putida or soil microbial activity. For DCA an inhibition of about 50% could be found. A growth test *in vitro* showed no inhibition by 3,4-DCA. The *in vitro* results are in contrast to the data obtained by radiorespirometry. The authors interpret that this may show an influence of physical property of soil (Rashid and Mayaudon, 1974).

The effects of 3,4-DCA and other mono- and dichloroanilines on nitrification in Guelph loam was investigated by Thompson and Corke (1969). 3,4-DCA was shown to be inhibitory against the autotrophic oxidation of ammonium-nitrogen to nitrite-nitrogen, but not nitric-nitrogen to nitrate-nitrogen. Nitrification was inhibited with a lag phase of 1, 2 and 17 days by the application of 2.5, 5 and 25 mg/kg of soil of 3,4-DCA, but the eventual appearance of nitrate was almost identical with that of the control. It could be shown that two patterns of reestablishment of the nitrification process were evident. At 100 mg/kg the recovery pattern for 3,4-DCA was slightly different to the control soil. It is not possible to derive an IC_{50} from the available data. Dennemann and van Gestel (1990) derived a 10-day NOEC for prolongation of the lag phase of 5 mg/kg soil. The data can be used only for an indication of inhibition of nitrification. The objective of the following experiment was to determine the influence of freshly applied and aged residues of 3,4-DCA on nitrogen mineralisation.The effect of freshly applied and 5 weeks aged residues of 3,4-DCA was investigated in a loamy sand soil with an organic carbon content of 0.7% (Bayer 2000). In the experiment with fresh residues, a loamy 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 NO_3 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 NO_3 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 NO_3 than in the untreated control. The quantities of NO_3 increased as the concentration of 3,4-DCA increased. In soil

treated with 100 mg/kg soil there was 91% less NO_3 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 mineralisation to release NO_3-N.

In the aged-residue test, after 5 weeks incubation, only the soil containing 100 mg/kg dw soil contained 40% less NO_3 than the control. 14 days after addition of the plant meal, 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 ID_{50} could not be calculated. However, the data show that the ID_{50} for 3,4-DCA lies between 32 and 100 mg/kg dw soil (Bayer 2000).

From these studies a 28-day 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-day NOEC of 32 mg/kg and a 28-day 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 mineralisation in soil.

3.2.3.1.2 Invertebrates

Results from short-term tests

Eisenia fetida andrei 24-hour/48-hour LC_{50} = 3.1 µg/cm^2

(filter-paper contact test, nominal concentration) (van Gestel and van Dis, 1988)

7-day/14-day LC_{50} = 240 (>180<320) mg/kg soil dw

(artificial soil, organic matter 7.2%, nominal concentration; van Gestel and van Dis, 1988)

7-day/14-day LC_{50} = 130 (>100 < 180) mg/kg soil dw

(Gilze soil and Gilze soil adjusted to pH 7, organic matter 1.7%, nominal concentration) (van Gestel and van Dis, 1988)

The data by the filter paper contact test do not predict the toxicity of chloroanilines in soil (Payà-Pérez and Mannone, 1997).

Results from long-term tests:

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 hours 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.

3.2.3.1.3 Plants

Results from short-term tests

Lactuca sativa	7-day/14-day EC_{50}	> 10.0 (almost 10)[7] mg/kg soil dw
	16-21-day EC_{50} =	1.7 mg/l nutrient solution

(Effect: growth (fresh weight), organic matter 1.4%, pH 7.5, nominal concentration; Hulzebos et al., 1993)

The test was performed according OECD Guideline 208. Nominal concentrations from 0.32 to 100 mg/kg dw soil were spaced by a factor of 3.2. Duplicate trays were used for the control and at least three test concentrations. Immediately after DCA treatment seeds were put into the soil. After four days, only the first five germinated seedlings were required, and any additional seedlings were discarded in order to exclude the effect of the binding of 3,4-DCA to soil organics. Seeds could possibly have germinated after four days as 3,4-DCA was less available and this effect was outside of the scope of this test. After 7 and 14 days, shoots were harvested by cutting them off at soil level. The fresh weight of each plant was determined immediately after harvesting. Water content and pH of the soil were checked at the start and at the end of the experiment. The concentration of 3,4-DCA was analysed by extraction with toluene, followed by analysis of the extract. The concentration of 3,4-DCA has dropped to a low value ($\leq$ 30%) of the initial concentration. The EC_{50} obtained for 3,4-DCA is slightly above 10 mg/kg soil dw. One concentration higher (32 mg/kg dry soil), seeds did not germinate, and therefore it is set equal to 10.

In laboratory tests, the bioavailability of 3,4-DCA for terrestrial plants was investigated. From a nutrient solution, radiolabelled 3,4-DCA was taken up by the roots. In tomatoes, oat, barley and wheat, 90-95% of the absorbed radioactivity remained in the roots, while in carrots the substance was found to be constantly distributed (Fuchsbichler, 1978b).

In a further test with two different soils, the uptake of covalently bound 3,4-DCA by oats was investigated. Three parallel tests were performed:

1. the test substance was pre-incubated 16 weeks before sowing
2. the test substance was pre-incubated 6 hours before sowing
3. the test substance was applied after sowing

After 3 and 6 weeks, the radioactivity which had been taken up by the plants was measured. While test 2 and 3 revealed similar results, the uptake in test 1 was reduced by a factor of 2 to 4 compared with the others (Fuchsbichler, 1978c).

Results from chronic tests

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 days 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 EC_{50} 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 EC_{50} 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 EC_{50} 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.

Determination of the $PNEC_{soil}$

For 3,4-DCA results from short-term tests with species from 3 trophic levels (plants, earthworms, microorganisms) and long-term/chronic tests from 3 trophic levels (plant, earthworm, microorganisms) are available. The lowest acute toxicity was recorded for the plant *Lactuca sativa* with a 14-day $EC_{50} > 10$ mg/kg soil, the experiment was conducted in fresh treated soil.

At this point it has to be remembered that the 3,4-DCA released from plant protecting agents under environmental conditions always exists in a covalently bound form. It is doubtful whether the toxicity tests on soil organisms in freshly treated soil are appropriate for the environmental

risk assessment. When applied during a test in soil, the 3,4-DCA will be mobile only for a few hours. After one or two days the substance is almost quantitatively bound and the same DCA-humic acid-complexes will be formed as under environmental conditions. The bioavailability of these complexes is reduced by a factor 2 to 4, as shown by the investigation of Fuchsbichler (1978b). Effect tests in which the test substance is pre-incubated several weeks before starting the test would be more appropriate for the risk assessment. Therefore, long-term tests in which the test substance is pre-incubated in soil several weeks before starting the tests were performed.

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 hours 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). From these long-term 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 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 with a storage period of 14 day after application of 3,4-DCA. 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.

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.

The effect of freshly applied and 5 weeks aged residues of 3,4-DCA on nitrification was investigated in a loamy sand soil with an organic carbon content of 0.7% (Bayer 2000). In the experiment with fresh residues, a loamy sand soil was treated with 0, 1, 3.2, 10, 32 and 100 mg/kg dw soil a.i. 3,4-DCA and immediately amended with lucerne-grass-green meal. 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. From these studies a 28-day 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-day NOEC of 32 mg/kg and a 28-day NOEC of 100 mg/kg can be deduced.

The tests show that aged residues of 3,4-DCA in soils, up to 100 mg/kg soil, do not have long-term influence on nitrogen mineralisation in soil.

All available long-term tests with aged residues of 3,4-DCA shows that bioavailability can be reduced by pre-incubation. Therefore, for an appropriate risk assessment the determination of the PNECsoil should be based on the 28-day NOEC of 100 mg/kg soil for inhibition of nitrification obtained with aged residues. The assessment factor is set to 10, because 3 short-term and 3 long-term tests are available.

$$PNECsoil = 100 \text{ mg/kg}/10 = 10 \text{ mg/kg}$$

3.2.4 Secondary poisoning

A biomagnification via food chain is not expected via the route water - fish. Due to the high bioaccumulation and bioconcentration factors for sediment dwelling organisms, biomagnification may occur for the route sediment - sediment dwelling organisms - worm-eating mammal or bird.

On the basis of mammalian toxicity data, 3,4-DCA is classified as toxic (T) and harmful (Xn; R 48). According TGD it is assumed that the available mammalian toxicity data can give an indication on the possible risks of the chemical to higher organisms in the environment. There are only scanty test data available which can be used for the effects assessment of secondary poisoning. For 3,4-DCA a short-term investigation with birds determining the acute lethal effect after oral application is available. After 18 hours a LD_{50} of 237 mg/kg bw/day for Red-winged Black birds were obtained. 3,4-DCA was usually dosed by gavage with solutions or suspensions in propylene glycol (Schafer et al., 1983). This study cannot be used for the assessment.

For the derivation of the $PNEC_{oral}$ results from mammal toxicity tests can be taken into account as well. After oral application to female rats an oral LD_{50} of 530 mg/kg was found (Marty and Wepierre, 1979).

According to TGD, effects on birds and on mammal populations are rarely caused by mortality after short-term exposure. Therefore, results from long-term studies are preferred, such as NOECs for mortality, reproduction or growth.

The NOAELs found in these studies have to be converted into a food concentration by using the ratio between body weight and daily food intake as conversion factor. In the TGD conversion factors for several laboratory test species (rats, mice) are given. However, as in case of the secondary poisoning assessment several uncertainties additional to the “basic” effects assessment should be considered (e.g. biological stress due to seasonal fluctuation in food intake, different body weight/food intake ratios for wildlife species and laboratory species) it is proposed to use an additional factor of 10 for the derivation of the PNECoral.

For mammals a 28-day repeated dose study for 3,5-DCA is available. After oral application to Wistar rats a NOAEL of 30 mg/kg bw/day could be obtained (Hoechst AG, 1989). According to TGD, this value must be converted to the concentration in the food using a factor of 10, which results in 300 mg/kg. With an assessment factor of 100 · 10, a $PNEC_{oral}$ of 0.3 mg/kg could be calculated.

Using a study on maternal and developmental toxicity with pregnant rats (gestational days 6-15), a NOAEL for maternal toxicity of 5 mg/kg bw/day were derived (Clemens and Hartnagel, 1990). According to TGD, this value must convert to the concentration in the food using a factor of 10, which results in 50 mg/kg. With an assessment factor of 10 · 10 a $PNEC_{oral}$ of 0.5 mg/kg can be calculated.

For the further assessment of secondary poisoning the $PNEC_{oral}$ of 0.3 mg/kg obtained using the 28-day repeated dose study with rats will be used. According to the TGD, the $PNEC_{oral}$ deduced from acute tests with birds and rats are not used.

3.3 RISK CHARACTERISATION

3.3.1 Aquatic compartment (incl. sediment)

3.3.1.1 Production and processing of 3,4-DCA

In the following table, the PEC/PNEC ratios for the point sources are presented (PNECmicroorg. = 0.44 mg/l, PNECaqua = 0.2 µg/l, PNECsed = 0.04 mg/kg ww):

Table 3.15 Risk characterisation from point sources

Site	Ceffluent [mg/l]	Ceffl/ PNECstp	PECaqua [µg/l]	PECaqua/ PNECaqua	PECsed [mg/kg ww]	PECsed/ PNECsed
Generic scenario	160	360	60	300	13	325
Company A	confidential	0.09	0.07	0.35	0.015	0.375
Company D	no WWTP	-	22	110	4.8	120

On the basis of site-specific information for company D, a PEClocal/PNECaqua ratio of 110 and a PECsed/ PNECsed of 120 is calculated which both indicate a hazard for the aquatic environment including sediment. However, since September 2001, the production at company D is at a standstill because of an explosion at a neighbouring factory. In July 2002, it was definitely decided by company D to close the production site (industry information from July 22, 2002; Besset 2002, Marcaillou 2002)[8]. Therefore, the risk identified for this site is no longer present.

Conclusion (ii)

3.3.1.2 Releases during manufacture of plant protection agents

Diuron

One production site is identical with a DCA producer, and the emissions are covered by the scenario above. At a further site, no releases occur.

Conclusion (ii)

Linuron

One site has no emissions. For a further site, the resulting PEC/PNEC ratios are

Table 3.16 PEC/PNEC ratios for linuron

Ceffluent [mg/l]	Ceffl/ PNECstp	PECaqua [µg/l]	PECaqua/ PNECaqua	PECsed [mg/kg ww]	PECsed/ PNECsed
$3.3 \cdot 10^{-4}$	$7.5 \cdot 10^{-4}$	0.0049	0.02	0.001	0.025

Conclusion (ii)

[8] Information available by request to the rapporteur Member State in Germany

Propanil

The emission was calculated with site specific data. The resulting PEC/PNEC ratios are:

Table 3.17 PEC/PNEC ratios for propanil

Ceffluent [mg/l]	Ceffl/ PNECstp	PECaqua [µg/l]	PECaqua/ PNEC	PECsed [mg/kg ww]	PECsed/ PNECsed
$2.9 \cdot 10^{-3}$	$6.6 \cdot 10^{-3}$	0.12	0.6	0.026	0.65

Conclusion (ii)

3.3.1.3 Releases from the use of plant protecting agents

The analysis of the available monitoring data revealed that the non-agricultural use of diuron as total herbicide on sealed areas is the source of frequent DCA detections. For the risk characterisation, the latest 90%ile figures (if not available, the maximum) are considered:

Table 3.18 Risk characterisation for rivers

River (Site)	Year	Measured Conc. [µg/l]	Meas. Conc./ PNEC
Donau (Ulm)	1996	< 0.05	< 0.25
Elde (Dömitz)	1995	< 0.05	< 0.25
Erft (Neuss)	1997	90%ile 0.36	1.8
Havel (Krughorn)	1996	< 0.05	< 0.25
Ijsselmeer (Andijk)	1996	Max. 0.08	0.4
Lippe (Wesel)	1997	90%ile 0.11	0.55
Main (Bischofsheim)	1997	90%ile 0.05	0.25
Meusse (Eijsden)	1996	Max. 0.56	2.8
Meusse (Keizersveer)	1996	Max. 0.68	3.4
Neckar (4 sites)	1997	< 0.05	< 0.25
Rhein (Mannheim)	1997	< 0.05	< 0.25
Rhein (Mainz)	1997	< 0.05	< 0.25
Rhein (Koblenz)	1997	< 0.05	< 0.25
Rhein (Bad Honnef)	1997	90%ile 0.17	0.85
Rhein (Kleve-Bimmen)	1997	< 0.1	< 0.5
Rhein (Lobith)	1997	90%ile 0.07	0.35
Ruhr (Duisburg)	1997	90%ile 0.29	1.5
Sieg (Bergheim)	1997	90%ile 0.46	2.3
Spree (Spandau)	1996	< 0.05	< 0.25
Teltowkanal (Kohlhasenbrück)	1996	< 0.05	< 0.25
Wupper (Leverkusen)	1997	90%ile 0.26	1.3

In a series of rivers the measured concentrations are above the PNEC. A risk to aquatic organisms has to be expected.

Conclusion (iii)[9]

For the maximum concentration measured at Meusse (Kreizersveer) of 0.68 µg/l with a sediment concentration of 0.15 mg/kg ww a PECsed/PNECsed ratio of 3.75 could be calculated. Therefore a risk to sediment organisms has to be expected as well. The data basis for the sediment can be improved by performing long term tests with a third sediment organisms representing a further exposure pathway (*Hyalella azteca*). However, this requirement for further testing was awaiting the outcome of the risk reduction strategy for the aquatic compartment. Because the measures recommended are expected to sufficiently reduce concentrations in the aquatic compartment, the test is now no longer deemed necessary.

Conclusion (i) [10]

3.3.1.4 Releases during production and use of Trichlorocarbanilide (TCC)

The releases of one site are covered by a DCA production scenario. Both further sites have no emissions.

Conclusion (ii)

For the use of TCC in household products, the ratios are:

Table 3.19 Risk characterisation for use of TCC in household products

Ceffluent [µg/l]	Ceffl/ PNECstp	PECaqua [µg/l]	PECaqua/ PNEC	PECsed [mg/kg ww]	PECsed/ PNECsed
0.5	$1.1 \cdot 10^{-3}$	0.054	0.27	0.012	0.3

Conclusion (ii)

It has to be considered that if the use of TCC as biocide in the EU will increase again a risk to the aquatic and sediment compartment cannot be excluded.

According to the Biocides Directive (98/8/EC) a biocidal product shall not be placed on the market and used unless it has been authorised in accordance with the Directive. By way of derogation biocides which are already on the market will be assessed in a 10-year work programme after entry into force of the Biocides Directive. TCC is included in the list of notified substances for product groups 1,2 and 4, i.e. 'human hygiene biocidal products', 'private area and public health area disinfectants and other biocidal products' and 'food and feed area disinfectants' (see <http://ecb.jrc.it/biocides/>). If TCC will be authorised for these product groups under the Biocided Directive, the application area for the substance might be broadened. The potential new application areas would result in discharges of TCC to the aquatic environment also.

[9] Commission Recommendation 2006/283/EC of 13th April 2006 on risk reduction measures for various substances including 3,4-DCA, OJ L 104/46.

[10] Commission Communication 2006/C 90/04 of 13th April 2006 on the results of the risk evaluation and the risk reduction strategies for various substances including 3,4-DCA (90/07).

3.3.1.5 3- and 4-monochloroaniline

In the anaerobic sediment layer 3- and 4-monochloroaniline are formed from 3,4-DCA. The ecotoxicity of both compounds is similar to 3,4-DCA, so their risk assessment should be covered by the present assessment.

3.3.2 Atmosphere

As there are no significant releases of 3,4-DCA into the atmosphere known, a risk assessment for this compartment is not necessary.

Conclusion (ii)

3.3.3 Terrestrial compartment

In the following table, the PEClocal_soil/PNEC ratios for 3,4-DCA releases from various plant protection agents are presented (PNECsoil = 10 mg/kg dw):

Table 3.20 Release of plant protection products

Use	PEClocal_soil [mg/kg dw]	PEClocal_soil/ PNECsoil
diuron, agricultural	3.9	0.390
diuron, total herbicide	5.3	0.530
linuron, present use	2.6	0.260
proposed future use	0.85	0.085
propanil	4.4	0.440

The assessment for soil organisms leads to a ratio below 1. Thus, a risk has not to be expected.

Conclusion (ii)

3.3.4 Secondary poisoning

For the food chain sediment - worm - bird or mammals various $PECs_{oral,worm}$ were calculated (see Section 3.1.5). Comparison with the lowest determinated PNECoral of 0.3 mg/kg results in the following ratios:

Table 3.21 Risk characterisation for the food chain

Site	$PEC_{oral,\ worm}$ [mg/kg]	PEC/PNEC
DCA production and processing, generic	17	57
DCA producer A	0.02	0.07
DCA producer D	6.3	21
Production of Linuron	0.0026	0.009
Production of Propanil	0.035	0.12

Use of trichlorocarbanilide	0.016	0.05

The PEC/PNEC ratios for DCA producer D is above 1, indicating a risk for the food chain. However, as the production of 3,4-DCA at this site has stopped, the risk identified for this site is no longer present

Conclusion (ii)

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 General discussion

3,4-DCA is employed as a chemical intermediate. The pattern of use of 3,4-DCA in the Federal Republic of Germany is as follows: the main quantity of 3,4-DCA (99.8%) is converted, with the help of phosgene, to 3,4-dichlorophenyl isocyanate, an initial product for the manufacture of phenylurea herbicides. The remaining 0.2% is used in the production of an azo disperse dye for polyester fibres.

3,4-DCA may be formed by decomposition of diuron, being an antifouling agent used for ships. According to information provided by the member states, the maximum concentration of diuron in the corresponding formulations amounts to 8 w/w. Based on the available information on half-lives of degradation under different conditions (see Section 3.1.2.4) and the high water solubility it is concluded that exposure to 3,4 DCA at the workplace during the application of the antifouling formulations can be neglected.

3,4-DCA is not known to be used in consumer products.

4.1.1.2 Occupational exposure

Presently there are two production sites in the EU. A third company has terminated the production of 3,4-DCA in 1994.

Occupational exposure limits (OEL) have not been established.

4.1.1.2.1 Production and use in the chemical industry

3,4-DCA is synthesised from 3,4-dichloronitrobenzene in a closed system using a continuous hydrogenation process with a distillation as a final purification step (Bayer AG, 1994; BUA; 1994; Rhone-Poulenc, 1998). According to one manufacturer the distillation residue contains approximately 20% 3,4-DCA. At normal work the substance is handled in a molten state at about 90°C (melting point = 72°C). According to one producer filling into tankers takes place using the gas-displacement method, drums are filled on suction-cleaned scales. For the other site the process is described as continuous, automated and closed. It is assumed that the technical equipment of both sites is comparable.

In the production of 3,4-dichlorophenyl isocyanate, 3,4-DCA is converted in a closed system using phosgene. On account of the toxicity of phosgene, particularly high demands are made on the leak proofness of this plant.

In the production of the azo disperse dye, the molten 3,4-DCA is drawn by suction into a reaction vessel and converted as an azo component. According to information provided by the manufacturers, as a precaution, the workers wear respiratory equipment and protective

clothing in case of possible exposure e.g. during the transfer and the cleaning of the apparatus and of the filter press.

The steps in the process are characterised as continuous and discontinuous without further differentiation.

In the above-mentioned areas, exposures to 3,4-DCA are to be expected during sampling and sample analysis, transfer and drumming activities as well as during cleaning, maintenance and repair work in particular. Although differing levels of exposure are to be expected in dependence on the activities concerned, it is not possible to undertake a differentiated, individual assessment of all of the activities on the basis of the currently available exposure data.

The possible routes of exposure are by inhalation of vapours or mainly of small particles formed by condensation from the vapour because of the different temperature of handling (at about 90°C; melting point at 72°C) and of the working environment (assumed: 20-30°C) and by skin contact.

At one site 3,4-DCA is produced in campaigns (3 campaigns per year; 1 campaign is lasting one month), the duration and frequency of exposure is 8 hours but not on a daily scale. For the other site the lack of information leads to the assumption that the duration and frequency of exposure of the workers is assumed to be 8 hours daily.

According to the information of the producers 265 workers could be exposed during different activities. At one site, 3,4-DCA is handled by approximately 155 workers on a permanent basis (8 hours assumed) and by approximately 82 workers on an occasional basis. Of these, 7 are women. For the other site detailed information were not submitted.

Inhalative exposure - Workplace measurements

According to one producer an in-company method of analysis is used to determine 3,4-dichloroaniline. Sampling is performed for total dust at a suction rate of 1.25 m/s onto silica gel which has been pre-treated with hydrochloric acid. The analytical detection limit amounts to 0.07 mg/m^3 (0.01 ml/min) for 120 l sample air and a volumetric flow rate of 1 l/minute.

In the case of the production of 3,4-dichloroaniline and the conversion to 3,4-dichlorophenyl isocyanate, 9 person-related measurements (8-h time-weighted averages) and 3 short-term values (approximately 1 hour) were taken at one site during the period 1990 - 1994 for work involving the connection and disconnection of transfer lines to tankers. All of the results are located below the detection limit of 0.07 mg/m^3 (see **Table 4.1**).

For the processing of 3,4-dichloroaniline in dye manufacture five short-term measurement results with an exposure time of less than one hour relating to the transfer of the molten product and the cleaning of the filter press are available. 4 values are located below the detection limit. The highest measured value (0.57 mg/m^3) determined during a sampling duration of less than half an hour produces, after conversion, an 8-hour time-weighted average of < 0.07 mg/m^3 (in addition, the worker was protected by a respiratory equipment).

On the basis of the current information on sampling and analysis methods and on measurement strategy (TRGS 402, 1986), the measurement results can be regarded as valid.

Table 4.1 3,4-DCA exposures at workplaces during production and further processing

Work area/ activities	Year of measure-ments	Number of measure-ments	Measure-ment range [mg/m³]	Geom. mean [mg/m³]	95% Value [mg/m³]	Duration [hrs/day] (1)
8-hour Time-weighted averages						
Production and conversion	1990-1994	9(p)(2)	< 0.07	no information	no information	no information
Short-term values						
Production and conversion, connection to and disconnection from tankers	1990-1994	3	< 0.07	no information	No information	app. 1
Dye manufacture, transfer of molten product and cleaning of the filter press	1990-1994	4(p)(2) 1(p) (2)	< 0.07 0.57	no information	No information	0.57 - 1.0 0.5

1) Information about the frequency [days/year] were not available
2) Personal sampling

Since the process technologies and the levels of protection are similar for both production sites, the measurement data submitted by one company are taken as representative.

Dermal exposure

Since the substance is handled in closed systems, dermal exposure is limited. With regard to potential dermal exposure of workers against 3,4-DCA, handling of the molten substance at elevated temperatures (90°C, drumming, connecting/disconnecting transfer lines) and handling of mixtures of 3,4-DCA (cleaning and maintenance, repair, after the substance is cooled down and solidified) in the chemical industry must be considered.

On account of the dangerous properties of 3,4-DCA (see Section classification) use of personal protective equipment (PPE) is recommended. The amount of 3,4-DCA that reaches the skin (actual dermal exposure) depends on the efficacy of PPE, which is determined by several factors, e.g. by the probability of use of PPE, the use habits and the performance (e.g. type, material: penetration, permeation of substance/mixtures, degradation).

In general, use of (functioning) PPE is assumed to be highly accepted in the large-scale chemical industry. Dermal contacts with chemicals handled at elevated temperatures (here 90°C) would immediately lead to severe health effects and thereby force the worker directly to the proper use of PPE or to avoid any contacts with the hot substance. In addition, the workforce of the chemical industry ought to be well trained to correctly use PPE As a conclusion, during handling of molten 3,4-DCA at elevated temperature (approximately 90ºC) skin and eye contact is seen to be restricted to single events (see EASE estimate in Section 4.1.1.2.2), which rarely occur. Taken into consideration the highly accepted use of proper gloves daily dermal exposure is assessed to be low.

The substance is applied at elevated temperature (transferring, drumming). Dermal contacts after the substance is cooled down are possible during cleaning, maintenance and repair tasks. For contacts to the solid, pure substance, the used gloves are regarded as suitable and dermal contacts are reduced to single events (see EASE estimate in Section 4.1.1.2.2). But workers are potentially exposed not against pure 3,4-DCA, but to its mixtures, which are quite complex in nature (e.g. distillation residues). The use of PPE (here: gloves) is an essential part of usually applied workers protection strategy for these kind of tasks. Nevertheless it has to be

mentioned, that the actual system for testing permeation properties of protective gloves usually refers only to the pure substance or determined solutions. There is a general lack of information about the performance of protective gloves under working conditions, where complex mixtures could be involved. An EASE-model calculation for an unprotected worker, gives a worst-case estimate for occasional dermal exposure (see Section 4.1.1.2.2).

4.1.1.2.2 Estimation of exposure according to the EASE model

The estimation of the level of exposure by inhalation according to the EASE model (version EASE for Windows 2.0, Aug. 1997) produces the following results:

Inhalative exposure

Exposure by inhalation to vapours during production and processing in the area of the large-scale chemical industry:

Input parameters:	t = 90°C (vapour pressure 20 Pa at 90°C, calculated) closed system significant breaching LEV present
Estimated level of exposure:	3.3 - 6.6 mg/m^3 (0.5 - 1 ml/m^3)

The exposure level includes also exposure to small particles which are formed by condensation of the vapour.

Dermal exposure

Potential dermal exposure during handling of the molten substance at elevated temperatures (approximately 90°C) within the chemical industry, incidental contacts to the solid substance although suitable gloves are worn:

Input parameters:	t=90°C closed system significant breaching direct handling incidental
Estimated level of exposure:	0-0.1 mg/cm^2/day. Assuming an exposed area of 420 cm², dermal exposure results in 0-42 mg/person/day.

Potential dermal exposure during cleaning, maintenance and repair work within the chemical industry

Input parameters:	t=20°C closed system significant breaching non dispersive use direct handling intermittent
Estimated level of exposure:	0.1 - 1 mg/cm^2/day.

Considering a concentration of 20% 3,4-DCA in the distillation residue and an exposed area in the order of magnitude of 1,300 cm^2 (hands and part of the forearms) the exposure level amounts to 26 - 260 mg/day.

4.1.1.2.3 Other exposure data

No other exposure data are available.

4.1.1.2.4 Integrated assessment

3,4-DCA is only produced and processed further as a chemical intermediate in large chemical companies.

Results of workplace measurements concerning the production and further processing of 3,4-DCA were submitted by 1 of 2 actual producers. Both companies described the process technology as “closed system”. On account of the similar processes, the data provided by one company are regarded to be representative for both sites. The company which did not submit measurement data carries out the production of 3,4 DCA not during the whole year but within 3 campaigns/year lasting 1 month each. The duration and frequency of exposure are assumed as 8 hours daily as a reasonable worst case although one company stated that exposure occurs not daily.

For the purpose of assessing the risks of daily inhalative exposure on the basis of the measured data during the production and the further processing within the large-scale chemical industry 0.07 mg/m^3 (0.01 ml/m^3; detection limit) should be used as a worst case, since all of the measured 8-hour TWA’s are located below it. Higher exposures (0.57 mg/m^3, 0.08 ml/m^3) may occur for short periods (0.5 hours) during particular activities as transfer or cleaning of the filter press. A shift average calculated on the basis of the short-term value of 0.57 mg/m^3 (0.08 ml/m^3, duration 0.5 h) amounts to 0.035 mg/m^3 and confirms the measured shift averages.

With regard to potential dermal exposure of workers against 3,4-DCA, handling of the molten substance (90°C, drumming connecting/disconnecting transfer lines) and handling the substance as a component of mixtures after cooling down (cleaning and maintenance, repair) in the chemical industry must be considered (see Section 4.1.1.2.1).

During handling of the molten 3,4-DCA (approximately 90ºC) skin and eye contact is assumed only to single contacts, which rarely occur. For the fields of production and further processing the highly accepted use of functioning PPE is assumed. Daily dermal exposure is assessed as low.

For handling the cooled and solidified substance, cleaning, maintenance and repair tasks are regarded to be the most probable tasks with possible dermal exposure. For contacts to the solid, pure substance, the used gloves are regarded as suitable and dermal contacts are reduced to single events. An EASE estimation results in 0 – 42 mg/person. But workers are potentially exposed not against pure 3,4-DCA, but to its mixtures, which are quite complex in nature (e.g. distillation residues). The use of PPE (here: gloves) is an essential part of usually applied workers protection strategy for these kind of tasks. Nevertheless it has to be considered that there is a general lack of information about the efficiency of protective gloves for cleaning, maintenance and repair tasks, where complex mixtures could be involved. Since the exposure limiting effect of gloves for handling the complex mixture cannot be estimated,

occasional dermal exposure (not daily) is assessed for the unprotected worker as a worst case situation. The corresponding EASE prediction results in 26-260 mg/p/day (occasional dermal exposure, worst case situation).

For assessing the risks of potential dermal contacts to the cooled substances (pure and in mixtures) 26-260 mg/p/day (occasional dermal exposure, worst case situation) should be taken.

Table 4.2 Summary of inhalative exposure data of 3,4-DCA for occupational risk assessment

Inhalative exposure								
Area of production and use	**Form of exposure**	**Activity**	**Duration [hs/day]**	**Frequency [days/year]**	**Shift average [mg/m³]**	**Method**	**Short term [mg/m³]**	**Method**
Chemical industry								
Production and further processing:	vapour/small particles(1)	drumming, filling, transfer cleaning(3), maintenance(4), repair work(4)	8	daily assumed (for one site)	< 0.07(2)	workplace measurements	--	--
		transfer, cleaning	0.5	not known	--	--	0.57(2,5)	workplace measurements

1) Vapour and particles formed by condensation from the vapour
2) Assessed on the basis of the submitted data
3) Frequency not known
4) Frequency not assumed on a daily scale
5) Workers wear respiratory equipment

Table 4.3 Summary of dermal exposure data of 3,4-DCA for occupational risk assessment

Dermal exposure							
Area of production and use	**Form of exposure**	**Activity**	**Frequency [days/year]**	**Level of exposure [mg/cm²/day]**	**Exposed area [cm²]**	**Shift average [mg/p/day]**	**Method**
Chemical industry							
Production and further processing:	molten substance (90°C)	handling (drumming, filling, transfer)	daily	low	--	low	exp. judgement
	cooled substance (pure and in mixtures / ambient temperature)	cleaning, maintenance, repair work	not daily	0.02 – 0.2	1,300	26 – 260(1)	EASE

1) Worst case estimate, the limited protection of gloves cannot be considered.

4.1.1.3 Consumer exposure

4.1.1.3.1 Use of 3,4-dichloroaniline

Since reliable information on 3,4-DCA in consumer products is missing, calculation of consumer exposure cannot be carried out.

Presumably, direct use of 3,4-DCA by consumer does not exist. The herbicide Diuron and paint formulations with this substance do not contain 3,4-DCA (Industry, November 30, 1999).

4.1.1.3.2 Possible release of 3,4-dichloroaniline from products used by consumers

Use of Trichlorocarbanilide

The formation of 3,4-DCA is possible from the use of Trichlorocarbanilide which is used as a deodorant and soap bactericide in household products (see Section 4.1.1.4).

According to the BgVV product register this substance was found in one product with a content of < 1%.

Use of Diuron

In the BgVV product register TRIC, 6 Diuron-containing products are listed (content varying from 3 to 80%). The current use pattern is that Diuron is used in anti-fouling products, as preservative in paints for facades and plasters and as an algicide.

Anti-fouling products

Primarily information came from DK and Sweden on use of Diuron in anti-fouling products (no quantitative data).

There is further information given by UK on use of Diuron in anti-fouling products (104 products are mentioned; maximum concentrations up to 8% (w/w)).

Preservative in paints for facades and plasters

According to the information by Industry (November 30, 1999) the concentration of Diuron in paints amounts between 0.2 and 0.5%.

Chemical for swimming pools

There is information that Diuron may be used as an algicide in chemicals for swimming pools. However, no further details of the extent of this use are available.

In conclusion

The possibility of an internal exposure to 3,4-DCA due to its formation from Diuron-containing products is considered to be negligible for the following reasons:

1. short time of exposure
2. low Diuron contents
3. low dermal absorption (see Industry, November 30, 1999)
4. only small amounts of 3,4-DCA are metabolically formed from Diuron.

Remarks

On the whole it is assumed that Diuron is metabolised in humans in the same manner as in the rat and dog by partial or complete demethylation and hydroxylation. The main metabolite is N-(3,4-dichlorophenyl)-urea and 3,4-dichloroaniline. Quantification of the metabolites showed that the concentration of 3,4-DCA in humans was 900 times lower than that of the main metabolite (van Boven, 1990).

4.1.1.4 Indirect exposure via the environment

Intake from inhalation

As there are no significant releases of 3,4-DCA into the atmosphere, this exposure pathway is assumed to be negligible.

The EUSES calculation of the human intake results for the regional scale (see Appendix C):

$$\Rightarrow DOSE_{air} = 4.0 \cdot 10^{-11} \text{ mg} \cdot \text{kg bw}^{-1} \cdot \text{d}^{-1}$$

Intake from drinking water gained from river water

During production, 3,4-DCA is emitted into surface waters where it can reach water works gaining drinking water by bank filtration. According to the Technical Guidance Documents, a purification factor of 1 (log Pow of < 4, H = < 100 $Pa \cdot mol^{-1}$, biodegradation rate > 10 days) has to be chosen for both storage in open reservoirs and dune recharge. However, a monitoring study shows that elimination during the purification processes occurs. The substance was measured in 3 drinking-water works where the water is gained from bank filtrate. The concentrations and the applied purification techniques are given in **Table 4.4** (Kussmaul et al., 1975):

Table 4.4 Measured concentration in water

River	River water [µg/l]	Bank filtrate [µg/l]	Drinking water [µg/l]	Purification techniques
Rhine	0.72	0.265	0.013	ozone, activated carbon filtration,
Rhine	0.679	0.235	0.011	treatment with ClO_2
Main	0.166	0.082	0.017	flocculation, accelerator, silica filter, activated carbon filtration, soaking in the underground

From the measured values, total removal rates of 98.2, 98.4 and 89.2% are calculated. For the following calculations, a purification factor of 90% is taken. The doses are estimated for each production site and the yearly average of the measured values from the river Rhine. With a daily intake of 2 l drinking water and a body weight of 70 kg, the doses are calculated as

Table 4.5 Indirect exposure by emission sources

Scenario	PEClocal [µg/l]	C_{drw} [µg/l]	$DOSE_{drw}$ [mg·kg bw^{-1}·d^{-1}]
Production site A (see Section 3.1.2.2)	0.07	0.007	$2.0 \cdot 10^{-7}$
Production site (see Section 3.1.2.2)	22	2.2	$6.3 \cdot 10^{-5}$
Production of Linuron	0.005	0.0005	$1.4 \cdot 10^{-8}$
Production of Propanil	0.12	0.012	$3.4 \cdot 10^{-7}$
Use of trichlorocarbanilide	0.054	0.0054	$1.5 \cdot 10^{-7}$
PECregional (see Section 3.1.6)	0.0042	0.00042	$1.2 \cdot 10^{-8}$

Intake from drinking water gained from groundwater

3,4-DCA is formed as a metabolite from different plant protection agents (see Section 3.1.4). A model calculation for the application of diuron resulted in a pore water concentration in agricultural soils of 21 µg/l. According to the TGD, the pore water concentration has to be set equal to the drinking water concentration. With this figure, the doses is

$$DOSE_{drw} = 6.0 \cdot 10^{-4} \text{ mg} \cdot \text{kg bw}^{-1} \cdot \text{d}^{-1}$$

However, we assume that this figure is unrealistic high. Drinking water is gained from groundwater. During the leaching process, the 3,4-DCA content should strongly decrease because of the high sorption (chemisorption, see Section 3.1.1.3). This is supported by the following investigation:

^{14}C-labelled 3,4-DCA was incubated into a sandy loam in a concentration corresponding to 1.43 kg/ha and stored under outdoor conditions. In the leaching water (70 cm depth), 3,4-DCA could not be detected suggesting that it contained less than 0.1% of the applied radioactivity (Viswanathan et al., 1978). A relevant contamination of groundwater is not expected by regular application of the plant protecting agents.

Intake from fish

The human intake from fish is calculated with a BCF of 45 l/kg, a daily consumption of 115 g fish and different exposure scenarios:

Table 4.6 Intake from fish

Scenario	PEClocal [µg/l]	C_{fish} [µg/kg]	$DOSE_{fish}$ [mg·kg bw^{-1}·d^{-1}]
Production site A (see Section 3.1.2.4)	0.07	3.15	$5 \cdot 10^{-6}$
Production site D (see Section 3.1.2.4)	22	990	$1.6 \cdot 10^{-3}$
Production of Linuron	0.0049	0.2	$3.2 \cdot 10^{-7}$
Production of Propanil	0.12	5.4	$8.7 \cdot 10^{-6}$
Use of trichlorocarbanilide	0.054	2.43	$3.9 \cdot 10^{-6}$
PECregional (see Section 3.1.6)	0.0042	0.19	$3 \cdot 10^{-7}$

Intake from plants

3,4-DCA can reach plants by two pathways:

- The plant protection agents which are its subsequent products are degraded in soil (see Section 3), and 3,4-DCA is taken up by the plant.
- The plant protection agent is taken up by the plant where it is metabolised.

There are several investigations dealing with the 3,4-DCA content as a consequence of the application of plant protecting agents which are its subsequent products:

Propanil is used as selective herbicide for weed control in rice fields. 3,4-DCA has been detected in all tested marketed rice samples. It has been estimated that marketed rice might contain as much as 1µg 3,4-DCA/g (Still and Mansager, 1969). This could be confirmed by experiments (Still et al., 1980) were plants treated with ^{14}C-3,4-DCA quantities to approximate those that would result from propanil treatment at recommended field application levels. Leaf and soil treatment resulted in the same amount of radioactivity in the whole plants. No significant radioactivity was detected in grains of the leaf-treated rice plants whereas in the grains of soil-treated plants 0.4 µg 3,4-DCA/g could be found after 120 days. The authors interpret the radioactivity in the rice grains as 3,4-DCA that was temporarily immobilised in soil as complex with humic substances and was made bioavailable for root uptake during the grain ripening period by the microbial cleavage of these humic complexes. This interpretation is also supported by the results of Still and Mansager (1969) who found 3,4-DCA in the grain of rice plants that were never treated with herbicide, but grew in soil that had a history of propanil treatment.

In pre-emergence application studies with linuron residue data for different plants from supervised trial were obtained (Stumpf, 1995). The herbicide was applied by spraying. The residues were calculated as linuron and analysed as 3,4-DCA (DCA is formed from linuron by alkaline hydrolysis during sample preparation). All metabolites which were cleaved by alkaline hydrolysis to 3,4-DCA were obtained. To differentiate between 3,4-DCA pre and post hydrolysis is not possible from this study.

The maximum residue amount was found for parsley, onions and celery. Application rates were 0.475 and 0.713 kg a.s./ha for parsley, from 1.5 to 3 kg a.s./ha and 0.71 to 1.9 kg a.s./ha for celery. The residue levels in the leaves of parsley were 0.37 mg/kg at most after 90 days, in the root residues ranged between 0.1 and 0.29 mg/kg after 180 days. For onions concentrations of residues in bulb and leaves were 0.1 and 0.2 mg/kg after 89 and 66 days. After 105 days the residues in the bulb were below the determination limit of 0.02 mg/kg. For celery 42 days after treatment maximum values of residues of 1.15 mg/kg in the leaves and 0.72 mg/kg in the bulb could be found. At latest 48 days after treatment residues in the leaves of celery were below 0.5 mg/kg. In the bulb of rooted celery residues after 42 days exceeded 0.5 mg/kg in some trials.

In other studies similar results for linuron could be found (Maier-Bode, Härtel, 1981). Additionally high residues were found after application of linuron in soybeans (after 65 days 1.3 mg/kg in the forage), carrots (after 82 days 0.41 mg/kg in the roots) and in clover (after 163 days 1.1 mg/kg in the dried).

In a study of diuron in rotational crop the soils were treated with 2,600 g a.i./ha (A) and 1,900 g a.i./ha (B) (Stevenson, 1989). After treatment, the soils were aged aerobically in greenhouse for either 4 months (A), or 12 months (B). At the end of the aging periods, lettuce,

wheat, and turnips were planted and grown to maturity. Soil samples at treatment, planting and plant harvest were extracted with different organic solvents, 1N HCl and 0.1 N NaOH.

It could found that the extractable soil concentration of ^{14}C-diuron decreased from 1.12 (B: 1.01) ppm at treatment to 0. 253 (B:0.212) ppm at planting, and to 0.209-0.237 (B: 0.078-0.194) at harvest. The soil concentration of extractable radioactive residues in diuron equivalents were 1.126 (B: 1.113) ppm at treatment, 0.675 (0.511) ppm at planting, and 0.651-0.735 (0.31-0.448) ppm at harvest. The unextractable radioactive residues in the soil increased from 5 - 6% at treatment to 11 - 16% at planting to 20 - 28% at the time of harvest.

The crops were harvested and analysed for total radioactive residues. The concentrations of extractable radioactive diuron in edible portions of the harvested plants were 0.041 (B: 0.04) ppm. The extractable radioactive residues in the edible portions of the harvested plants were 0.235 (B: 0.157) ppm. Unextractable radioactive residues could be found in the range of 10%.

No radioactive TCAB or TCAOB were found in the soil or the plants with the detection limit of 0.002 ppm.

A study of the residue pattern in rotational crops and the degradation behaviour in soil under field conditions was also performed with a linuron application rate of 950 g linuron/ha, (nominal) (Sochor and Wrede, 1997). The initial concentrations determined experimentally were between 0.55 mg/kg and 0.29 mg/kg on the day of application.

The residues in the crops were determined by the method of Bleidner. The results of degradation and binding of the degradation products of urea-herbicides in the soil are in relation with the other findings. An uptake of the active ingredient from soil occurred only at the early crop stage in carrots and lettuce in the range of 0.02 - 0.19 mg linuron/kg.

Summarising, it must be said, that the results of the studies are very different. More or less amounts of 3,4-DCA were found in the different parts of the plants. But all investigation with radioactive material show that only 50% of the starting radioactivity could be found, that means the other part is bound in an unextractable manner to the matrix - as also found for the soil compartment (see Section 3.1.1). The results of Still et al. (1981) on the identity of bound chloroaniline residues in plants indicate that the chloroanilines may be bound covalently to lignin via 1,6-addition to a quinone method intermediate during the lignin synthesis as also could be shown for the soil.

This can be confirmed by an animal bioavailable study (Sandermann et al., 1992). Treatment of intact wheat plants and excised shoot tissues with [^{14}C]-3,4-DCA led to 55-65% incorporation of the radioactivity into the „insoluble„ residue. A sequential solubilisation revealed that approximately 85% of the ^{14}C-label was associated with the operationally defined lignin fraction. When the insoluble wheat metabolite residue was fed to rats and lambs, 11-20% of the bound radioactivity was released in soluble form. Re-feeding the lamb fecal residue to rats released another 7% of the bound radioactivity. A 3,4-DCA-lignin metabolite prepared enzymatically has been previously shown to be more extensively solubilised by rats (66%). Mild acid hydrolysis under simulated stomach conditions resulted in a strong release of free 3,4-DCA (30%) only from the previously used lignin metabolite.

A review of monitoring data of 3,4-DCA in plants treated with linuron is available (Maier-Bode and Härtel, 1981). The measured DCA-concentrations in more than 200 samples were generally in the range between lower detection limit to 0.1 mg/kg, in some cases up to 0.7 mg/kg. The respective application amounts were between 0.5 and 4.5 kg linuron/ha (in

two cases 7.5 kg/ha). It has to be considered that the results may overestimate the 3,4-DCA-concentration: due to the analytical method (which starts with alkaline hydrolysis), residual linuron as well as mono- and desmethyllinuron are hydrolysed to 3,4-DCA.

As this publication gives the best available database, it is used for the exposure estimation. As a worst case approach, it is assumed that the contribution of linuron, mono- and desmethyllinuron to the detected 3,4-DCA concentrations can be neglected. An average concentration of 0.1 mg/kg plant (leaf and root crops) is assumed:

- $DOSE_{stem} = 1.7 \cdot 10^{-3}$ mg · kg bw^{-1} · d^{-1}
- $DOSE_{root} = 5.5 \cdot 10^{-4}$ mg · kg bw^{-1} · d^{-1}
- $DOSE_{stem} + DOSE_{root} = 2.3 \cdot 10^{-3}$ mg · kg bw^{-1} · d^{-1}

This scenario is based on the assumption that all food plants are treated with linuron. On the other hand, further plants are treated with diuron and propanil. These agents are used in the same concentration range, so the respective 3,4-DCA concentrations are assumed to be similar.

The scenario could be improved if it would be based on a more precise consume pattern for the different plants and their specific 3,4-DCA concentrations.

The EUSES calculation of the human intake results for the regional scale (see Appendix C):

- $DOSE_{stem} = 8.8 \cdot 10^{-8}$ mg · kg bw^{-1} · d^{-1}
- $DOSE_{root} = 1.3 \cdot 10^{-11}$ mg · kg bw^{-1} · d^{-1}

Intake from meat and milk

With a log Pow of 2.7, very low biotransfer factors for meat and milk are expected. Compared with the uptake from plants, these exposure pathways seem to be of no importance.

The EUSES calculation of the human intake results for the regional scale (see Appendix C):

- $DOSE_{meat} = 1.3 \cdot 10^{-11}$ mg · kg bw^{-1} · d^{-1}
- $DOSE_{milk} = 1.5 \cdot 10^{-11}$ mg · kg bw^{-1} · d^{-1}

Total daily intake for humans

For the estimation of the total daily intake for humans, the different life-cycle steps of 3,4-DCA are considered. Only the important exposure pathways are considered:

Table 4.7 Total daily intake for humans

Scenario	Relevant pathways	$DOSE_{total}$ [mg·kg bw^{-1}·d^{-1}]
Production site A (see Section 3.1.2.2)	Drinking water, fish	$5.2 \cdot 10^{-5}$
Production site D	Drinking water, fish	$1.7 \cdot 10^{-3}$
Production of Linuron	Drinking water, fish	$3.3 \cdot 10^{-7}$
Production of Propanil	Drinking water, fish	$9 \cdot 10^{-6}$

Table 4.7 continued overleaf

Table 4.7 continued Total daily intake for humans

Scenario	Relevant pathways	$DOSE_{total}$ [mg·kg bw^{-1}·d^{-1}]
Use of trichlorocarbanilide	Drinking water, fish	$4 \cdot 10^{-6}$
Use of plant protecting agents	Plants (leaf and root crops)	$2.3 \cdot 10^{-3}$

The exposure scenarios for the production propanil and TCC are based on default parameters as no emission data are available. The respective figures could be improved.

Additionally, DCA metabolites like 3,3',4,4'-tetrachloroazobenzene (TCAB), 3,3',4,4'-tetrachloroazoxybenzene (TCAOB) and related compounds were taken up. For an initial approach, for TCAB a PEC of 60 µg/kg was determined from a monitoring study.

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

4.1.2.1 Toxicokinetics, metabolism and distribution

Mechanism of action

3,4-dichloroaniline and the 2,4 and 2,5-isomers are substances producing methaemoglobin. Their methaemoglobin-producing effect is based on hydroxylated compounds which are formed as intermediates (Lenk and Sterzl, 1984; McMillan et al., 1991; Singleton and Murphy, 1973).

In vitro, the methaemoglobin-producing effect of N-hydroxy-3,4-dichloroaniline was found to be high in rat and cattle erythrocytes, that of 6-hydroxy-3,4-dichloroaniline was found to be lower. *In vitro*, these hydroxyl derivatives were confirmed to be metabolites of 3,4-dichloroaniline (Lenk and Sterzl, 1984; McMillan et al., 1990).

Absorption

Based on the excretion study with ^{14}C-3,4-dichloroaniline (Worobey and Shields, 1991) it can be concluded that the substance is absorbed via the gastrointestinal tract. Approximately 80% of the radioactivity (5.04 µg ^{14}C-3,4-dichloraniline/animal) administered orally to rats was excreted in the urine within 24 hours after administration.

Further studies on the quantification of absorption of 3,4-dichloroaniline are not available. It can be concluded from studies on their acute systemic effects of dichloroanilines, in particular on methaemoglobin formation, that dichloroanilines are absorbed through the skin and the lungs.

Distribution in tissue

After oral administration of ^{14}C-3,4-dichloroaniline to rats (5.04 µg/animal, no dosage given), liver, kidney, muscle, and blood contained ≤ 1%, adrenals, thyroid, and spleen ≤ 0.1% of the dose after 72 hours. From data on ^{14}C-content it may be concluded that there is no bioaccumulation (Worobey and Shields, 1991).

Studies in rats, mice and guinea pigs have revealed that, after acute i.p. administration (296 mg/kg bw), concentrations of 3,4-dichloroaniline in liver and plasma had reached their maximum 30-60 minutes after administration, in parallel with the formation of methaemoglobin levels of 75%, 55% and 32%, and had decreased again considerably within a few hours (no further data) (Chow and Murphy, 1975).

Biotransformation

Only one *in vivo* study is available on the biotransformation of 2,4-, 2,5- and 3,4-dichloroaniline. After feeding dichloroanilines to rabbits ortho- or para- hydroxylated compounds were detected in urine.

As a metabolite of 2,4-dichloroaniline, 6-hydroxy-2,4-dichloroaniline was detected by analysis and it was not possible to clearly identify the metabolite of 3,4-dichloroaniline as 2-hydroxy- or 6-hydroxy-3,4-dichloroaniline. Other data, in particular on quantification, cannot be taken from the study (Bray et al., 1957).

In *in vitro* studies, both ring hydroxylated (2- and 6-hydroxy-3,4-dichloroaniline) and N-hydroxylated (N-hydroxy-3,4-dichloroaniline) and N-acylated (N-(3,4-dichlorophenyl) acetamide and N-(3,4-dichlorophenyl) formamide metabolites of 3,4-dichloroaniline were detected (Lenk and Sterzl, 1978 and 1984; McMillan et al., 1990).

In female rats, haemoglobin adducts were found 24 hours after oral administration of 81 mg (0.5 mmol) 3,4-dichloroaniline/kg bw. The haemoglobin binding index (in mmol/mol Hb/dose (mmol/kg bw)) was 9 (Birner and Neumann, 1988). The comparative haemoglobin binding indices for aniline were 22, for 3-chloroaniline 12 and for 4-chloroaniline 569 (Sabbioni and Neumann, 1990; Birner and Neumann, 1987). The 2,4- and 2,5-isomers were not investigated.

Elimination

Excretion of orally administered ^{14}C-3,4-dichloroaniline (5.04 µg/animal) in rats was found to be 81% in urine and 26% in faeces, where the largest share was excreted already within the first 24 hours after administration. 3 days after administration, the substance had been excreted completely (Worobey and Shields, 1991).

Substances with a similar structure which may be considered for reference

Other dichloroaniline isomers e.g. 2,4- or 2,5-dichloroaniline and monochlorinated aniline compounds such as 2-, 3- or 4-chloroaniline were considered in the evaluation of harmful effects of 3,4-dichloroaniline, however to a varying degree.

4-Chloroaniline

The main metabolites of 4-chloroaniline are 4-chloroacetaniline and 2-amino-5-chlorophenyl sulfate. The radioactivity reaches a maximum in plasma one hour after administration of ^{14}C-labelled 4-chloroaniline, and can be found in erythrocytes within 24 hours (Ehlhardt and Howbert, 1991). In the urine of mice, rats and monkeys, 80, 81 to 87 and 55% of 4-chloroaniline and its metabolites were formed within 24 hours, while in the faeces, only 5.2, 8.3 and 1.2% are excreted within 4 days. The main metabolite in the urine at 49% (mice), 54% (rats) and 36% (monkeys) is 2-amino-5-chlorophenyl sulfate. The total excretion in 24-hour urine is 80% in mice, 88% in rats and 56% in monkeys, and the total excretion after

4 days (urine and faeces) is 91% (mice), 101% (rats) and 82% (monkeys) (Ehlhardt and Howbert, 1991). 4-Chloroaniline is eliminated from the tissues in 2 phases with half-lives of less than 10 minutes and 3 to 4 hours (Perry et al., 1981; NTP, 1989). In humans, 4-chloraniline is mainly excreted in the urine. Conjugated 4-chloroaniline and conjugated 6-hydroxy-3-chloroaniline (2-amino-5-chlorophenol) have also been found as major metabolites in the urine in a case of acute poisoning (Yoshida et al., 1991, 1992a,b).

In vitro 4-chloroaniline is N-hydroxylated to 4-chlorophenylhydroxylamine by rat liver microsomes (Ping Pan et al., 1979) and to a small amount to 4-aminophenole by rabbit liver microsomes (Daly et al., 1968; Ichikawa et al., 1969).

After induction with phenobarbital rabbit liver microsomes showed hydroxylation of 4-chloroaniline to 4-chlorophenylhydroxylamine and 2-hydroxy-4-chloroaniline (Lenk and Sterzl, 1981, 1984).

2-Chloroaniline

In the hydrolysed urine of female rabbits 24 hours after oral uptake of 2-chloroaniline metabolites found were 4-hydroxy-2-chloroaniline (4-amino-3-chlorophenol) and traces of 6-hydroxy-2-chloroaniline (2-amino-3-chlorophenol) (no further information) (Bray et al., 1956). Thus, as in the case of 3-chloroaniline, ring hydroxylation is the predominant phase I metabolic reaction in the rabbit.

In an *in vitro* study on liver, kidney and lung microsomes of albino rabbits and on the adrenal microsomes of cattle it has been shown that 2-chloroaniline is hydroxylated mainly in the microsomes of the liver, to a lesser extent in the kidney and lung, but not in the adrenal gland (Ichikawa et al., 1969).

3-Chloroaniline

For 3-chloroaniline the main metabolites were found to be derivatives of 4-hydroxy-3-chloroaniline (4-amino-2-chlorophenol) and, on a smaller scale, those of 2-hydroxy-5-chloroaniline (2-amino-4-chlorophenol). Derivatives of 1-hydroxy-2-chloroaniline (2-amino-6-chlorophenol) occurred only in traces. There was no indication of N-hydroxylated compounds. After hydrolysis 39-50% of the administered 3-chloroaniline dose was identified in the urine as 4-hydroxy-3-chloroaniline (4-amino-2-chlorophenol) and 16-25% as 2-hydroxy-5-chloroaniline (2-amino-4-chlorophenol) (Böhme and Grunow, 1969).

In vitro, two hours after the incubation of rat hepatocytes with 3-chloroaniline, small amounts of conjugated 3-chloro-4-hydroxyacetaniline and 4-aminophenylsulfate ester were identified (no further data given) (Alary et al., 1986).

In vivo only ring hydroxylated derivates are detected as metabolites of dichloroanilines as well as for 2- and 3-chloroanilines, whereas for 4-chloroaniline also N-acetylated metabolites are formed. The elimination rates of 3-chloroaniline (3-CA), 4-chloroaniline (4-CA) and 3,4-DCA are comparable and found to be 45-75% (3-CA), 88% (4-CA) and 81% (3,4-DCA) in the urine of rats.

Summary

Data are available which show that 3,4-dichloroaniline is absorbed from the gastrointestinal tract, whereas absorption through skin and lungs has not investigated by kinetic studies.

However, from the toxic effects which are observed it can be concluded that 3,4-dichloroaniline is also absorbed through skin and lungs. *In vivo* hydroxylation of 3,4-dichloroaniline leads to the formation of ortho- and para-hydroxylated compounds. N-hydroxylation was additionally observed *in vitro*. Rats completely excreted orally administered 3,4-dichloroaniline predominantly in the urine and to a lesser content in faeces. Thus, there seems to be no concern for bioaccumulation.

4.1.2.2 Acute toxicity

Studies in animals

Like other chloroanilines, the primary toxic effect of 3,4-dichloroaniline is methaemoglobin formation. Acute intoxication is indicated by the symptoms of methaemoglobinemia (cyanosis), fatigue, dyspnoea, and muscle weakness.

3,4-dichloroaniline has demonstrated moderate acute toxicity after oral application to rats: For male rats oral LD_{50} values of 570 mg/kg (Bayer AG, 1981a) and of 880 mg/kg (Marty and Wepierre, 1979) are documented, for female rats an oral LD_{50} of 530 mg/kg was found (Marty and Wepierre, 1979). Diarrhoea, cyanosis, narcosis, reduced reflexes and paralysis of the extremities were observed, but no visible lesions were seen at necropsy.

An oral LD_{50} of 570 mg/kg was calculated for male rats in a study with "pure" 3,4-dichloroaniline performed similar to OECD guideline 401 (10 male rats/dose with vehicle lutrol, no data on number of doses). Result: 300 mg/kg caused no mortality and no clinical signs. Clinical signs at doses of 500-800 mg/kg included diarrhoea, paralysis of the hind extremities, ventral recumbency, cyanosis, narcosis, reduced reflexes, red staining around eyes and nose. These signs appeared 15 minutes after application of the substance and remained till the end of the study (after 14 days). Deaths were observed at days 2-3. No visible lesions at gross necropsy were documented (Bayer AG, unpublished report, 1981a).

Oral LD_{50} values of 880 mg/kg for male rats and 530 mg/kg for female rats were detected in a study with 3,4-dichloroaniline (Marty and Wepierre, 1979). Data on the method used, on the purity of the test substance or on toxicological observations are not documented. In a similar test with mice LD_{50} values of 510 mg/kg for male mice and of 470 mg/kg for female mice were calculated (no further data given, Marty and Wepierre, 1979).

In a test with > 99% pure „technical" 3,4-dichloroaniline doses of 50 mg/kg and 500 mg/kg were given to 6 male rats each dose (vehicle 0.5% methylcellulose in aqueous solution). No mortality occurred. Clinical signs documented included lethargy, ataxia, cool to touch, pale appearance of skin, red staining around eyes, loss of righting and corneal reflexes, and shallow respiration. No visible lesions were seen at gross necropsy (Rohm and Haas, unpublished report, 1978).

Concerning acute inhalation toxicity, 3,4-dichloroaniline has proven moderately toxic for rats.

Following aerosol exposure the LC_{50} value was found to be greater than the highest tested concentration of 0.631 mg/l/4 hours. The aerosol was generated by solving the test substance in a vehicle (ethyl alcohol/polyethylene glycol 1:1) and by spraying this solution into the exposure chamber (no mortalities and only unspecific symptoms like reduced motility and bradypnoea, no visible changes at gross necropsy; Bayer AG, 1990a). Following exposure to gas/aerosol atmosphere (MMAD 1.89 µm, GSD 1.52, Mass Fraction < 5 µm 99%) mortality

was found to be 4/10 to 10/10 in a concentration range from 2.8 to 4.7 mg/l/4 hours, corresponding to a LC_{50} value of 3.3 mg/l/4 hours (concentrations of 0.047 to 2.2 mg/l did not cause any mortality). The test atmosphere was generated by pumping molten 3,4-dichloroaniline into a tube enclosed in a furnace heated to 140-205°C. The evaporated test material was blown into the exposure chamber by nitrogen. Clinical observations: During or immediately following exposure, rats in most groups had red nasal and ocular discharges, and had test material crystallised on their whiskers. Rats exposed to 0.84, 2.2 and 2.8 (50% particulate) mg/l were lethargic and/or limp and were staggering. At the 4 highest exposure concentrations, some rats died during exposure. All deaths occurred either during exposure or 1-2 days post exposure. Methaemoglobin analysis: Rats survived methaemoglobin values of approximately 28% while deaths occurred at methaemoglobin values of 47-62%. After 24 hours recovery, methaemoglobin values were still highly elevated. Average percent methaemoglobin had returned to baseline by approximately 9 days post exposure (Du Pont de Nemours, 1984).

In another test with evaporated molten 3,4-DCA (purity 98.8%) approximately 30 g of 3,4-dichloroaniline was weighted into a flask and heated to 160^{o}C. Nitrogen as carrier gas was bubbled directly into the molten material at a controlled rate, houseline air was used to dilute the gas, atmosphere was held at a chamber concentration of approximately 20% oxygen. Groups of 6 rats each were exposed for 4 hours to 32, 39, 50, 65, 70, 101, and 253 mg/m^3 3,4-dichloroaniline in air.

2/6 animals died at an exposure concentration of 0.065 mg l/4 hours. At the higher test concentrations of 0.070, 0.101 or 0.253 mg/l/4 hours all animals died within 5 days. Clinical observations: salivation, rapid and shallow breathing, ataxia, lens opacity with red discharge from the eyes, prostration, cyanosis, unresponsive to sound. During all exposures the test material sublimed on the defection shield and the walls of the exposure chamber. Under the conditions of this experiment the Approximate Lethal Concentration (ALC) was reported to be 0.065 mg/l/4 h (Du Pont de Nemours, 1976a). This study was later on criticised by the same lab because of study design and generation of test atmosphere (Du Pont de Nemours, 1976a res. 1983). Therefore the result seems to be very questionable and cannot be validated.

The reported data on acute dermal toxicity demonstrated species differences: In studies with rats dermal application of 1,000 mg/kg did not cause mortalities and no toxic symptoms (Rohm and Haas Company, 1978; Bayer AG, 1981b). In a study with male rabbits the animals died from dose of 300 mg/kg and upwards: Male albino rabbits weighing between 2 and 3 kg were clipped over the trunk area and fitted with plastic collars. Proper doses of 50% (wt/vol) test material in acetone were applied to the backs when wrapped with elastic and with adhesive bandage. After 24 hours, the rabbits were unwrapped, washed and caged for observations for 14 days, or until death. Doses of 130, 200, 300, 450, 670, 1,000 and 1,500 mg/kg in acetone were used (1 animal/dose). Clinical signs: At 18 hours, all rabbits demonstrated cyanosis, salivation, lacrymation, prostration; at 24 hours in animals dosed 450 mg/kg and higher cyanosis and ataxia were observed. Skin on all rabbits was slightly red and swollen 24 hours after substance application. No clinical signs at 2 days or duration of test; no sign of acne on back at 14 days. Mortalities occurred at doses of 300 mg/kg and higher. Necropsy: Doses of 300 mg/kg and above caused kidney and liver involvement, 1,500 mg/kg lung and liver congestion. (Du Pont de Nemours, 1976b). In another test on rabbits a dose range from 400 to 2,500 mg/kg was tested. The animals died at 1,000 and 2,500 mg/kg. Therefore the dermal LD_{50} was regarded to be $631 < LD_{50} < 1{,}000$ mg/kg under the conditions of this test (Younger Labs, 1974).

Studies in humans

No data are available.

Mechanism of methaemoglobin formation

Hemoglobin is an iron-containing tetrameric protein, consisting of four globin chains and four identical haem groups with iron in the ferrous state. Each haem is able to reversibly bind an oxygen molecule to its ferrous group. The iron can be oxidised from the ferrous to ferric state leading to the so-called methaemoglobin. Hemoglobin consequently loses its property of binding reversibly with oxygen.

Methaemoglobin formation *in vivo* by arylamines as an expression of acute toxicity is rarely an effect of the administered compound itself, but mostly produced by one or more metabolites. The methaemoglobin forming ability of different chloroaniline compounds *in vivo* is based on the metabolic activation by liver microsomal cytochrome P450 enzymes resulting in the formation of hydroxylated compounds as reactive intermediates (Marquardt and Schäfer, 1994).

In case of feeding of 2,4-, 2,5- and 3,4-dichloroaniline to rabbits ortho- or para-hydroxylated compounds were detected in urine. As a metabolite of 2,4-dichloroaniline, 6-hydroxy-2,4-dichloroaniline was detected by analysis and it was not possible to clearly identify the metabolite of 3,4-dichloroaniline as 2-hydroxy- or 6-hydroxy-3,4-dichloroaniline (Bray et al., 1957). In *in vitro* studies, both ring hydroxylated (2- and 6-hydroxy-3,4-dichloroaniline) and N-hydroxylated (N-hydroxy-3,4-dichloroaniline) and N-acylated (N-(3,4-dichlorophenyl) acetamide and N-(3,4-dichlorophenyl) formamide metabolites of 3,4-dichloroaniline were detected (Lenk and Sterzl, 1978, 1984; McMillan et al., 1990).

Interspecies differences have been investigated only in a limited number of studies, mainly *in vitro* studies. Although these studies show that species differences are present a distinct ratio of animal species to humans can not be established since the extent of methaemoglobin formation of different substances *in vivo* depends on the individual chemical structure as well as on further factors such as reducing capabilities in the blood (mainly NADH-dependent reductase) and intraspecies differences (Blom, 2000).

For example, although 3,4-dichloroaniline and 4-chloroaniline cause the same kind of effects there are distinct differences not only in methaemoglobin-forming capabilities, but also in initial hemoglobin oxidation rates as well as in duration of action. *In vivo*, the additional chlorine atom in 3-position of the benzene ring of 3,4-dichloroaniline decreases the methaemoglobin-forming activity of 4-chloroaniline by more than 50% in rats after i.p. injection (Lenk and Sterzl, 1982).

Various *in vitro* studies with erythrocytes yielded a rough dichotomy for the formation of methaemoglobin: Rat/mouse/rabbit/guinea pig/monkey are less sensitive to methaemoglobin formation and generally show a more effective reduction of induced methaemoglobin than do man/dog/cat (Blom, 2000). The cat is most sensitive to methaemoglobin formation, primarily because of a different type of hemoglobin.

Other information

Immunotoxicity

3,4-Dichloroaniline had toxic effects on Nk-cell activity and induced a dose-related immunomodulation on T-cell dependent and T-cell independent B-cell immune responses in mice after a single intraperitoneal injection (Barnett et al., 1992). The relative spleen weight and spleen cellularity were elevated at 150 mg/kg bw. No disturbance of T-cell function was evident.

Conclusion

Data on acute toxic effects caused by exposure of humans to 3,4-dichloroaniline are not available. In animal experiments the acute toxicity of the substance was moderate in rats (by the oral and inhalation way of exposure) and more pronounced in rabbits (when applied dermally): Oral LD_{50} values of 570-880 mg/kg for male (Bayer AG, 1981a; Marty and Wepierre, 1979) and of 530 mg/kg for female rats (Marty and Wepierre, 1979) and an inhalation LC_{50} value (substance vapours) of 3.3 mg/l/4 hours for male rats (du Pont de Nemours, 1984) were detected; the dermal LD_{50} for male rabbits was approximately 300 mg/kg (du Pont de Nemours, 1976a). Clinical signs like diarrhoea, paralysis of the hind extremities, cyanosis, narcosis and reduced reflexes mostly appeared on the day of application of the substance, these signs remained till the end of the study (after 14 days) in an acute oral test with male rats (Bayer AG, 1981a). Methaemoglobin analysis in an acute inhalation toxicity study revealed that rats survived methaemoglobin values of approximately 28% while deaths occurred at methaemoglobin values of 47-62%. After 24 hours recovery, methaemoglobin values were still highly elevated in the test animals; average percent methaemoglobin had returned to baseline by approximately 9 days post exposure (du Pont de Nemours, 1984).

Like other chloroanilines, the primary toxic effect of 3,4-dichloroaniline is methaemoglobin formation. Taking into account that humans are much more sensitive to methaemoglobin producing substances than rats (Lester, 1943) and that results of studies with cats, better suited to judge the level of toxicity for humans, are not available, 3,4-dichloroaniline 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

Studies in animals

After occlusive application, 3,4-dichloroaniline has proven slightly irrative on rabbit skin: Slight reversible erythema (grade 1, reversible within 2 days) but no edema were seen in a Draize skin test (Hoechst AG, 1986a). In one test for eye irritation moderate irritation of the conjunctivae and iris were seen which were reversible within 14 days (vascularisation of the cornea in 2/3 animals began at day 7 and were still present at day 14) (Rohm and Haas, 1978). In another eye irritation test the effect seen on conjunctivae, iris and cornea were mild (redness of the conjunctiva with mean scores for the observation times 24, 48 and 72 hours of 1.7/2/2, conjunctival oedema 0,7/0.7/1.3, irritation of the iris 1/0.7/0.3 and corneal opacity 1.3/1/0; Hoechst AG, 1986b). However, vascularisation of the cornea was observed in

1/3 animals, beginning as late as 7 days after test application and being still present after 14 days.

Vascularisation of the cornea is to be considered a serious damage to the eye since "Corneal transparency is dependent on a special arrangement of cells and collageneous fibrils in acid mucopolysaccharide environment, an absence of blood vessels, and deturgescence (the state of relative dehydration of corneal tissue). Any toxin interfering with any of these factors may result in corneal opacification" (Wallace Hayes, 1985).

No data are available regarding the respiratory irritating properties of 3,4-dichloroaniline.

Studies in humans

Cases of chloracne recorded in previous years after exposure to industrial 3,4-dichloroaniline are attributed to the hyperkeratogenous and acnegenic effects of 3,3',4,4'-tetrachloro-azobenzene and 3,3',4,4'-tetrachloroazoxybenzene, impurities formerly present in industrial 3,4-dichloroaniline. Since the introduction of 3,4-dichloroaniline containing virtually none of these impurities, there has been no more cases of chloracne in Germany. (Nach. Chem-Tech., 1976; Taylor, 1979; Taylor and Lloyd, 1982; Morse et al., 1979; Poland et al., 1976; Scarisbrick and Martin, 1981).

Conclusion

Human data on local irritation/corrosion caused by 3,4-dichloroaniline are not available. Based on the submitted animal data on Draize tests with rabbits, the substance is only slightly irritating to skin (no oedema, erythema grade 1 reversible within 2 days, (Hoechst AG, 1986a) but causes serious damage to eyes: The scores detected in a well documented Draize eye test (Hoechst AG, 1986b) after 24/48,72 hours per animal were as follows: Redness of the conjunctivae 1.7/2/2; chemosis 0.7/0.7/1.3; iris 1/0.7/0.3 and cornea 1.3/1/0. Vascularisation of the cornea starting 7 days after treatment was observed but data on reversibility of that effect were not documented. Similar corneal vascularisation was detected in 2/3 rabbits within a further study where this effect was still present at day 14 (Rohm and Haas, 1978). Vascularisation of the cornea is seen in two Draize eye tests with rabbits and in the summary of the study performed by Hoechst AG in 1986 is stated that allocation of EU risk phrase R 41 is appropriate based on the observations in this study. Thus, based on these data, 3,4-dichloroaniline is classified as "Xi, Irritating" and labelled with "R 41, Risk of serious damage to eyes".

4.1.2.4 Corrosivity

Studies in animals

3,4-dichloroaniline is not corrosive to skin or eyes of rabbits (see information within Section 4.1.2.3).

Studies in humans

No data available.

4.1.2.5 Sensitisation

Studies in animals

The maximisation test on the guinea pig revealed a skin sensitising potential of 3,4-dichloroaniline. In a Magnusson Kligman test up to 75% of the guinea pigs exhibited a positive reaction (Bayer AG, 1990b). Twenty test and 10 control animals were used. The test animals received an intradermal injection of 2.5% and a topical application of 50% substance. A first challenge was conducted with 50% and a second challenge with 5% and 25% substance (vehicle: propylenglykol). Though control animals exhibited dermal reactions, the frequency and intensity of the dermal reactions observed in test animals demonstrated a sensitisation potential: up to 75% (15/20) of the test animals showed a positive response (Bayer AG, 1990b).

Studies in humans

No data available on skin or inhalation sensitisation.

Conclusion

Human data on sensitisation by inhalation or skin contact are not available. There is one animal test available. In a Magnusson Kligman test up to 75% of the test animals demonstrated a positive reaction (Bayer AG, 1990). Based on this result 3,4-dichloroaniline is labelled with “R 43, May cause sensitisation by skin contact”.

4.1.2.6 Repeated dose toxicity

Studies in animals

Only few data of limited reliability are reported on 3,4-dichloroaniline. There are no studies on repeated dose toxicity for all exposure routes that have been performed according to the minimum requirements of the EU existing chemical program. Additional data on structurally similar chloroaniline compounds were accepted by the Rapporteur to add to the required data on subacute toxicity according to the regulation 793/93. These aromatic amino compounds which have similar physico-chemical characteristics demonstrate similar toxicokinetic behaviour as compared with 3,4-DCA (see Section 4.1.2.1). Although some studies with these compounds were not in compliance to actual standard methods and guidelines, results demonstrated identical main toxic effects – haemolytic anemia and methaemoglobinemia with compensatory responses in the erythropoetic system of bone marrow, spleen, liver and/or kidney. Of course, considerations of the respective toxicological data have taken into account that the levels of the effects are species-, chemical- and dose-specific.

3,4-dichloroaniline

Inhalation

Test atmospheres containing both vapour and solid particles of 0, 10, 45 or 200 mg/m^3 of 3,4-dichloroaniline (99.35% purity) were exposed for 2 weeks (nose-only, 6 hours/day, 5 days/week) to male Crl:CD BR rats (Kinney, 1986). From a total of 20 rats per group 10 rats were used to determine toxic effects, 5 of which were killed after 10th exposure and

5 rats per group were allowed to recover for 14 days post-exposure. Testing parameters included urinalysis after the 9th exposure, haematology and clinical chemistry examinations on day 10 of treatment and on day 14 of recovery, weighing of 6 organs and histopathological examinations of 28 organs/tissues. The remaining 10 rats per groups were used to monitor methaemoglobin level alternately on each second day for half of each group. For these rats no other data are reported due to the potentially confounding effects of repeated bleeding. Time points of methaemoglobin analyses immediately after blood sampling are of critical value (Beutler et al., 1995), but were not reported in this study.

At the end of treatment, 3,4-dichloroaniline caused dose-dependent methaemoglobinemia in all exposed rats, dose-related increased incidences of minimal accumulation of hemosiderin in the spleen of mid and high dose rats (5/5 rats at high dose, 2/4 rats at mid dose), anemia, significantly elevated spleen weights (absolute and spleen/body weight) and mild extramedullary haematopoiesis in high dose rats. Anemia was characterised by significantly depressed erythrocyte counts (-17% at 200 mg/m³), reduced concentrations of haemoglobin and hematocrit, elevated platelet count and elevated mean corpuscular volume (MCV), MCH, and MCHC after the 10th exposure. Mean methaemoglobin levels were 12.2% (minimum-maximum 10.0-18.4%, except values on treatment-free weekend) in the high and 1.6% (minimum - maximum 1.0-2.1%) in the mid dose group. In the low dose group a significantly raised methaemoglobin level of 0.8% (minimum - maximum 0.7-1.1%) was found. Although these values are within the range of normal values, they are corresponding to an approximately 2.5-fold increase above control (mean methaemoglobin value 0.3%, minimum - maximum. 0.2-0.5%). No other treatment-related changes were found in the 10 mg/m^3 group at the end of the treatment period.

No significant clinical signs or body weight changes were observed in rats exposed to 10 or 45 mg/m³ of 3,4-dichloroaniline. Rats exposed to 200 mg/m³ had significantly depressed body weights compared to controls after the 1st and 6th exposures; body weights for these rats were generally lower than controls throughout the exposure period. Various non-specific clinical signs (low incidences of dry red nasal and ocular discharges, brown discoloured fur, stained perineum, hair loss) were observed during the exposure period (mainly in the 1st treatment week).

During the recovery period, the body weight of rats exposed to 200 mg/m³ remained (non-significantly) lower than that of other groups. Hemosiderosis was still found in mid and high dose animals after 14 days of recovery being more pronounced and more frequent than after the 10th exposure. All rats were affected; the severity was mild in high dose rats and minimal in mid dose animals. Some blood cell parameters (depressed erythrocyte counts (-9.5%), increased MCV) were still changed at 200 mg/m^3. The number of erythrocytes were significantly lower in the low and mid dose group (-7.2% and –7.4%) than those of the control groups after recovery. Methaemoglobin level of the low dose rats returned to the range of control animals three days after the end of exposure, while methaemoglobin levels in mid and high dose groups remained significantly elevated through the recovery period. No adverse effect was seen in the lungs, trachea and nose examined histopathologically.

In this study, the NOAEC for systemic effects was not derived; the NOAEC for local effects on the respiratory tract was 200 mg/m^3. With respect to systemic toxic effects at the end of treatment, the concentration of 3,4-dichloroaniline effective to induce hemosiderosis and methaemoglobin concentrations above the normal range was 45 mg/m^3. However this concentration is not considered to be the LOAEC, because the erythrocyte counts in the low and mid dose decreased during recovery. Although their values represented only mild anemia, they were significantly below those of control groups after recovery time. The significant

increase of methaemoglobin levels at the low dose group in comparison to the control values, the return of increased methaemoglobin levels after the third day of recovery and the delayed effect on erythrocytes during recovery support that 10 mg/m³ represents the LOAEC.

- In a study of low reliability, repeated inhalation of 0.015, 0.03, and 0.08 mg/m^3 of 3,4-dichloroaniline of rats (15-18 rats/group, no data on sex and strain) up to 100 days revealed reversible neurofunctional disorders of the motor chronaxy of antogonistic muscles from the 40th day of treatment (≥ 0.03 mg/m³) and reduced hemoglobin (at 0.08 mg/m³, maximum decrease –20% on day 55 of exposure) and increased sulfhaemoglobin concentrations (at 0.08mg/m³ (Andreeshcheva, 1970, no more data).

Dermal

- In a skin absorption subacute test on male rabbits (10 animals/group), daily application on the dorsal skin of 60 mg/kg bw 3,4-dichloroaniline (99.9%) (10% solution in acetone, 1.6 ml) on 10 consecutive days for 6 hours a day resulted in anemia and increased methaemoglobin levels (Du Pont de Nemours, 1976c). Animals from a vehicle control group received acetone only (100%, 5ml). Five animals of each group were necropsied on day one post-treatment and the remaining five rabbits were killed on thirteen days post-treatment. Blood was obtained from each animal prior to the start of test, on day five and day ten of applications. Parameters measured were counts of WBC, erythrocytes, hemoglobin, hematocrit, methaemoglobin, and activities of ALAT. Clinical signs and gross pathology data were reported; histopathology was not performed.

Marked effects on the spleen were reported for rabbits treated with 3,4-dichloroaniline on day 1 after treatment. A dark brown spleen was observed in all five rabbits of the 3,4-dichloroaniline group; two rabbits showed enlarged swollen and heavy spleens. All rabbits treated developed moderate skin effects consisting of thickening, crust formation and necrosis. Comparable skin effects were seen in the acetone group at the end of treatment and after recovery. No other effects than those on the skin were observed in the acetone group. After the 5th and 10th treatment with 3,4-dichloroaniline average erythrocyte counts, haemoglobin, and hematocrit were lower than the pre-treatment values and lower than the controls. An increase in methaemoglobin was observed at day 5 and day 10 of treatment.

Table 4.8 3,4-dichloroaniline effects after repeated dermal exposure to male rabbits

Parameter	Test compound	Day 0	Day 5	Day 10
Erythrocytes*	Acetone	5.95**	5.84	5.74
	3,4-DCA	5.47	4.46	3.43
Hematocrit (%)	Acetone	39	41	39
	3,4- DCA	38	33	35
Haemoglobin (%)	Acetone	12.4	12.2	12.0
	3,4- DCA	11.6	9.6	9.2
Methaemoglobin	Acetone	0.3	0	1.0
(%)	3,4- DCA	0.9	9.5	5.9

* x10^6/mm³,
** mean values of 10 animals, 3,4-DCA 3,4-dichloroaniline

All rabbits from the recovery group exposed to 3,4-dichloroaniline showed thickened skin on day 13 of recovery dry skin and crust formation was seen in some animals. These effects were comparable to those reported for the recovery group of acetone treated animals. Enlarged and

heavy spleens were still present in the 3,4-dichloroaniline recovery group, but were not seen in the vehicle (acetone-) group.

4.1.2.6.1 Data from structurally related substances

2,5-dichloroaniline

Oral administration of 30, 150 and 750 mg 2,5-dichloroaniline per kg bw on 28 days to Wistar rats resulted in haemolytic anemia with increased medullary and extramedullary erythropoietic activity and hemosiderin deposits in the spleen at dose levels of 150 mg/kg bw/day and higher (Hoechst AG, 1989). There were no premature deaths. High dose animals showed non-specific clinical symptoms (reduced spontaneous activity, hunched posture, retracted flanks), respiratory distress, uncoordinated movements, trembling and salivation. At this dose level, water consumption was increased in both sexes and growth was retarded in males. A brown-yellow discoloration of urine was seen in high dose males and females. Haematological examinations revealed decreased red cell counts, decreased haemoglobin levels and increased reticulocyte counts in the 750 mg/kg dose group. A dose-related increase of the mean total bilirubin level was registered in the mean total bilirubin level was registered in females of mid and high dose groups. The relative weight of the spleen increased in high dose animals of each sex. At mid and high dose level erythropoiesis was enhanced in the bone marrow, and hemosiderosis was found in the spleen. Additionally high dose animals presented an extramedullary erythropoietic activity in the spleen. The NOAEL of this study was 30 mg/kg bw/day.

Methaemoglobin formation was not seen in any group. However all methaemoglobin levels of this study were estimated to be 0 g/l in all dosed groups and in control groups. Because normal values of methaemoglobin concentrations up to 1-2% are expected in untreated animals, false negative results due to methodical problems are suspected. Therefore this parameter was not considered for effect assessment.

2-chloroaniline

In a 4-week inhalation study which is conform to test protocol of OECD 412, 10 male and 10 female Wistar rats were exposed on 5 days/week, 6 hours/day to 2-chloroaniline concentrations of 39, 217, and 886 mg/m³ air (corresponding to 7, 41, and 169 ppm, analytical concentrations) in a head-only exposure chamber (Bayer AG, 1992b). Clinical symptoms were cyanosis and tremor in mid and high dose females and cyanosis in high dose males. Two high dose females showed prostration, staggering gait and reduced startle and myostactic reflexes. Mean body weight gain was lower in high dose males compared to the controls. In all o-chloroaniline vapour exposed female groups and in males at the mid and high concentrations dose-related reduced values RBC, haemoglobin, hematocrit and higher numbers of Heinz' bodies, and reticulocytes were observed. At the high dose concentration, mean cell volume of RBC was increased and mean corpuscular haemoglobin concentration was lower than in control groups. Methaemoglobin production was increased in high dose male and female groups, minimal but non-significant increase was evident in mid dose males and in mid and high dose females (no data on time point of measuring). Mean Plasma concentration of total bilirubin was higher in mid and high dose groups of each sex. O-chloroaniline exposed females also had higher levels of magnesium, lower triglyceride values, and lower serum cholinesterases activity (high dose) and dose-dependently reduced cholesterol values (all dose groups). Monooxygenase activity of O-demethylase and

N-demethylase were higher in high dose females in comparison to control animals. Urinalysis in this group showed higher excretion of protein and bilirubin and increased pH-values. After the end of treatment, animals exposed at mid and high concentrations showed black-red discoloration of the spleen. A dose-related increase of relative and absolute spleen weights was evident in all dosed males and mid and high dose females, relative liver weights were higher in high dose males and females than in control groups. Corresponding to macroscopic spleen changes, an increased mean severity of hemosiderosis was seen in mid and high dose males and all female dose groups. Mid and high dose groups of each sex showed higher frequencies of splenic congestion. A higher cell number of late erythropoietic maturity stages (normal blasts, macro blasts) was seen in bone marrow smears at all dose levels in each sex, the number of lymphocytes was reduced in high dose males and females. A NOAEC for systemic toxicity was not derived in this study; the LOAEC was 39 mg/m³. The NOAEC regarding to the local effects on the respiratory tract was 886 mg/m³.

In a 13-week subchronic gavage study on F344 rats and B6C3F1 mice (10 animals/sex/group), 2-chloroaniline was administered at dose levels 0, 10, 20, 40, 80, and 160 mg/kg bw/day (Eastin, 1992). Treatment was associated at $\geq$ 40 mg/kg in rats and at 160 mg/kg in mice with a transient cyanosis (rats only) and tremors. Methaemoglobinemia was evident in a dose-related manner in all (rat) groups at study termination (no data whether methaemoglobin formation was examined in mice). Anemia and Heinz bodies were detected in high dose animals (rat and/or mice?). Spleens of high dose rats and mice $\geq$ 80 mg/kg were dark-red and enlarged. The 40 mg/kg (males only), 80, and 160 mg/kg groups of both species showed higher spleen weights than control animals. Microscopic examination revealed increased haematopoiesis in the spleen ($\geq$ 80 mg/kg), erythroid cell hyperplasia in the bone marrow (rats $\geq$ 80 mg/kg), and hemosiderosis of the renal cortex (high dose rats). From this study, a 2-chloroaniline NOAEL was not derived, the LOAEL for rats was 10 mg/kg bw/day. Effect levels for mice could not be derived on the actually presence of the abstract only.

3-chloroaniline

A 13-week subchronic gavage study (abstract only, Eastin, 1993) was also conducted with 3-chloroaniline with similar study design and test dosages as reported in the 2-chloroaniline study by Eastin (1992). Dosing in rats $\geq$ 80 mg/kg and in high dose mice was associated with a transient cyanosis (rats only) and tremors and ataxia (mice only). A dose-related increase in methaemoglobin was evident after the third dose administration in additional (clinical pathology) rats. A significant increase in Heinz bodies was detected in animals at $\geq$ 40 mg/kg in rats and $\geq$ 80 mg/kg in mice. Those dose groups also showed haematological changes indicative of anemia and enlarged spleens. All rat dose groups and mice $\geq$ 40 mg/kg showed increased spleen weights. Microscopic examination revealed increased haematopoiesis in spleen (male mice $\geq$ 20 mg/kg, and in female mice and both sexes of rats $\geq$ 10 mg/kg), erythroid cell hyperplasias of the bone marrow (rats $\geq$ 40 mg/kg), pigmentation (hemosiderin) of the renal cortex (rats $\geq$ 20 mg/kg) and pigmentation of the liver (rats $\geq$ 40 mg/kg). A NOAEL was not derived; the LOAEL for rats and mice is 10 mg/kg bw/day.

4-chloroaniline hydrochloride

In subchronic studies (NTP, 1989; Chabra et al., 1986) on 4-chloroaniline hydrochloride 0, 5, 10, 20, 40, and 80 mg/kg bw/day of the test substance was orally administered to F-344 rats and B6C3F1 mice received 0, 7.5, 15, 30, 60, and 120 mg/kg bw/day (5 days/week, 13 weeks) (doses calculated as 4-chloroaniline). One high dose female rat died before the end of the studies of unknown causes, premature deaths of mice were not related to the test substance. A

dose-related enlargement of the spleen was observed in rats and mice. High dose male rats only gained lower body weight. Cyanosis was evident at rats of high dose levels. Dose-related increases in the severity of the methaemoglobinemia and anemia were observed in both species of all dose groups. In both species, hemosiderosis in the spleen, kidneys, and liver was observed histologically in rats of all dose groups and in mice treated with 30 mg/kg and higher. Bone marrow hyperplasia (rats) and increased haematopoiesis (rats and mice) in liver and spleen occurring at all dose groups was interpreted to reflect the response to the haemolytic anemia and possible reduction in oxygen carrying capacity attributable to the methaemoglobinemia. Histological examination was performed on all vehicle control animals and high dose animals.

In a sixteen-day dose-finding study with 0, 25, 50, 100, 200 and 400 mg/kg bw/day 4-chloroaniline hydrochloride rats and mice that received 200 or 400 mg/kg died within six days. In mice deaths occurred in all dosed groups. Rats that received 100 mg/kg had lower mean body weights at the end of the study. Compound-related clinical signs included blue extremities and eyes, indicative for cyanosis, at 200 and 400 mg/kg lethargy, and at 25 or 50 mg/kg labored breathing. Splenic enlargement was observed in rats at 25, 50, and 100 mg/kg. Sinusoidal congestion of the spleen and hemosiderin deposition in the renal cortical tubular epithelial cells was observed in rats that received 100 mg/kg. In mice treated with 100 mg/kg livers showed Kupffer cell hemosiderosis and congestion of the spleen. In both 16-day studies histological examinations were done on two males and two females from the control group and the 100 mg/kg dose group.

Based on these results, groups of 50 rats of each sex were administered 2, 6, or 18 mg/kg 4-chloroaniline in aqueous hydrochloride acid by gavage, 5 day per week for 103 weeks. Groups of 50 mice of each sex were administered 3, 10 or 30 mg/kg on the same schedule. Body weight gain in high dose rats and mice were comparable to the vehicle controls. The survival rates of the low and mid dose groups of the male rats and the low and high dose groups of female rats was greater than that of the controls and was attributed to a lower incidence of mononuclear cell leukaemia. In mice survival rates were decreased in the mid dose males.

Haematologic and methaemoglobin measurements were made on blood samples collected from 15 randomly selected male and female rats per dose group at 6, 12, 18, and 24 months. In general, high dose group at various intervals showed mild haemolytic anemia and dose-related increases in methaemoglobin. Altered red blood parameters indicative for a regenerative anemia and methaemoglobinemia was found transiently in all male and female dose groups and in the mid and high dose groups at the end of the study.

In rats, compound-related non-neoplastic lesions were seen in the bone marrow, spleen and liver. This lesions included bone marrow hyperplasia (males of all dose groups, mid and high dose females), hepatic hemosiderosis (high dose males), and splenic fibrosis (males of all dose groups, high dose females) and lipocytic infiltration in the spleen (high dose males and females).

Treatment-related non-neoplastic changes in mice were hemosiderin pigmentation of the Kupffer cells in the liver (high dose males and females), proliferation of hemopoietic cells in the liver (females of all dose groups), multifocal renal tubular hemosiderin pigmentation (high dose females). For toxic (non-neoplastic) effects, no NOAEL could be derived; the LOAEL is 2 mg/kg bw/day for rats and 7.5 mg/kg bw/day for mice.

(Parameters of clinical chemistry were not investigated in the NTP-studies; tumour response was evident in the high dose groups in rats and all dose groups in mice, detailed tumour data were not reported here, see NTP, 1989.)

Although 4-chloroaniline and 3,4-dichloroaniline causes the same kind of effects there are distinct differences not only in methaemoglobin-forming capabilities, but also in initial haemoglobin oxidation rates as well as in duration of action. *In vivo*, the additional Cl-atom in 3-position of the benzene ring of 3,4-dichloroaniline decreases the methaemoglobin-forming activity of 4-chloroaniline by more than 50% in female Sprague Dawley rats after i.p. injection (Lenk and Sterzl, 1982).

Summarising the data on structurally related substances (2,5-dichloroaniline, 2-chloroaniline, 3-chloroaniline, and 4-chloroaniline (hydrochloride)) similar main effects as by 3,4-dichloroaniline were induced consisting in dose-dependent haemolytic anemia and methaemoglobinemia with compensatory responses in the erythropoietic system of bone marrow, spleen, liver, and kidneys. Although some studies on the analogues were not or not fully in compliance with actual method and documentation standards, hepatotoxic effects were consistently evident at all exposure routes.

In vitro studies

In an *in vitro* study (Valentovic et al., 1995) on rat liver and kidney slices 3,4-DCA, 2,5-DCA and other DCA isomers increased the lactate dehydrogenase leakage and diminished the pyruvate directed gluconeogenesis. The kidney slices were more sensitive than the liver slices.

Studies in humans

Following reports of limited reliability (no quantitative data on the exposure level and duration, multiple chemicals exposure, no detailed data on the methods and investigated population) represent the few information after repeated human exposure to 3,4-dichloroaniline:

A high hospitalisation rate of workers surveyed in a pesticide plant were found to be caused by intoxication of carbonate pesticide methomyl and 3,4-dichloroaniline (methaemoglobinemia, cyanosis, eye and skin irritation) (Morse et al., 1979). Workers exposed to 3,4-dichloroaniline and 3,4-dichloropropionanilide had chloracne which was discussed to be caused by the contaminant 3,3',4,4'-tetrachloroazobenzene. Effect on the liver enzymes or lipids of workers with or without chloracne could not be associated to the 3,4-dichloroaniline exposure by Scarisbrick and Martin (1981).

No effect on methaemoglobin and haemoglobin levels were indicated in a surveillance of 151 workers exposed to 3,4-dichloroaniline (Rohm and Haas, 1982). In a British report only few cases of cyanotic symptoms were attributed to dichloroaniline exposure in 1961-1980 (Sekimpi and Jones, 1986).

Summary

Repeated dose studies on rats and rabbits (Du Pont de Nemours, 1976c, Kinney, 1986) indicated that 3,4-dichloroaniline has erythrotoxic properties, produces increased concentrations of methaemoglobin, enhances erythropoiesis, and as a sequence of erythrotoxicity induces persistent hemosiderosis. These effects were seen at concentrations of

10 mg/m³ after inhalation exposure on 10 days and 60 mg/kg bw after daily application to the dorsal skin on 10 days. From another inhalation study there is some concern that 3,4-dichloroaniline may also affect the neurofunction (Andreeshcheva, 1970). Altered enzyme activities on liver and kidney cells may give some indication on possible cytotoxic effects *in vitro* (Valentovic et al., 1995), but actually no support was obtained from *in vivo* studies. Studies from structurally related substances as reported above reveal consistent effects to that of 3,4-dichloroaniline.

The reports from human exposure gave indications on methaemoglobinemia, cyanosis, eye and skin irritation after repeated exposure to 3,4-dichloroaniline; however data could not clearly be attributed to this substance mainly because of mixed chemical exposure.

4.1.2.7 Mutagenicity

3,4-dichloroaniline

Bacterial in vitro assay

Bacterial mutation tests with S. typhimurium strains TA97, TA98, TA100, TA1535, and TA1537 were negative with and without S9-mix for doses up to 2,000 µg/plate (Bayer AG, 1984; BG Chemie, 1985a; McMillan et al., 1988; Zeiger et al., 1992). The Ames test was also negative in the presence of norharman (Suzuki et al., 1986). A gene mutation test with Aspergillus nidulans (only without S-9 mix) was positive from 5 µg/ml upwards. The effect was dose-dependent and the survival of spores was 32% at the highest tested dose of 200 ug/ml. Only one experiment was carried out (Prasad, 1970).

In vitro assays with mammalian cells

The result of a mammalian cell gene mutation test with CHO cells (HPRT test) was negative for doses up to 250 µg/ml with and without S-9 mix. There was a weak toxic effect at the highest dose without S-9 mix (McMillan et al., 1988).

A chromosomal aberration test with human lymphocytes was negative for concentrations up to 1 mmol/l with and without S-9 mix. There were no toxic effects up to the highest dose tested (Bauchinger et al., 1989).

A test for sister chromatid exchanges (SCE test) with human lymphocytes was weakly positive with S-9 mix from 0.125 mmol/l upwards. There was a 3-fold increase at 0.5 mmol/l and 1.0 mmol/l. The effect was dose-dependent. Without S-9 mix there was a negative result for doses up to 1.0 mmol/l. No toxic effects were found (Bauchinger et al., 1989).

Tests for unscheduled DNA synthesis (UDS test) with rat liver cells were negative for doses up to 10 µg/ml. No toxic effects were found up to the highest tested dose (McMillan et al., 1988; Yoshimi et al., 1988). Another UDS test (Andrae, 1986) had an equivocal result because of inconsistent findings for doses ranging from 0.16 to 47.7 µg/ml (weak “effects”, no dose-dependency).

A mitotic spindle damage test with V79 cells was positive after 3-hour exposure to 0.25 and 0.5 mmol/l (Salassidis and Bauchinger, 1990); the main effect was an induction of monopolar metaphases. Information on toxic effects at these concentrations was not given, however, higher concentrations led to drastic toxic effects.

In vivo assays with mammals

An *in vivo* micronucleus test was negative in bone marrow cells of mice after oral gavage of 980 mg/kg bw. There were lethal effects and a reduction of the PCE/NCE ratio (BG Chemie, 1985b).

Another *in vivo* micronucleus test was also negative in bone marrow cells of mice after intraperitoneal injection of doses up to 200 mg/kg bw two times 24 hours apart. Clinical symptoms and lethal effects were induced; furthermore, the PCE/NCE ratio was reduced (Du Pont de Nemours, 1989).

Data from structurally related compounds:

For the structurally related compound 4-chloroaniline positive *in vitro* data related to several endpoints are described: gene mutations in bacterial systems (Zeiger E., 1990) and in mammalian cells (Mitchell et al., 1988; Myhr and Caspary, 1988; Wangenheim and Bolcsfoldi, 1988), chromosomal aberrations (Anderson et al., 1990), sister chromatid exchanges (Anderson et al., 1990) and unscheduled DNA synthesis in primary rat liver cells (Williams et al., 1982). *In vivo* there is described a positive result in a somatic mutation test in Drosophila melanogaster (Graf et al., 1990).

For m-chloroaniline and o-chloroaniline *in vitro* data to several endpoints are described: negative bacterial mutation tests (Thompson et al., 1983; Zeiger et al., 1987), positive gene mutation tests in fungi (Prasad, 1970), negative chromosomal aberration tests only without S-9 mix (Ishidate M., 1988) and negative UDS tests (Thompson et al., 1983). Data for *in vivo* assays are not available for these compounds.

Concerning structurally related compounds of dichloroaniline only p-chloroaniline shows a mutagenic potential for mammalian cells. However, from *in vivo* tests only a positive somatic cell mutation test in Drosophila melanogaster is available.

Conclusion

Although *in vitro* genotoxicity tests were negative for gene and chromosome mutations, there is limited evidence for a mutagenic potential mainly due to a weakly positive SCE test *in vitro* and a positive test for induction of spindle damage *in vitro*. The clearly negative *in vivo* micronucleus tests indicate that this potential is unlikely to be expressed *in vivo*.

4.1.2.8 Carcinogenicity

No experimental animal data are available on 3,4-dichloroaniline.

4.1.2.8.1 Results from other studies on chemical analogues

4-chloroaniline (hydrochloride)

4-chloroaniline and 4-chloroaniline hydrochloride have carcinogenic properties in rats and mice (see NTP, 1989, and NTP, 1979) leading mainly to sarcomas of the spleen and/or hemangiomas/hemangiosarcomas in spleen and liver. Actually the classification and labelling has been revised from the EU Commission Group on the classification and labelling of

dangerous substances on July, 10-12, 1996 in Ispra (Italy) to "Carcinogen, Cat. 2, Toxic, T, R 45" (document ECB l/12/96).

According to the IARC classification system (IARC, 1993) 4-chloroaniline was classified as possibly carcinogenic in humans (group 2B).

No carcinogenicity data are available on other chloroanilines (2,5-dichloroaniline, 2- and 3-chloroaniline).

Studies in humans

No data available on 3,4-dichloroanilines or structurally similar chloroanilines.

Conclusion

Related to carcinogenicity, there are no data from long term studies on 3,4-dichloroaniline available. *In vivo* genotoxicity data did not give concern on carcinogenic properties of 3,4-dichloroaniline itself.

Whereas no carcinogenicity data are available on 2,5-chloroaniline, 2-chloroaniline and 3-chloroaniline, 4-chloroaniline is carcinogenic in rats and mice. Structural similarity of 4-chloroaniline may give some concern that 3,4-dichloroaniline may have carcinogenic properties, too. However, the available metabolic data give no evidence for an *in vivo* dehalogenation of 3,4-DCA to 4-chloroaniline thus this suspicious fact is considered to be negligible. In the absence of further supporting data, it is concluded that database is not sufficient for classification of 3,4-dichloroaniline as a category 3 carcinogen.

4.1.2.9 Toxicity for reproduction

Studies in animals

Fertility impairment

Hazard identification with respect to fertility is not possible since data from investigations on reproductive capacity and/or reproductive function from animal studies are not available for 3,4-dichloroaniline. Likewise, there are no data available from adequate 90-day repeated dose toxicity studies sufficient to supplement the developmental toxicity study in the sense of screening for reproductive toxicity.

Limited information with respect to male reproductive organs may be obtained from an inhalation study (see Section 4.1.2.6) with 3,4-dichloroaniline in rats (10 exposures during 2 weeks) where no changes in absolute and relative testes weights or histopathological appearance of testes and epididymides were reported (Kinney, 1986).

Developmental toxicity

3,4-dichloroaniline was evaluated for maternal and developmental toxicity in a teratology study in pregnant (Charles River Crl:CD BR) rats (Clemens and Hartnagel, 1990). The substance was administered as a suspension at doses corresponding to 5, 25, or 125 mg/kg bw/day by gavage at a volume of 10 ml/kg bw during the period of major organogenesis (gestational days 6-15). Controls received comparable amounts of the aqueous carboxymethylcellulose/Tween 80 vehicle. Each group was comprised of 28 inseminated

dams. They were monitored for food consumption, body weights and clinical signs of toxicity during the investigation. Signs of cyanosis were not observed. At sacrifice on gestational day 20 the parameters evaluated included: number of dams with live progeny, corpora lutea, implantations, resorptions, litter size, placental weights, and pre- and post-implantation loss. Live fetuses (348 - 425 per group) were examined for weight, sex, and gross external, visceral, and skeletal dysmorphogenic changes.

With the experimental conditions of this study signs of maternal toxicity were observed at the 25 and 125 mg/kg dose levels substantiated by significantly reduced food consumption and significantly reduced average body weight gain. Borderline developmental toxicity occurred at the clearly maternally toxic high dose level (125 mg/kg bw/day) only, in form of a slight but not statistically significant increase in resorptions and consequently post-implantation loss. Also a significant delay in ossification of a few skeletal elements was observed. No significant or toxicologically relevant effects were produced on any of the maternal reproductive or fetal parameters studied with doses up to 25 mg/kg bw/day. A specific teratogenic potential was not identified.

Conclusion

For 3,4-dichloroaniline no significant adverse effects on embryonic/fetal development were revealed from an OECD Guideline according teratology study in rats. From this study the following values were derived:

NOAEL (embryo-/fetotoxicity)	25 mg/kg bw/day
NOAEL (maternal toxicity)	5 mg/kg bw/day

Other information

Data from short term repeated dose toxicity studies on structurally related compounds (see Section 4.1.2.6) such as 2-chloroaniline (28-day, inhalatory; Bayer AG, 1992b) and 2,5-dichloroaniline (28-day, oral; Hoechst AG, 1989) did not reveal apparent changes in male reproductive organs. Furthermore, in a study with an *in vivo* model proposed as a “rapid-test” to identify carcinogenic activity, the i.p. administration of 2,4-dichloroaniline, 2- and 3-chloroaniline during five days at doses of 25, 50, 250, 400, or 500 mg/kg bw/day to groups of five mice each did not increase the rate of sperm head abnormalities (Topham, 1980).

3,4-dichloroaniline is one of the metabolites which are formed *in vivo* from the crop protective herbicide diuron. In a replicate 3-generation feeding study on rats with 0 or 125 ppm diuron in the diet any obvious differences between treated and untreated animals had not been revealed (Hodge et al., 1967). Under the experimental conditions of the study the main metabolite was N-(3,4-dichlorophenyl)-urea. 3,4-dichloroaniline was also detected in the urine in small amounts (< 0.2% of the dose given = 0.02 mg/kg bw/day).

In the same study Hodge et al. (1967) administered up to 1,250 mg/kg bw food (equivalent to 62.5 mg/kg bw/day diuron; assuming a 10-kg bw and a food consumption of 500 g/day) to dogs for 2 years. Following the treatment, 3,4-dichloroaniline was found in urine (1.2% of the total metabolites). Therefore it can be assumed that the animals treated with diuron were internally exposed to 3,4-dichloroaniline. Following the highest dose, adverse effects were reported in the spleen (increased haematopoiesis) and in the bone marrow (erythroid hyperplasia). However, no histopathological changes in gonads and uterus were reported. It is assumed that this dose of diuron would represent an internal exposure to 3,4-dichloroaniline of 0.75 mg /kg bw (1.2%).

Studies in humans

No data available.

Other information

It is assumed that diuron is metabolised in humans in the same manner as in the rat and dog by partial or complete demethylation and hydroxylation. The main metabolite is N-(3,4-dichlorophenyl)-urea and – in traces in the urine – 3,4-dichloroaniline. Quantification of the metabolites showed that the concentration of 3,4-DCA in humans was 900 times lower than that of the main metabolite (van Boven, 1990).

4.1.3 Risk characterisation

4.1.3.1 General aspects

3,4-dichloroaniline may be absorbed through the gastrointestinal tract, the skin and the lungs. *In vivo* hydroxylation of 3,4-dichloroaniline leads to the formation of ortho- and para-hydroxylated compounds. N-hydroxylation was additionally observed *in vitro*. Rats completely excreted orally administered 3,4-dichloroaniline predominantly in the urine and to a lesser content in faeces. Thus, there is no reason to assume concern for bioaccumulation.

The primary toxic effect of 3,4-dichloroaniline is methaemoglobin formation. Acute intoxication is indicated by methaemoglobinemia (cyanosis), fatigue, dyspnoea, and muscle weakness.

3,4-dichloroaniline has demonstrated for rats moderate acute toxicity after oral application and after inhalation (LD_{50} values of 530 – 880 mg/kg and LC_{50} of 3.3 mg/ml/4 hours). Acute dermal toxicity demonstrated great species differences: In tests with rats the dermal LD_{50} was detected to exceed 1,000 mg/kg (no mortalities and no toxic signs) whereas a test with male rabbits demonstrated a dermal LD_{50} of 300 mg/kg.

Data on acute toxicity to humans are not available.

Immunomodulating and immunotoxic effects of 3,4-dichloroaniline was observed after single intraperitoneal injection to mice. However, the limited data do not allow to draw firm conclusions regarding human health.

Cases of chloracne recorded in previous years after exposure to industrial 3,4-dichloroaniline are attributed to the hyperkeratogenous and acnegenic effects of 3,3',4,4'-tetrachloro-azobenzene and 3,3',4,4'-tetrachloroazoxybenzene, impurities formerly present in industrial 3,4-dichloroaniline. Since the introduction of 3,4-dichloroaniline containing virtually none of these impurities, there have been no more cases of chloracne in Germany. In Draize tests with rabbits, 3,4-dichloroaniline caused mild irritation but corneal vascularisation 7-14 days after instillation of the substance into rabbit eyes.

No data are available regarding the respiratory irritating properties of 3,4-dichloroaniline.

3,4-dichloroaniline has no local corrosive properties.

3,4-dichloroaniline demonstrated skin sensitising properties in guinea pigs. Skin or inhalation sensitisation of humans is not reported.

In animals, repeated exposure to 3,4-dichloroaniline or structurally related compounds resulted primarily in methaemoglobinemia and haemolytic anemia. Spleen weight increase, microscopically hemosiderosis in the spleen, liver, and/or kidneys and increased compensatory erythropoiesis were observed as main effects. From the 14-day inhalation study (6 hours/day, 5 days/week) a LOAEC of 10 mg/m^3 was derived. Scarcely documented indications on neurofunctional disorders on rats were reported after repeated inhalation of 3,4-dichloroaniline.

In humans, cyanosis, methaemoglobinemia, eye and skin irritation were observed after prolonged occupational exposure to mixed chemicals including 3,4-dichloroaniline.

Although *in vitro* genotoxicity tests were negative for gene and chromosome mutations, there is limited evidence for a mutagenic potential mainly due to a weakly positive SCE test *in vitro* and a positive test for induction of spindle damage *in vitro*. The clearly negative *in vivo* micronucleus tests indicate that this potential is unlikely to be expressed *in vivo*.

No data are available on the carcinogenic potency of 3,4-dichloroaniline. *In vivo* genotoxicity data did not give concern on carcinogenic properties of 3,4-dichloroaniline. A possible concern that 3,4-dichloroaniline may have carcinogenic properties might result from the structurally similar substance 4-chloroaniline which is carcinogenic in rats and mice. However, the available metabolic data give no evidence for an *in vivo* dehalogenation of 3,4-DCA to 4-chloroaniline thus this suspicious fact is considered to be negligible.

There are no fertility studies following treatment with 3,4-DCA. Likewise, data from adequate 90 day repeated dose toxicity studies sufficient to supplement the developmental toxicity study with rats for the purpose of screening for reproductive toxicity, are neither available. Thus, information from studies in rats and dogs with diuron from which 3,4-dichloroaniline is metabolically formed were used to supplement the fertility data. The assessment of data from the available teratology study with rats did not indicate any significant developmental toxicity. The NOAEL for developmental toxicity is 25 mg/kg bw/day. There are no human data available on toxicity for reproduction.

4.1.3.2 Workers

4.1.3.2.1 General remarks on calculations and extrapolation, summary of relevant effects

The assessment of 3,4-dichloroaniline is based on animal data, since human data are not available for most endpoints. The calculations to adjust the animal data to human exposure scenarios follow the idea of a central tendency estimate. The derivation of factors necessary for route-to-route extrapolation and extrapolation from animals to humans is described in the next two sections.

4.1.3.2.2 Route-to-route extrapolation

The LD_{50}- and LC_{50}-values can be compared to get some information about the influence of the exposure route on systemic availability. In rats an oral LD_{50}-value in the range of 530-880 mg/kg and an LC_{50}-value of 3,300 mg/m^3 (4 h) were determined. Assuming a respiratory minute volume of 0.8 l/min/kg (Snipes, 1989) the exposure to the

LC_{50}-concentration leads to an inhaled dose of 634 mg/kg (3.3 mg/l · 0.8 l/min/kg · 240 minutes), which is in the dose range of the oral LD_{50}. A similar external body burden leads to an equivalent toxicity. Dermal exposure up to 1,000 mg/kg led to no lethality and no sign of toxicity in rats. Comparing this information with the oral LD_{50} the assumption of an at least 3-fold lower systemic availability for the dermal route seems to be justified. Overall equipotent doses for oral, inhalation and dermal exposure are assumed to be related by the factors of 1:1:3.

4.1.3.2.3 Interspecies differences

As other aromatic amines 3,4-dichloroaniline is a methaemoglobin generating substance. Information about interspecies differences (rat/human) are not available, but comparative oral studies with acetanilide indicates a 10 times greater sensitivity of man concerning methaemoglobinemia. Corresponding studies with aniline suggest a 10 - 100 times greater sensitivity. The extent to which the documented differences already take into consideration inter-individual differences in sensitivity among humans (acetyliser status) is not known (Jenkins et al., 1972 and Lester, 1943).

Based on the knowledge of the metabolism of aromatic amines, the formation of methaemoglobin is considered to be a biological indicator for the potential formation of different reactive metabolites via N-hydroxylation. These reactive metabolites and not necessarily methaemoglobin itself are considered to be the starting point for toxic effects of aromatic amines. According to this argumentation species differences in methaemoglobin formation thus similarly indicate sensitivity differences concerning other endpoints of toxicity.

In the case of 3,4-dichloroaniline species extrapolation from rat to human at oral or dermal route will generally use an overall factor of 1/10 for all toxicological endpoints. At the inhalative route using air concentrations this factor reduces to ½.5 because of the approximately 4 times higher respiratory minute volume of rats compared to humans on the basis of bodyweight.

It has to be added, that the scientific bases for the introduction of these species extrapolation factors is weak. However, if data from rats and rabbits are compared significant species differences are obtained concerning 3,4-dichloroaniline toxicity, which clearly show that rats belong to a less sensitive species.

4.1.3.2.4 Summary of effects relevant for workplace risk assessment

Table 4.9 Summary of effects of 3,4-dichloroaniline relevant for workplace risk assessment

	Inhalation	**Dermal**
Acute toxicity (lethality)	Anticipated human LC_{50}: 1,320 mg/m^3 (1)	Anticipated human LD_{50}: 8,750 mg/person (2)
Acute toxicity (cyanosis)	Anticipated human effect concentration range : 18-80 mg/m^3 (3)	Anticipated dose range for human effects: 540-2,400 mg/person (3)

Table 4.9 continue overleaf

Table 4.9 continued Summary of effects of 3,4-dichloroaniline relevant for workplace risk assessmen

	Inhalation		Dermal	
Irritation/Corrosivity	Inhalation No local effects described(4)	Eye 3,4-dichloroaniline may result in serious damage to the eyes		Dermal Slightly irritating
Sensitisation	No case reports		Skin sensitiser	
Repeated dose toxicity (local effects)	No local effects observed(3)		Slightly irritating (based on acute irritation testing)	
Repeated dose toxicity (systemic effects)	Anticipated human NAEC: 1 mg/m³(3)		Anticipated human NAEL: > 30 mg/person/day(3)	
Mutagenicity	Genotoxicity is unlikely to be expressed *in vivo*			
Carcinogenicity	No data on 3,4-dichloroaniline			
Fertility impairment	No indication for specific toxicity on fertility: NOAEL (dog, oral): 0.75 mg/kg/day (6)			
Developmental toxicity	No specific developmental toxicity NOAEL (rats, oral) for embryo/fetotoxicity: 25 mg/kg/day NOAEL for maternal toxicity: 5 mg/kg/day			

1) Based on a rat LC_{50} of 3,300 mg/m³
2) Based on a rabbit LD_{50} of 300 mg/kg
3) Based on a 2-week inhalation study in male rats
4) No data on respiratory irritation
5) Based on acute irritation testing
6) According to limited information based on a 2-year study in dogs using one dose of diuron which showed no histopathological changes in gonads and uterus.

4.1.3.2.5 Occupational risk assessment

For the purpose of risk assessment, it is assumed, that inhalation of vapour/particles and skin exposure are the main routes of exposure. Oral exposure is not considered to be a significant route of exposure under normal working practices. The workplace exposure is restricted to the production and further processing in the chemical industry (see **Table 4.2** and **Table 4.3**).

4.1.3.2.6 Acute toxicity

Inhalation

Lethality

The LC_{50} was 3,300 mg/m³ (4 hours) in rats. Taking into account an interspecies factor of ½. 5 (derivation see above under "Interspecies differences") an LC_{50} of 1,320 mg/m³ is estimated for humans.

Comparing the estimated human LC_{50}-value of 1,320 mg/m³ with the highest exposure concentration of 0.57 mg/m³ that was measured as a short-term value (0.5 hours) a MOS value of 2,316 is calculated. The direct use of the rat LC_{50}-value of 3,300 mg/m³ for the calculation results in a MOS value of 5,789. A risk of lethality after acute inhalation exposure is not expected at these workplaces.

Conclusion (ii).

Cyanosis

In a 2-week inhalation study in male rats the highest concentration of 200 mg/m^3 led to a methaemoglobin level of 12.2%. An air concentration of 45 mg/m^3 resulted in a methaemoglobin level of 1.6%. Methaemoglobinemia is considered to represent an acute effect and it is assumed that the reported levels have been reached shortly after onset of exposure. In humans a methaemoglobin concentration in the range of 10% can lead to cyanosis although it has to be mentioned, that this effect as such was not reported as clinical symptom in the rat study. Using the interspecies factor of ½. 5 it is estimated that air concentrations in the range of 18-80 mg/m^3 might lead to cyanosis in humans.

In **Table 4.10** the corresponding MOS values are listed in a dual way: in column (a) calculation of MOS values uses directly the concentrations from the 2-week inhalation study that led to methaemoglobin formation whereas in column (b) the MOS values are based on the above derived human effect concentration range. The resulting MOS values in columns (a) and (b) differ by a factor of 2.5, which is the result of the interspecies adjustment.

Table 4.10 MOS values for acute toxicity (cyanosis, inhalation) of 3,4-dichloroaniline

Form of exposure	Inhalative exposure (mg/m3)	(a) Direct MOS with 45-200 mg/m^3 (approximately 2-10% methaemoglobin in rats)	(b) Adjusted MOS with 18-80 mg/m^3 (estimated 2-10% methaemoglobin in humans)	Conclusion
Production and further processing in the chemical industry				
Vapour/ small particles(1)	< 0.07(2) (3)	> 643-2,857	> 257-1143	(ii)
	0.57(2) (4)	79-351	32-140	(ii)

1) Vapour and particles formed by condensation from the vapour
2) Assessed on the basis of the submitted data
3) Shift average
4) Short-term value

A risk of cyanosis after acute inhalation exposure is not expected at these workplaces.

Conclusion (ii).

Dermal

Lethality

In rats a dose of 1,000 mg/kg led to no lethality and no signs of toxicity. The lowest LD_{50} in rabbits is reported to be 300 mg/kg. The more sensitive species with more precise data is used for a further assessment. A human LD_{50} of 125 mg/kg is derived by metabolic rate scaling (factor ½.4), without application of an additional factor for substance specific sensitivity differences between rabbits and humans. For a body weight of 70 kg a human LD_{50} of 8,750 mg/person is calculated. Without species adjustment a LD_{50} of 21,000 mg/person is obtained.

The dermal exposure during production and further processing of the molten 3,4-dichloroaniline is assessed to be low, because highly accepted use of functioning PPE is assumed. For cleaning, maintenance and repair tasks however, occasional dermal exposures cannot be ruled out. As a worst-case estimate an exposure level of 26-260 mg/person is given for the unprotected worker. The lowest possible MOS value calculates to 34 (8,750/260). The

lowest direct MOS-value would be 81 (21,000/260). Thus a risk concerning lethality due to acute dermal exposure is not expected.

Conclusion (ii).

Cyanosis

Regarding cyanosis two new data are available. In skin absorption subacute test on male rabbits a daily dose of 60 mg/kg led to anemia and increased methaemoglobin levels up to 10% at days 5 and 10 of treatment. Extrapolated to humans, using only metabolic rate scaling (factor ½. 4, see under lethality above), the rabbit dose corresponds to a dose of 1,750 mg/person in humans. Additionally the information from the 2-week inhalation study as outlined under acute toxicity inhalation could be used as starting point for short term risk assessment concerning cyanosis by the dermal route. With a route-to-route extrapolation factor of 3 (see Section 4.1.3.2.1) and a human respiratory volume of 10 m^3/8 hours the inhalative dose range of 18-80 mg/m^3 corresponds to a dermal dose range of 540-2,400 mg/person. The according direct values without species extrapolation are 4,200 mg/person (60 mg/kg · 70 kg/person) from the dermal rabbit study and 1,350-6,000 mg/person (45-200 mg/m^3 · 10 m^3/person · 3) from the inhalative rat study.

Using the worst-case estimate for dermal exposure during cleaning, maintenance and repair works of 26-260 mg/person in combination with the possible effect range calculated for humans of 540-2,400 mg/person, MOS calculation reveals a range of 2-92. The range for the direct MOS-values is 5-230 (1,350/260 – 6,000/26).

At the upper end of the calculated MOS spectrum values are clearly out of the concern range. However, the value at the low end suggests, that human dermal exposure might be only a factor of 2 lower than the possible effect level. It has to be taken into account though, that by this estimation some worst-case aspects may come together. Most importantly, dermal exposure is assessed as worst-case scenario with EASE-estimation without taking in consideration the exposure reducing effect of personal protection equipment (PPE). In addition effects assessment relies on deviations of haematological parameters in a 14-day study which would not be detectable with the protocol of an acute standard test. Related clinical symptoms have only been reported at the high end of the dose range.

In summary the MOS-values are not judged to indicate concern. This decision takes into account that with the proper use of suitable PPE, here gloves, control measures exist, which should be able to efficiently reduce exposure. However, it has to be taken in mind, that acceptance and functioning of gloves during cleaning, maintenance and repair works clearly represent a critical risk factor under the aspect of acute dermal toxicity.

Conclusion (ii).

4.1.3.2.7 Irritation/corrosivity

Dermal

3,4-Dichloroaniline was only slightly irritant to the skin of rabbits after acute irritation testing. Because the slight irritation effects are not considered sufficient for classification no concern for workers is derived.

Conclusion (ii).

Eyes

Based on rabbit data 3,4-Dichloroaniline may result in serious damage to the eyes. Even though suitable PPE usually is worn in the chemical industry eye contact critically depends on the proper use of eye glasses. 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, 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.

Conclusion (ii).

Inhalation

This section provides no additional information compared with the following section "Repeated dose toxicity: Inhalation (local effects)". Please refer to the according passage.

Conclusion (ii).

Sensitisation

Dermal

No human data are reported, but based on animal data 3,4-dichloroaniline is considered to be sensitising to the skin. An effect threshold for sensitisation cannot be estimated.

Dermal exposure during production and further processing in the chemical industry is reported to critically depend on the proper use of suitable gloves. Highly accepted use of functioning gloves is assumed for handling of the molten substance, minimising exposure to a level close to zero. In that case the risk of skin sensitisation is anticipated to be negligible. However during cleaning, maintenance and repair work relevant dermal exposures against 3,4-dichloroaniline cannot be excluded. The risk of workers to develop contact allergies is considered to be of concern.

Conclusion (iii). [11]

Inhalation

Data on respiratory sensitisation in man (e.g. case reports) and in animals is not available. For preliminary risk assessment inhalation exposure is not suspected to result in respiratory tract sensitisation.

Conclusion (ii).

[11] Commission Recommendation 2006/283/EC of 13th April 2006 on risk reduction measures for various substances including 3,4-DCA, OJ L 104/46.

4.1.3.2.8 Repeated dose toxicity

Inhalation (systemic effects)

Repeated dose studies on rats and rabbits indicate that 3,4-dichloroaniline has erythrotoxic properties, increases methaemoglobin levels, enhances erythropoiesis and induces persistent hemosiderosis as a consequence of erythrotoxicity. As starting point for risk considerations the lowest tested air concentration in the two-week inhalation study in rats of 10 mg/m^3 is used which represents the LOAEC (see Section 4.1.2.6).

Although the effects were minimal at 10 mg/m^3 an additional extrapolation step is considered to be justified to achieve a concentration, that is assumed to be effect free. An extrapolation factor of 1/3 is used as default value (ECETOC, 1995). The erythrotoxicity seen in the study is interpreted as an early indicator of chronic toxicity, which is assumed to occur soon after onset of exposure. Thus it is not expected that the estimated effect level would dramatically decrease with elongation of study duration, instead an increase of the severity of the chronic effects seems likely. Thus a duration adjustment step for long term exposure on the background of the 14-day study is not indicated. For interspecies adjustment the factor of ½. 5 is used (see Section 4.1.3.2.1).

In summary concerning chronic inhalative toxicity of 3,4-dichloroaniline a human NAEC of 1 mg/m^3 (10 mg/m^3/3/2.5) is calculated.

In **Table 4.11** the corresponding MOS values are listed. In column (a) calculation of MOS values uses directly the minimal effect concentration from the 2-week inhalation study whereas in column (b) MOS values are based on the anticipated human NAEC. The resulting MOS values in columns (a) and (b) differ by a factor of 10, which is the result of the use of adjustment factors as outlined above.

Table 4.11 MOS values for repeated dose toxicity (systemic, inhalation) of 3,4-dichloroaniline

Form of exposure	Shift average value (mg/m^3)	(a) Direct MOS with the minimal effect level in rats of 10 mg/m^3	(b) Adjusted MOS with an estimated human NAEC of 1 mg/m^3	Conclusion
Production and further processing in the chemical industry: drumming, filling and transfer(1)				
Vapour/ small particles(2)	< 0.07(3)	> 143	> 14	(ii)

1) Only daily exposure scenarios included
2) Vapour and particles formed by condensation from the vapour
3) Assessed on the basis of the submitted data

Results of workplace measurements during production and further processing within the large-scale chemical industry generally remained below the detection limit of 0.07 mg/m^3. So based on the adjusted MOS-value of > 14 concern is not derived for the daily exposure during drumming, filling and transfer activities. However, repeated dose toxicity of 3,4-dichloroaniline is considered to be significant. Corresponding toxicological data principally could be used for the establishment of a health-based occupational exposure limit.

Conclusion (ii).

Dermal (systemic effects)

In a dermal rabbit study of low reliability anemia and increased methaemoglobin levels were observed at 60 mg/kg/day (only one dose reported). A threshold cannot be derived, but the study indicates, that systemic availability has to be expected.

Because of the lacking dose response relationship and the poor quality of the dermal study the above mentioned inhalation study is used as a starting point for an assessment. The human NAEC as calculated above is modified by an additional route-to-route extrapolation factor of 3 (see Section 4.1.3.2.1).

Starting with the concentration of 1 mg/m^3 and assuming a respiratory volume of 10 m^3/8 hours a human NAEL for chronic dermal toxicity of 30 mg/person/day (10 mg/m^3/3 · 10 m^3/8 hours) is estimated. Alternatively on the basis of the minimal effect level the direct LAEL without interspecies adjustment may be calculated to 300 mg/person/day.

Daily dermal contact to 3,4-dichloroaniline during production and further processing is assessed to be low for the molten substance under the assumption of highly accepted use of functioning gloves. Increased exposure levels could result during cleaning, maintenance and repair work. This contact however is reported to occur only occasionally and is therefore not considered under the aspect of risk assessment for chronic toxicity.

In summary systemic risks from chronic toxicity by skin contact are not anticipated to occur at workplaces.

Conclusion (ii).

Inhalation (local effects)

Data on irritation via inhalation is not reported in Section 4.1.2.3. Signs of respiratory irritation are not described in the acute inhalation studies. The 2-week exposure of 3,4-dichloroaniline up to 200 mg/m^3 led to no effects in the respiratory tract of male rats. The NOAEC (local effects) might be higher, since a LOAEC was not determined. Comparing the NOAEC (local effects) of higher than 200 mg/m^3 with the minimal effect concentration of systemic toxicity of 10 mg/m^3 respiratory irritation and local effects via repeated inhalation are not considered to be of concern.

Conclusion (ii).

Dermal (local effects)

This section provides no additional information compared with the preceding section “Irritation/Corrosivity: Dermal.” Please refer to the according passage.

Conclusion (ii).

4.1.3.2.9 Mutagenicity

Although *in vitro* genotoxicity tests were negative for gene and chromosome mutations, there is limited evidence for a mutagenic potential mainly due to a weakly positive SCE test *in vitro* and a positive test for induction of spindle damage *in vitro*. The clearly negative *in vivo*

micronucleus tests indicate that this potential is unlikely to be expressed *in vivo*. Corresponding risks are not expected.

Conclusion (ii).

4.1.3.2.10 Carcinogenicity

No data on 3,4-dichloroaniline is available. The class of mononuclear aromatic amino compounds comprises carcinogenic and non-carcinogenic substances. The structure activity relationship of these compounds is quite complex and a speculation as to the potential of 3,4-dichloroaniline is uncertain. However a carcinogenic property of 3,4-dichloroaniline cannot be excluded with certainty. Concerning the mechanism of a possible tumour development it should be mentioned that the *in vivo* genotoxicity data are not indicative of a genotoxic mechanism. A possible tumour development that is based on non-neoplastic effects (threshold mechanism) has to be kept in mind for the assessment of the MOS of "Repeated dose toxicity". A risk of carcinogenicity via a genotoxic mechanism is not expected.

Conclusion (ii).

4.1.3.2.11 Reproductive toxicity

Fertility impairment

Studies on the reproductive function or capacity of 3,4-dichloroaniline are not available. Limited information is given by the 2-week inhalation study in male rats. No effects were observed in testes and epididymides up to the highest concentration of 200 mg/m^3. Thus for short-term exposure a human NAEC for male reproductive organs in the range of 80 mg/m^3 (interspecies extrapolation ½.5) can be assumed, which is in the range of the anticipated human effect concentration for acute toxicity (cyanosis).

From a 2-year feeding study in dogs with diuron, a herbicide that is partly metabolised to 3,4-dichloroaniline, no histopathological changes in gonads and uterus were reported at the highest dose tested, which may be assumed to correspond to an internal exposure to 3,4-dichloroaniline of 0.75 mg/kg/day. Using metabolic rate scaling for species extrapolation to humans (factor ½) leads to a human NAEL of 26.3 mg/person/day (oral). This would correspond to a daily inhalative exposure with air concentrations of 2.6 mg/m^3. Without species extrapolation this value would be 5.3 mg/m^3.

Because of the limitations in the data base a valid risk characterisation for fertility is not possible. The following preliminary assessment is used to decide on the request of further data.

For short-term exposures at the workplace effects at male reproductive organs are not to be expected at dose levels without symptoms of acute toxicity. From the fact that there is no scenario with concern under the aspect of acute toxicity no indication for concern with respect to fertility impairment after short-term exposure is given.

Concerning repeated exposure there is only one inhalative exposure scenario to be assessed. Daily air concentrations below the detection limit of 0.07 mg/m^3 may be compared with the human NAEC of 2.6 mg/m^3 derived from the dog study. The resulting adjusted MOS-value

would be > 38, which is too high to give reason for concern. For comparison the direct MOS using 5.3 mg/m^3 would be > 75.

In summary from the perspective of occupational risk assessment the request of a study on fertility impairment is not considered to be essential.

Conclusion (ii).

Developmental toxicity

No significant adverse effects on embryonic/fetal development were revealed from a teratology study in rats. The NOAEL for embryo/fetotoxicity was identified at 25 mg/kg/day and that for maternal toxicity at 5 mg/kg/day. The maternal NOAEL roughly fits to the results of the 2 week inhalation study in male rats, since the minimal effect level of that study of 10 mg/m^3 (0.01 mg/l) is equivalent to an oral dose of approximately 3 mg/kg (0.01 mg/l · 0.8 l/min/kg · 360 minutes = approximately 3 mg/kg). A higher sensitivity of pregnant compared to non-pregnant rats is not expected. The fetus seems to be approximately 5-fold less sensitive than the dams and a risk of specific developmental toxicity at workplaces is not expected.

Conclusion (ii).

4.1.3.2.12 Conclusions of the occupational risk assessment

Table 4.12 Conclusions of the occupational risk assessment [1]

Area of exposure[2]	Activity[2]	Acute toxicity	Irrit./ Corros.	Sensitisation		Repeated dose toxicity		Muta-genicity	Carcino-genicity	Reproductive toxicity	
		inh./ derm.	(eyes/inh./ derm.)	inh.	derm.	inh.	Derm			Fertility	Dev. tox.
Chemical industry											
Production and further processing	drumming, filling, transfer (molten substance)										
	cleaning, maintenance, repair work (room temperature) [3]				(iii)						

1) Blank fields: conclusion ii is applied
2) For details see Table 4.2 and 4.3
3) In the chemical industry normally suitable gloves are worn, however occasional dermal contact against complex mixtures of the substance during cleaning, maintenance and repair work cannot be excluded

4.1.3.3 Consumers

Since there is no consumer exposure, a health risk of consumers regarding Acute toxicity, Irritation, Corrosivity, Sensitisation, Repeated dose toxicity, Mutagenicity, Carcinogenicity, and Reproductive toxicity is not expected.

Conclusion (ii).

4.1.3.4 Humans exposed indirectly via the environment

Indirect exposure via the environment is calculated using data for oral uptake via drinking water and fish as well as via vegetable food. Compared with this, the other pathways can be neglected.

A daily intake via drinking water and fish of $1.7 \cdot 10^{-3}$ mg/kg bw/day has been calculated (scenario production site D). However, the main contribution to the total daily intake of 3,4-dichloroaniline results from the contamination of plants which were treated with plant protecting agents. Following this scenario, a total daily intake of $2.3 \cdot 10^{-3}$ mg/kg bw/day can be assumed.

4.1.3.4.1 Repeated dose toxicity

Oral exposure

Because no data from an oral study following 3,4-dichloroaniline administration are available, data from 4-chloroaniline may be assessed for risk characterisation.

In 2-year studies on 4-chloroaniline hydrochloride in rats and mice a NOAEL was not established (NTP, 1989). A LOAEL of 2 mg/kg bw/day in rats and of 7.5 mg/kg bw/day in mice was derived.

Inhalation exposure

No NOAEC was derived from the 14-day inhalation study (6 hours/day, 5 days/week) on 3,4-dichloroaniline (Kinney, 1986). Conversion of the LOAEC of this study (10 mg/m^3) to the inhaled amount of the substance (respiratory minute volume (rat) 0.8 l/min/kg; exposure duration: 6 hours (360 minutes/day) to an oral dose yields:

$$0.01 \text{ mg/l} \cdot 0.8 \text{ l/minutes/kg} \cdot 360 \text{ minutes} = 2.88 \text{ mg/kg bw.}$$

This LOAEL is considered to be appropriate for the calculations of quantitative risk assessment on 3,4-dichloroaniline.

Dermal exposure

Sparse data from a rabbit dermal study are not considered to be sufficient to derive an appropriate NOAEL or LOAEL.

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 (see Section 4.1.2.6).

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 of 3,4-dichloroaniline and structurally related substances on rats and mice (anemia and non-neoplastic effects). The calculated LOAEL of 2.88 mg/kg bw/day from the 14-day inhalation study on male rats is used for risk assessment although limitations of this study are seen. The main findings on 3,4-dichloroaniline from the dermal 10-day study on rabbits which is not in full compliance to actual standard methods and guidelines and from the 2-year studies on 4-chloroaniline hydrochloride in rats and mice showed identical main toxic effects.

Taking into account that only a LOAEL could be derived from a 14-day study, there is concern which has to be expressed in the magnitude of the MOS.

Intra- and interspecies variation

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. However, the reported data on acute dermal toxicity demonstrated species differences. Furthermore, it cannot be excluded that humans are more sensitive regarding methaemoglobin formation than rats and mice.

Therefore, there is concern which has to be expressed in the magnitude of the MOS.

The nature and severity of the effect

The effects described in rats and mice as “low observed adverse effect” are haemolytic anemia and methaemoglobinemia with compensatory responses in the erythropoetic system of bone marrow, spleen, liver, and kidneys. These effects are considered to be serious health effects.

There are no reasons to assume that the effects shown in the animal experiments are limited to the species tested, thus being not of relevance for humans. Because of the seriousness of the effects 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 daily intakes with an assumed absorption of 100% are compared with a calculated oral LOAEL from the 14-day inhalation study on male rats.

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 indirect exposure scenario there is no reason to assume a special risk for elderly, children or other people suffering from special diseases like anemia.

Other factors

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

MOS for the exposure scenario

a) The daily intake for oral exposure via drinking water and fish has been calculated to be $1.7 \cdot 10^{-3}$ mg/kg bw/day (scenario production site D). The margin of safety between the

estimated exposure level of	$1.7 \cdot 10^{-3}$ mg/kg bw/day
and the	
oral LOAEL of	2.88 mg/kg bw/day

is judged to be sufficient, even if special considerations on intra- and interspecies variation as well as the nature and severity of the effects seen in a 14-day study are taken into account.

b) The main contribution to the total daily intake of 3,4-dichloroaniline results from plants with a calculated figure of $2.3 \cdot 10^{-3}$ mg/kg bw/day. The margin of safety between the

estimated exposure level of	$2.3 \cdot 10^{-3}$ mg/kg bw/day
and the	
oral LOAEL of	2.88 mg/kg bw/day

is judged to be sufficient, even if special considerations on intra- and interspecies variation as well as the nature and severity of the effects seen in a 14-day study are taken into account.

Conclusion (ii).

4.1.3.4.2 Reproductive toxicity

Fertility impairment

Studies on the reproductive function or capacity of 3,4-dichloroaniline are not available. Limited information is given by the 2 week inhalation study in male rats. No effects were observed in testes and epididymides.

3,4-dichloroaniline is one of the metabolites which are formed *in vivo* from the plant protecting agent diuron. In a replicate 3-generation feeding study on rats with 0 or 125 ppm diuron in the diet (= 10 mg/kg bw/day) no substance related effects were seen in the treated animals compared to the control (Hodge et al., 1967). Under the experimental conditions of the study 3,4-dichloroaniline was detected in the urine in small amounts (< 0.2% of the dose given = 0.02 mg/kg bw/day).

In a two-year study Hodge et al. (1967) administered up to 1,250 mg/kg food to dogs (equivalent to 62.5 mg/kg bw/day diuron). Following the treatment, 3,4-dichloroaniline was found in urine (1.2% of the total metabolites). Therefore it can be assumed that the animals treated with diuron were internally exposed to 3,4-dichloroaniline. Following the highest dose, adverse effects were reported in the spleen (increased haematopoiesis) and in the bone marrow (erythroid hyperplasia). However, no histopathological changes in gonads and uterus were reported. It is assumed that this dose of diuron would represent an internal exposure to 3,4-dichloroaniline of 0.75 mg 3,4-DCA/kg bw (1.2%).

Developmental toxicity

For 3,4-dichloroaniline no significant adverse effects on embryonic/fetal development were revealed from an OECD Guideline according teratology study in rats. From this study a NOAEL for embryo-/fetotoxic effects of 25 mg/kg bw/day was derived.

MOS for the exposure scenario

Reproductive toxicity - fertility

The total daily intake of 3,4-dichloroaniline for oral exposure via drinking water and fish and from plants has been summed up to about $4 \cdot 10^{-3}$ mg/kg bw/day (see Section 4.1.1.4).

From the diuron study on dogs an internal exposure to 3,4-DCA of 0.75 mg/kg bw/day was derived.

The margin of safety between the

calculated exposure level of	$4 \cdot 10^{-3}$ mg/kg bw/day
and the	
NOAEL (internal exposure) of	0.75 mg/kg bw/day

is judged to be sufficient, especially, if additionally interspecies variations in metabolism of Diuron with respect to a lower 3,4-DCA formation in humans are taken into account.

Conclusion (ii).

Reproductive toxicity – developmental toxicity

The total daily intake of 3,4-dichloroaniline for oral exposure via drinking water and fish has been summed up to about $4 \cdot 10^{-3}$ mg/kg bw/day (see Section 4.1.1.4).

From the OECD guideline study with DCA on rats (Clemens and Hartnagel, 1990) a NOAEL of 25 mg/kg bw was derived for embryo-/fetotoxic effects. Thus, the margin of safety between the

calculated exposure level of	$4 \cdot 10^{-3}$ mg/kg bw/day
and the	
NOAEL of	25 mg/kg bw/day

is judged to be sufficient,

Conclusion (ii).

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

4.2.1 Exposure assessment

4.2.1.1 Occupational exposure

See Section 4.1.1.2.

4.2.2 Effects assessment: hazard identification and dose (concentration) - response (effect) assessment

4.2.2.1 Explosivity

3,4-dichloroaniline is not explosive.

4.2.2.2 Flammability

3,4-dichloroaniline is not highly flammable.

4.2.2.3 Oxidising potential

Due to its chemical structure, 3,4-dichloroaniline is not expected to possess any oxidising properties.

4.2.3 Risk characterisation

4.2.3.1 Workers

Not applicable.

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

5 RESULTS

5.1 ENVIRONMENT

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account. [12]

This conclusion is reached because of

– the non-agricultural use of diuron on sealed areas as total herbicide,

which is expected to cause a risk to the aquatic environment.

An environmental pollution of 3,4-dichloroaniline from the use of diuron as antifouling agent and as algicide in the construction sector has to be expected. These releases could not been taken into account in the risk characterisation, as neither sufficient exposure relevant information nor an appropriate exposure model are available. Diuron is more toxic than 3,4-DCA and probably occurs in higher concentrations, thus the 3,4-DCA exposure from these applications should be covered by a diuron assessment. It is recommended to perform an assessment for diuron in the frame of the Biocide Directive 98/8/EU.

Conclusion (i) There is need for further information and/or testing. [13]

For the releases of 3,4-DCA from the non-agricultural use of diuron on sealed areas as a total herbicide the PEC/PNEC ratio for sediment is above 1. The data basis can be improved by performing a long term test with a third sediment organism representing a further exposure pathway (*Hyalella azteca*). However, this requirement for further testing was awaiting the outcome of the risk reduction strategy for the aquatic compartment. Because the measures recommended are expected to sufficiently reduce concentrations in the aquatic compartment, the test is now no longer deemed necessary.

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. [13]

Based on the available information the exposure of workers against 3,4-dichloroaniline generally is low with the exception of occasional dermal contact during cleaning, maintenance and repair work. On that background for skin sensitisation concern has to be raised.

[12] Commission Recommendation 2006/283/EC of 13th April 2006 on risk reduction measures for various substances including 3,4-DCA, OJ L 104/46.

[13] Commission Communication 2006/C 90/04 of 13th April 2006 on the results of the risk evaluation and the risk reduction strategies for various substances including 3,4-DCA (90/07).

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

For the other toxicological endpoints the risk orientated conclusions result in no concern with the consequence that risk reduction measures are of low priority. Although the hazard assessment revealed significant toxicological properties for 3,4-dichloroaniline, exposure levels reported at the workplace are below the concern range.

5.2.1.2 Consumers

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

5.2.1.3 Humans exposed indirectly via the environment

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

5.2.2 Human health (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

APPENDIX A MONITORING OF DIURON IN RIVERS (µg/l)

(References: LUA NRW, 1999; RIWA 1996,1997)

Table A1 Monitoring of Diuron In Rivers (µg/l)

River (Site)	1994	1995	1996
Aller (Verden)			< 0.1 n=4
Donau (Ulm)			< 0.05 n=13
Donau (Jochenstein)	<0.02 n=6	<0.02-0.02 ∅ <0.02 n=3	< 0.02 N=4
Elde (Dömitz)			< 0.05 N=12
Erft (Neuss)			< 0.05-0.37 ∅ <0.10 n=8
Fulda (Wahnhausen)			< 0.03-1.10 90%ile 0.23 n=25
Havel (Hennigsdorf)			< 0.05 n=13
Havel (Krughorn)			< 0.05 n=3
Ilm (Niedertreba)		< 0.05 n=13	
Inn (Kirchdorf)	< 0.02 n=4	< 0.02 n=4	< 0.02-0.04 ∅ <0.02 n=4
Lausitzer Neiße (Görlitz)			< 0.05 n=4
Lippe (Wesel)	0.06-0.14 ∅ 0.10 n=3	< 0.05-0.54 ∅ 0.22 n=8	
Main (Viereth)	< 0.02-0.12 90%ile 0.07 n=18	< 0.02-0.15 90%ile 0.06 n=17	< 0.02-0.07 90%ile 0.06 n=13
Main (Erlabrunn)	< 0.02-0.07 90%ile 0.07 n=14	< 0.02-0.07 ∅ 0.04 n=10	< 0.02-0.06 90%ile 0.05 n=13

Table A1 continued overleaf

Table A1 continued

River (Site)	1994	1995	1996
Main (Kahl)	< 0.02-0.06 90%ile 0.06 n=11	< 0.02-0.09 90%ile 0.07 n=13	< 0.02-0.07 90%ile 0.06 n=12
Main (Bischofsheim)	< 0.03-0.46 90%ile 0.34 n=33	< 0.03-0.32 90%ile 0.19 n=33	< 0.03-0.26 90%ile 0.14 n=33
Mosel (Palzem)		< 0.05-1.50 90%ile 0.92 n=12	< 0.05-0.82 90%ile 0.55 n=11
Mosel (Koblenz)			< 0.05-0.19 ∅ 0.14 n=6
Nahe (Grolsheim)			< 0.05-0.27 90%ile 0.24 n=15
Neckar (Kirchentellinsfurt)		< 0.05 n=6	
Neckar (Deizisau)	< 0.05-0.11 90%ile 0.11 n=12	< 0.05-0.05 90%ile <0.05 n=12	< 0.05-0.19 90%ile 0.09 n=13
Neckar (Poppenweiler)	< 0.05-0.17 90%ile 0.08 n=13	< 0.05-0.17 90%ile 0.10 n=11	< 0.05-0.12 90%ile 0.07 n=13
Neckar (Kochendorf)	< 0.05-0.15 90%ile 0.13 n=12	< 0.05-0.07 90%ile 0.06 n=11	< 0.05-0.10 90%ile 0.09 n=13
Neckar (Mannheim)	< 0.05-0.10 90%ile 0.10 n=13	< 0.05-0.10 90%ile 0.08 n=11	< 0.05-0.09 90%ile 0.07 n=13
Nidda (Frankfurt-Nied)	0.04-1.20 90%ile 1.02 n=14	0.05-1.60 90%ile 0.71 n=21	0.03-0.90 90%ile 0.70 n=18
Peene (Anklam)			< 0.05 n=12
Pleiße (Gößnitz)		< 0.05 n=13	
Regnitz (Hausen)	< 0.02-0.04 ∅ <0.02 n=3	< 0.02-0.11 ∅ 0.04 n=3	< 0.02-0.06 ∅ <0.02 n=5

Table A1 continued overleaf

Table A1 continued

River (Site)	1994	1995	1996
Rhein (Öhningen)		< 0.05 n=4	< 0.05 n=13
Rhein (Mainz)	< 0.05-0.09 90%ile 0.06 n=26	< 0.05-0.08 90%ile <0.05 n=26	< 0.05-0.11 90%ile 0.07 n=27
Rhein (Kleve-Bimmen)	< 0.05-0.10 ∅ <0.05 n=4	< 0.05-0.10 ∅ 0.06 n=4	< 0.05-0.11 90%ile 0.11 n=12
Rhein (Lobith)		< 0.01-0.13 90%ile 0.06 n=13	< 0.05-0.12 90%ile 0.10 n=14
Ruhr (Duisburg)	< 0.05-0.31 ∅ 0.15 n=8		
Rur (Einruhr)			< 0.1-0.19 90%ile 0.13 n=13
Rur (End-Steinkirchen)			< 0.1-0.40 90%ile 0.39 n=18
Saale (Camburg-Stöben)		< 0.05 n=13	
Saar (Kanzem)		< 0.08-0.71 90%ile 0.68 n=11	< 0.05-0.33 90%ile 0.24 n=12
Sächs. Saale (Joditz)	< 0.02 n=5	< 0.02-0.18 ∅ 0.05 n=4	< 0.02 n=1
Salzach (Laufen)	< 0.02 n=3	< 0.02 n=2	< 0.02 n=5
Sieg (Bergheim)	< 0.05-0.21 ∅ 0.08 n=4		
Spree (Neuzittau)		< 0.1 n=2	< 0.05 n=13
Steinach (Muppberg)		< 0.05 n=13	
Sude (Bandekow)			< 0.05 n=13

Table A1 continued overleaf

Table A1 continued

River (Site)	1994	1995	1996
Teltowkanal (Kohlhasenbrück)			< 0.05 n=3
Tollense (Demmin)			< 0.05 n=12
Uecker (Ueckermünde)			< 0.05 n=12
Unstrut (Straußfurt)		< 0.05 n=13	
Warnow (Kessin)			< 0.05 n=11
Weiße Elster (Gera-Langenberg)		< 0.05 n=13	
Werra (Gerstungen)		< 0.05 n=13	
Weser (Hemeln)			< 0.1 n=4
Weser (Bremen)			< 0.03-0.20 90%ile 0.16 n=24
Wipper (Hachelbich)		< 0.05 n=13	
Wupper (Leverkusen)	< 0.05-0.18 ∅ 0.10 n=4		

APPENDIX B CALCULATION OF ENVIRONMENTAL FATE

Table B1 Partitioning between soil and pore water

I/C	Model calculations for soil concentration	Use of diuron		
I	Name of chemical	3,4-Dichloroaniline		
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	2,7	-
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	10,000	l/kg
C		=	10	m^3_water/kg_oc
C	Organic matter-water partition coefficient	Kom	5,882352941	m^3_water/kg_om
C	Solids-water partitioning coefficient in soil	Kp_soil	0,2	m^3_water/kg_solid
C	Total soil-water partitioning coefficient	Ksoil_water	300,2	m^3_water/m^3_wet soil

Table B2 Partitioning between water and air

I/C	Model calculations for soil concentration	Use of diuron		
I	Name of chemical	3,4-Dichloroaniline		
I	Henry's law coefficient	Henry	0,0514	Pa.m^3/mol
C	Air-water partirion coefficient	Kair_water	2,16924E-05	-

Table B3 Characteristics of soil and soil use

I/C	Model calculations for soil concentration	Use of diuron		
I	Name of chemical	3,4-Dichloroaniline		
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

Table B4 Derivation of removal rate constants

I/C	Model calculations for soil concentration	Use of diuron		
I	**Name of chemical**	**3,4-Dichloroaniline**		
C	Pseudo first order rate constant for volatilisation			
C	from agricultural soil	kvolat_agri	9,51539E-07	d-1
C	from grassland	kvolat_grass	1,90308E-06	d-1
I	Pseudo first order rate constant for biodegradation	kbio_soil	6,93E-04	d-1
C	Pseudo first order rate constant for leaching			
C	in agricultural soil	kleach_agri	7,99467E-06	d-1
C	in grassland	kleach_grass	1,59893E-05	d-1
C	First order rate constant for removal			
C	from agricultural soil	k_agri	7,02E-04	d-1
C	from grassland	k_grass	7,11E-04	d-1

Table B5 Concentration in soil through aerial deposition

I	Annual average deposition flux	DEPtotal	0,00E+00	kg_chem/m^2/day
C	aerial deposition flux per kg of soil			
	in agricultural soil	Dair_agri	0	kg_chem/kg_soil/day
	in grassland	Dair_grass	0	kg_chem/kg_soil/day
	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

Table B5 Concentration in soil through sludge application

I	Concentration in dry sewage sludge	Csludge	5,18E-06	kg_chem/kg_dry sludge
	Initial concentration on the first year of application			
S	in agricultural soil	Csludge_agri1(0)	9,70E-07	kg_chem/kg_dry soil
S	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	0,773979133	-
C	in grassland	Facc_grass	0,771455929	-
	Initial concentration after 10 a of sludge application			
C	in agricultural soil	Csludge_agri10(0)	3,96057E-06	kg_chem/kg_dry soil
C	in grassland	Csludge_grass10(0)	0	kg_chem/kg_dry soil

Table B6 Total concentration in soil

	Initial total concentration after 10 a			
C	in agricultural soil	Csoil_agri10(0)	3,96057E-06	kg_chem/kg_dry soil
C	in grassland	Csoil_grass10(0)	0	kg_chem/kg_dry soil
D	averaging period for crops & 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	3,91916E-06	kg_chem/kg_dry soil
		=	3919,16183	µg/kg dry soil
C	in agricultural soil for indirect exposure	PECagri_ind	3,72058E-06	kg_chem/kg_dry soil
		=	3720,575885	µg/kg dry soil
C	in grassland for indirect exposure	PECgrass_ind	0	kg_chem/kg_dry soil
		=	0	µg/kg dry soil

Table B7 Total concentration in soil porewater

C	for terrestrial ecosystem	PEClocal_soil	2,21972E-05	kg_chem/m^3_water
C		=	22,19718219	µg/l
C	in agricultural soil for indirect exposure	PECagri_ind	2,10724E-05	kg_chem/m^3_water
C		=	21,07243955	µg/l
C	in grassland for indirect exposure	PECgrass_ind	0	kg_chem/m^3_water
C		=	0	µg/l

APPENDIX C CONTINENTAL AND REGIONAL CONCENTRATIONS, INDIRECT EXPOSURE

EUSES Full report Single substance

Printed on 2/26/04 6:13:30 PM
Study 3,4-DCA
Substance 3,4-DCA
Defaults Standard
Assessment types 1B, 3B
Base set complete No

Explanation status column'O' = Output; 'D' = Default; 'S' = Set; 'I' = Imported

Name	Reference	Value	Units	Status
Physico-Chemical Properties				
Molecular weight	162	162	[g.mol-1]	S
Melting point	72	72	[oC]	S
Boiling point	272	272	[oC]	S
Vapour pressure at 25 [oC]	0.184	0.184	[Pa]	S
Octanol-water partition coefficient.	2.7	2.7	[log10]	S
Water solubility	580	580	[mg.l-1]	S
Continental				
Total continental emission to air	0	33.3	[kg.yr-1]	S
Total continental emission to wastewater	0	0	[kg.yr-1]	S
Total continental emission to surface water	0	3.191E+03	[kg.yr-1]	S
Total continental emission to industrial soil	0	0	[kg.d-1]	S
Total continental emission to agricultural soil	0	0	[kg.d-1]	S
Regional				
Total regional emission to air	0	3.7	[kg.yr-1]	S
Total regional emission to wastewater	0	0	[kg.d-1]	S
Total regional emission to surface water	0	355	[kg.yr-1]	S
Total regional emission to industrial soil	0	0	[kg.d-1]	S
Total regional emission to agricultural soil	0	0	[kg.d-1]	S
Distribution				
Partition Coefficients				
Solids Water Partitionaing				
Organic carbon-water partition coefficient	193.807	1E+04	[l.kg-1]	S
Solids-water partition coefficient in soil	3.88	200	[l.kg-1]	O
Solids-water partition coefficient in sediment	9.69	500	[l.kg-1]	O
Solids-water partition coefficient suspended matter	19.4	1,000	[l.kg-1]	O
Solids-water partition coefficient in raw sewage sludge	58.1	3E+03	[l.kg-1]	O

Solids-water partition coefficient in settled sewage sludge	58.1	3E+03	[l.kg-1]	O
Solids-water partition coefficient in activated sewage sludge	71.7	3.7E+03	[l.kg-1]	O
Solids-water partition coefficient in effluent sewage sludge	71.7	3.7E+03	[l.kg-1]	O
Suspended matter-water partition coefficient	5.75	251	[m3.m-3]	O
Soil-water partition coefficient	6.01	300	[m3.m-3]	O
Sediment-water partition coefficient	5.65	251	[m3.m-3]	O
Air-Water Partitioning and Adsorption to Aerosol Particles				
Sub-cooled liquid vapour pressure	0.768	0.768	[Pa]	O
Fraction of chemical associated with aerosol particles	1.3E-04	1.3E-04	[-]	O
Henry's law constant	0.0514	0.0514	[Pa.m3.mol-1]	O
Air-water partitioning coefficient	2.17E-05	2.17E-05	[m3.m-3]	O
Biota-Water				
Bioconcentration factor for aquatic biota	39.355	45	[l.kg-1]	S
Degradation and Transformation Rates				
Environmental				
Specific degradation rate constant with OH-radicals	0	0	[cm3.molec-1.s-1]	D
Rate constant for degradation in air	0	1.84839	[d-1]	S
Rate constant for hydrolysis in surface water	6.93147E-07	0	[d-1]	S
Rate constant for photolysis in surface water	1E+06	18	[d] (Dt50)	S
Rate constant for biodegradation in surface water	0	0	[d-1]	O
Total rate constant for degradation in bulk surface water	5E+05	18	[d] (Dt50)	S
Total rate constant for degradation in bulk soil	1E+06	1,000	[d] (Dt50)	S
Rate constant for biodegradation in aerated sediment	6.93147E-07	6.93E-04	[d-1]	S
Total rate constant for degradation in bulk sediment	1E+07	1E+04	[d] (Dt50)	S
Continental and Regional				
Continental				
Continental PEC in surface water (total)	0	5.9E-07	[mg.l-1]	O
Continental PEC in surface water (dissolved)	0	5.75E-07	[mg.l-1]	O
Continental PEC in air (total)	0	3.49E-11	[mg.m-3]	O
Continental PEC in agricultural soil (total)	0	1.32E-08	[mg.kgwwt-1]	O
Continental PEC in pore water of agricultural soils	0	7.46E-11	[mg.l-1]	O
Continental PEC in natural soil (total)	0	4.91E-08	[mg.kgwwt-1]	O
Continental PEC in industrial soil (total)	0	4.91E-08	[mg.kgwwt-1]	O
Continental PEC in sediment (total)	0	2.1E-04	[mg.kgwwt-1]	O
Regional				
Regional PEC in surface water (total)	0	4.26E-06	[mg.l-1]	O

Regional PEC in surface water (dissolved)	0	4.2E-03	[ug.l-1]	O
Regional PEC in air (total)	0	1.86E-10	[mg.m-3]	O
Regional PEC in agricultural soil (total)	0	7E-08	[mg.kgwwt-1]	O
Regional PEC in pore water of agricultural soils	0	3.96E-10	[mg.l-1]	O
Regional PEC in natural soil (total)	0	2.61E-07	[mg.kgwwt-1]	O
Regional PEC in industrial soil (total)	0	2.61E-07	[mg.kgwwt-1]	O
Regional PEC in sediment (total)	0	1.49E-03	[mg.kgwwt-1]	O
Exposure				
Biocentration Factors				
Partition coefficient worm-porewater	20	20	[l.kg-1]	O
Bioconcentration factor for earthworms	5.67	0.114	[kg.kg-1]	O
Bioconcentration factor for fish	39.4	39.4	[l.kg-1]	O
Partition coefficient between plant tissue and water	4.32	4.32	[m3.m-3]	O
Partition coefficient between leaves and air	1.99E+05	1.99E+05	[m3.m-3]	O
Transpiration-stream concentration factor	0.554	0.554	[-]	O
Bioaccumulation factor for meat	1.26E-05	1.26E-05	[d.kg-1]	O
Bioaccumulation factor for milk	7.94E-06	7.94E-06	[d.kg-1]	O
Purification factor for surface water	1	1	[-]	O
Humas Exposed to or via the Environment				
Regional				
Concentrations in Fish, Plants and Drinking Water				
Regional concentration in wet fish	0	1.65E-04	[mg.kg-1]	O
Regional concentration in root tissue of plant	0	2.45E-09	[mg.kg-1]	O
Regional concentration in leaves of plant	0	5.13E-08	[mg.kg-1]	O
Regional concentration in grass (wet weight)	0	5.13E-08	[mg.kg-1]	O
Fraction of total uptake by crops from pore water	??	2.73E-03	[-]	O
Fraction of total uptake by crops from air	??	0.997	[-]	O
Fraction of total uptake by grass from pore water	??	2.73E-03	[-]	O
Fraction of total uptake by grass from air	??	0.997	[-]	O
Regional concentration in drinking water	0	4.2E-06	[mg.l-1]	O
Concentrations in Meat and Milk				
Regional concentration in meat (wet weight)	0	2.95E-09	[mg.kg-1]	O
Regional concentration in milk (wet weight)	0	1.86E-09	[mg.kg-1]	O
Fraction of total intake by cattle through grass	??	0.0148	[-]	O
Fraction of total intake by cattle through drinking water	??	0.985	[-]	O
Fraction of total intake by cattle through air	??	9.66E-05	[-]	O
Fraction of total intake by cattle through soil	??	1.39E-04	[-]	O
Daily Human Doses				
Daily dose through intake of drinking water	0	1.2E-07	[mg.kg-1.d-1]	O

Fraction of total dose through intake of drinking water	??	0.306	[-]	O
Daily dose through intake of fish	0	2.71E-07	[mg.kg-1.d-1]	O
Fraction of total dose through intake of fish	??	0.692	[-]	O
Daily dose through intake of leaf crops	0	8.79E-10	[mg.kg-1.d-1]	O
Fraction of total dose through intake of leaf crops	??	2.24E-03	[-]	O
Daily dose through intake of root crops	0	1.34E-11	[mg.kg-1.d-1]	O
Fraction of total dose through intake of root crops	0	3.42E-05	[-]	O
Daily dose through intake of meat	0	1.27E-11	[mg.kg-1.d-1]	O
Fraction of total dose through intake of meat	??	3.23E-05	[-]	O
Daily dose through intake of milk	0	1.49E-11	[mg.kg-1.d-1]	O
Fraction of total dose through intake of milk	??	3.8E-05	[-]	O
Daily dose through intake of air	0	3.98E-11	[mg.kg-1.d-1]	O
Fraction of total dose through intake of air	??	1.01E-04	[-]	O
Regional total daily intake for humans	0	3.92E-07	[mg.kg-1.d-1]	O

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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 tonnes/annum)
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	Oxidising (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 Organisation
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)

European Commission
DG Joint Research Centre, Institute of Health and Consumer Protection
European Chemicals Bureau

EUR 22235 EN **European Union Risk Assessment Report**
3,4-dichloroaniline

Editors: S.J. Munn, K. Aschberger, O. Cosgrove, S. Pakalin, A. Paya-Perez, B. Schwarz-Schulz, S. Vegro

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The report provides the comprehensive risk assessment of the substance 3,4-dichloroaniline. 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.

Part I - Environment

This part of the evaluation considers the emissions and the resulting exposure to the environment 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.

The environmental risk assessment concludes that there is concern for the aquatic environment as a consequence of exposure arising from the non-agricultural use of diuron as total herbicide on sealed areas.

There is a need for further information to adequately characterise the risks to the aquatic ecosystem arising from the release form non-agricultural use of diuron on sealed areas as total herbicide and performing a long term test with a third sediment organism representing a further exposure pathway (Hyalella azteca). However, this requirement for further testing was awaiting the outcome of the risk reduction strategy for the aquatic compartment. Because the measures recommended are expected to sufficiently reduce concentrations in the aquatic compartment, the test is now no longer deemed necessary.

Part II – Human Health

This part of the evaluation considers the emissions and the resulting exposure to human populations in all life cycle steps. The scenarios for occupational exposure, consumer exposure and humans exposed via the environment have been examined and the possible risks have been identified.

The human health risk assessment concludes that there is concern for workers with regard to skin sensitisation as a consequence of dermal exposure arising form cleaning, maintenance and repair work in the production and further processing of 3,4-dichloroaniline. For consumers and humans exposed via the environment there is no concern

For human health as far as physico-chemical properties are concerned there is no concern.

The conclusions of this report will lead to risk reduction measures to be proposed by the Commission’s committee on risk reduction strategies set up in support of Council Regulation (EEC) N. 793/93.

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