



SCOEL/REC/153

Aniline

Recommendation from the
Scientific Committee on Occupational Exposure Limits



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Adopted September 2015



EUROPEAN COMMISSION

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Scientific Committee on Occupational Exposure Limits

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**RECOMMENDATION FROM THE
SCIENTIFIC COMMITTEE ON OCCUPATIONAL
EXPOSURE LIMITS
FOR
ANILINE**

8-hour TWA:	7.74 mg/m³ (2 ppm)
STEL:	19.35 mg/m³ (5 ppm)
BLV:	0.2 mg aniline/l urine (after hydrolysis, sampling: end of shift)
Additional categorisation:	SCOEL carcinogen group C (carcinogen with a mode of action-based threshold)
Notation:	'Skin'

The present Recommendation was adopted by SCOEL on 2015-09-23, re-edited and accepted for publication on 2016-09-14.

This evaluation is based on Henschler (1973), IARC (1982), Henschler (1983), U.S. EPA (1985), Henschler (1992), HSE (1997), NRC (2000), ACGIH (2001), Greim (2002), ECB (2004), Bomhard and Herbold (2005), NLM (2005), Hartwig (2011), the references cited in these reviews and additional database search by SCOEL (August 2015 and July 2016).

RECOMMENDATION EXECUTIVE SUMMARY

For the evaluation of the toxicity of aniline, the following effects must be taken into account:

- carcinogenicity;
- methaemoglobin (Mhb) formation, linked with
- toxic effects on the haematopoietic system with erythrocyte toxicity and effects on the spleen.

Genotoxicity and carcinogenicity

Aniline is not mutagenic in standard bacterial tests. In mammalian cells *in vitro*, the results are not uniform, but positive results were observed with respect to chromosomal effects, SCE and mutagenicity. *In vivo*, induction of micronuclei was observed in bone marrow cells of rats and mice. However, the doses in these studies were high and caused marked Mhb-formation (Bomhard and Herbold, 2005). In general, the genotoxicity of aniline appears very low, if any. However, distinct metabolites of aniline are genotoxic, when individually tested. However, the balance of formation and detoxification of these metabolites has not been studied. Considering the distance between the high doses that produced genotoxicity in some tests and the proposed OEL, genotoxic effects of aniline appear negligible in practice.

Data on carcinogenicity of aniline in humans are inadequate for an evaluation. Experimentally, aniline is carcinogenic in rats, but not in mice. Tumours were mainly observed in the spleen of male rats, and the tumour incidence was clearly non-linear. It has been discussed whether the development of tumours is be connected to erythrocyte toxicity, which is indicated by the formation of Mhb and Heinz bodies with the histopathological effects in the spleen being a consequence of this effect (ECB, 2004). If this is the case, it can be argued that repetitive toxic effects play a decisive role for the development of tumours, and that no increased tumour risk should be expected in the absence of an increased erythrocyte turnover. This view is experimentally be supported by Mellert et al. (2004), corroborating the contention that experimentally carcinogenic doses of aniline cause early effects on haematological parameters, inflammatory reaction in the spleen and perturbations in iron metabolism as a result of haemolytic anaemia. Recent studies into the mechanism of the experimental splenotoxicity and carcinogenicity in rats (section 7.7.2.) have provided further confirmation for a categorisation into SCOEL group C of carcinogens (as a compound with a practical threshold). Therefore, it is now well established that chronic splenotoxicity and subsequent carcinogenicity is a secondary process following an increased breakdown of erythrocytes because of aniline-induced methaemoglobinaemia. It follows that avoidance of excessive methaemoglobinaemia will protect against carcinogenesis in the spleen.

Accordingly, the experimental carcinogenicity of aniline can reasonably be linked to a defined threshold-related process. *According to the delineations by SCOEL on the derivation of OELs for carcinogens and mutagens (Bolt and Huici-Montagud 2008), aniline is categorised into group C (carcinogens with a mode of action-based threshold).*

Developmental toxicity

In a developmental toxicity study with oral treatment of rats, maternal toxic effects were observed at 7.2 mg aniline/kg · d (Price et al., 1985). There was no evidence of foetotoxicity or teratogenicity at maternally non-toxic doses. Accordingly, no developmental effects are expected at concentrations that protect from haematotoxic effects as derived above.

Methaemoglobin formation and other haematotoxic effects

Following acute uptake of aniline, the critical toxic effect is the formation of methaemoglobin (MHb). Depending on the concentration of MHb, methaemoglobinaemia may have serious acute health effects. By analogy to tolerable levels of CO-Hb in carbon monoxide exposed persons (about 4% CO-Hb; see the SUM document on carbon monoxide), a MHb-level of about 5 % has been considered tolerable (Bolt et al., 1985). After repeated experimental exposure to aniline, the critical toxic effects are erythrocyte toxicity and toxic effects on the spleen. In a subacute inhalation study with rats, minimal splenic histopathological alterations developed at the lowest exposure concentration of 64.7 mg/m³ (17 ppm) (du Pont de Nemours and Co, 1982). In another subacute inhalation study with rats, a borderline increase in splenic extra-medullary hematopoiesis was seen at 32.4 mg/m³ (8 ppm), while 9.2 mg/m³ (2.4 ppm) was not associated with any significant effect (Pauluhn, 2004). In a subchronic study, a "doubtful" slight cyanosis but not any other effects were reported in rats, dogs, mice and guinea pigs at the only exposure concentration of 5 ppm, but the limited evaluation precludes definitive conclusions (Oberst et al., 1956). Inhalation exposure studies with rats have established a experimentally based NOAEC of 9.2 mg/m³ (2.4 ppm) upon subacute exposure in rats. Both available subacute inhalation studies (du Pont de Nemours and Co, 1982; Pauluhn, 2004) did not include additional aniline exposure via the skin. In general, the experimental data in rats appear compatible with those observed in humans.

In a subacute feeding study with rats, minimal erythrocyte toxicity with sporadic occurrence of Heinz bodies and minimal vascular congestion of the spleen were reported at the lowest dose of 4 mg/kg · d (Mellert et al., 2004). As the effects were slight, the NAEL does not appear to be much lower. In a chronic/carcinogenicity study (CIIT, 1982), the lowest dose of 7 mg/kg · d represented a LOAEL with respect to erythrocyte toxicity and effects on the spleen. No definite NOAEL is provided in either of these studies. With direct transformation into a corresponding air concentration (route-to-route extrapolation) a dose of 4 mg/kg · d corresponds to 28 mg/m³ (7 ppm) assuming a body weight of 70 kg, a breathing volume of 10 m³ during an eight hour exposure and 100% absorption. Comparison of the effects in the subacute and chronic feeding study does not support a strong increase in severity of the effects with prolonged exposure. Again, both oral feeding studies (Mellert et al., 2004; CIIT, 1982) did not include additional exposure via the skin.

Taking these data together, a health-based OEL may be derived, which protects against the relevant non-neoplastic effects, including methaemoglobinaemia. The experimental data in rats consistently point to beginning haematotoxic effects (MHb formation, associated with Heinz bodies) and spleen toxicity above repeated inhalation exposures of 5 ppm aniline.

Inhalation studies are reported in dogs (Pauluhn, 2002, 2005) and rats (Pauluhn, 2004), but there are large species differences in the quantities of MHb production due to aniline exposure between experimental animal species and humans. Therefore, the derivation of an OEL must rely on available data from humans. A preference for human data is in line with the key methodology of SCOEL.

A new experimental human exposure study by Käfferlein *et al* (2014) provides a strong basis for standard setting. The study indicates that after a 6-hour exposure to 2 ppm a level of MHb formation of 1.6 % was reached, which is not expected to further increase after 8 hours. This level is more than 2-fold below the critical MHb level. A comparison of the methaemoglobin levels reached under the conditions (moderate exercise) of this study (Figure 1) and the critical methaemoglobin levels of 4–5 % leads to the conclusion that an uncertainty factor to bridge the experimental conditions to those in industrial practice is not required. An OEL of 2 ppm aniline is derived. No additional uncertainty factor is needed to compensate for possible additional human inter-individual variation that exceeds the variations recorded in the study of Käfferlein *et al* (2014). Carry-over effects to the next shift must not be taken into account, as the half-life of MHb after cessation of aniline exposure is about 3.5 hours.

A STEL is a preferred means to limit short-term exposures with possible methaemoglobin formation. In view of the short half-life of aniline and the rapid decrease of methaemoglobin, an excursion factor of 2 will provide adequate protection. Applying the preferred value approach of SCOEL, a *STEL of 5 ppm is therefore recommended.*

Biological monitoring

Regarding biological monitoring, the recent studies of Käfferlein *et al* (2014) and Modick *et al* (2016) supersede the early study of Dutkiewicz and Piotrowski (1961). The time course of aniline in urine during and after the 6-hour exposure to 2 ppm aniline (Section toxicokinetics) showed a mean concentration of aniline in urine of 170 µg/l. Considering an 8-hour workshift, a *BLV of 0.2 mg aniline/l urine is therefore recommended (measured after hydrolysis).*

As far as analytical measurement (biological monitoring) is concerned, standard methods have been validated (Lewalter and Biedermann, 1994; Lewalter and Gries, 2001). According to Lewalter and Biedermann (1994) the detection limit for determination of aniline in urine is 1 µg/l. For the haemoglobin adduct of aniline, the detection limit is given as 1 ng/l blood (Lewalter and Gries, 2001).

Other assignments

Dermal absorption of aniline both in liquid and vapour form substantially contributes to total aniline uptake (Dutkiewicz, 1961; Korinth *et al.* 2008). *Therefore, a 'skin' notation is warranted.*

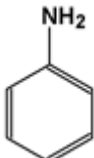
Aniline may cause contact allergy in humans, which is often associated with "para-group" cross-reactivity. Depending on the test protocol, skin sensitisation was also observed in animal tests with guinea pigs.

Measurement systems

Analytical measurement systems exist to determine the recommended levels with an appropriate level of precision and accuracy. This comprises both the measurement in air and the determination with respect to biological monitoring.

RECOMMENDATION REPORT

1 CHEMICAL AGENT IDENTIFICATION AND PHYSICO-CHEMICAL PROPERTIES

Name:	Aniline
Synonyms:	Aminobenzene, phenylamine
Molecular formula:	C ₆ H ₅ NH ₂
Structural formula:	

EC No.:	200-539-3
CAS No.:	62-53-3
Molecular weight:	93.127 g/mol
Conversion factors:	1 ppm = 3.87 mg/m ³
(20 °C, 101.3kPa)	1 mg/m ³ = 0.258 ppm

Freshly distilled aniline is a colourless oily liquid that turns dark on exposure to light and air. Aniline has a melting point of -6 °C and a boiling point of 184.1 °C. The vapour pressure at 25 °C is 0.65 hPa. Aniline is moderately soluble in water (about 35 g/l at 20 °C) and soluble in alcohol, benzene and most other organic solvents. A log P_{OW} of 0.90 is reported. The pK_a is 4.6 (25 °C), the density 1.0217 g/cm³ at 20 °C. The substance has a flash point of 76 °C (closed cup) (IARC 1982; ACGIH, 2001; NLM, 2005; ECB, 2004).

2 EU HARMONISED CLASSIFICATION AND LABELLING

Information about the EU harmonised classification and labelling for Aniline is provided by ECHA as summarised in Tables 1 and 2.

Table 1: EU harmonized classification and labelling for Aniline according to ECHA-notification-summary/115877 (ECHA, 2015)

Internat. Chemical Identification	EC no.	CAS no.	Classification		Labelling			Spec. Conc. Limits, M-factors
			Hazard Class & Category Code (s)	Hazard statement code (s)	Pictogram Signal Word Code (s)	Hazard statement code (s)	Suppl. Hazard statement code (s)	
aniline	200-539-3	62-53-3	Acute Tox. 3	H301	GHS06	H301		STOT RE 1; H372: C ≥ 1%
			Acute Tox. 3	H311	GHS09	H311		
			Skin Sens. 1	H317	GHS05	H317		
			Eye Dam. 1	H318	GHS08	H318		STOT RE 2; H373: 0,2% ≤ C < 1%
			Acute Tox. 3	H331	Dgr	H331		
			Muta. 2	H341		H341		
			Carc. 2	H351		H351		
			STOT RE 1	H372		H372		
Aquatic Acute 1	H400		H400					

Classification	Risk Phrases	Safety Phrases	Indication of danger	Concentration Limits	
				Concentration	Classification
Carc. Cat. 3; R40 Muta. Cat. 3; R68 T; R23/24/25- 48/23/24/25 Xi; R41 R43 N; R50	23/24/25	(1/2)	T	C ≥ 25 %	T; R23/24/25
	40	26		1 % ≤ C < 25 %	Xn; R20/21/22
	41	27	N	C ≥ 1 %	T; R48/23/24/25
	43	36/37/39		0,2 % ≤ C < 1 %	Xn;
	48/23/24/25	45			R48/20/21/22
	68	46			
50	61				
		63			

3 CHEMICAL AGENT AND SCOPE OF LEGISLATION

Aniline is a hazardous chemical agent in accordance with Article 2 (b) of Directive 98/24/EC and falls within the scope of this legislation.

Aniline is **not** a carcinogen or mutagen for humans in accordance with Article 2(a) and (b) of Directive 2004/37/EC.

4 EXISTING OCCUPATIONAL EXPOSURE LIMITS

Occupational exposure limits for aniline exist in a number of countries, as shown in the table below. The values presented below represent examples and are not an exhaustive listing of all limit values within the EU and other countries.

Table 3: Existing OELs for aniline; adapted from the GESTIS database (GESTIS, 2015).

EU-countries	TWA (8 hrs)		STEL (15 min)		References
	ppm	mg/m ³	ppm	mg/m ³	
Austria	2	8	10	40	GKV (2011)
Belgium	2	7.7			Royal Decision (2014)
Denmark	1	4	2	8	BEK (2011)
European Union	2	7.74	5	19.35	SCOEL (2014)
Finland	2	7.7	4	15	MoSH (2012)
France	2	10			INRS (2012)
Germany (AGS)	2	7,7	4	15.4	BAUA (2006)**
Germany (DFG)	2	7.7	4	15.4	DFG (2015)
Hungary		8		32	MHSFA (2000)
Ireland	1	3.8			HSA (2011)
Latvia		0.1			GESTIS (2015)
Poland		5		20	MLSP (2002)**
Spain	2	7.7			INSHT(2011)
Sweden	1	4	2	8	SWEA (2011)
United Kingdom	1	4			GESTIS (2015)
Non EU-countries	ppm	mg/m ³	ppm	mg/m ³	
Australia	2	7.6			Safe Work Australia (2011)
Canada (Ontario)	2				Ontario Ministry of Labour (2013)
Canada (Québec)	2	7.6			IRSST(2010)
China		3			GESTIS (2015)

Japan	1	3.8			JSOH (2014)
New Zealand	1	4			HS (2013)
Norway	1	4			GESTIS (2015)
Singapore	2	7.6			GESTIS (2015)
South Korea	2	10			GESTIS (2015)
Switzerland	2	8	4	16	SUVA (2015)
USA (NIOSH)	LFC *				NIOSH (2007)
USA (OSHA)	5	19			OSHA (2006)

* Lowest Feasible Concentration

** Updates show no data on aniline, therefore the value is still considered valid

In addition to the above OELs, there are also biological threshold limit values established in the following countries:

EU: Biological Limit Value (BLV): 30 mg/l, measured as mg of p-aminophenol per liter of urine, measured 0-2 h after the end of exposure or at end of shift (SCOEL, 2010).

Germany: The following BAT values ("Biologischer Arbeitsstoff-Toleranz-Wert": biological tolerance value for occupational exposures), defined as the maximum permissible quantity of a chemical substance or its metabolites or the maximum permissible deviation from the norm of biological parameters induced by these substances in exposed humans, were established DFG (2015) , BAUA (2013).

- BAT=500 µg/l measured as µg of aniline (after hydrolysis) per liter of urine, measured for long-term exposures: after several shifts;
- BLW = 100 µg/l measured as µg of aniline (released from aniline-haemoglobin conjugate) in B_E (erythrocyte fraction of the whole blood).

Spain: Biological Limit Value (BLV): 30 mg/l, measured as mg of p-aminophenol per liter of urine, measured 0-2 h after the end of exposure or at end of shift; INSHT Instituto Nacional de Seguridad e Higiene en el Trabajo (INSHT 2011).

USA: A Biological Exposure Index (BEI) of 50 mg/L of p-aminophenol in urine measured at the end of shift has been established by ACGIH (2013).

According to Drexler and Greim (2008), the Senate Commission for the Investigation of Health Hazards of Chemical Compounds (MAK Commission) of the German Research Foundation also considered a limit of 5 % Met-Hb in post-shift blood samples, however, without formally establishing a BAT value.

5 OCCURRENCE, USE AND OCCUPATIONAL EXPOSURE

5.1 Occurrence and use

Aniline is used as a parent substance in the chemical industry for the syntheses of many compounds, e.g., dyes, antioxidants, rubber accelerators, drugs, photographic chemicals, isocyanates, herbicides and fungicides (ACGIH, 2001).

Besides being a compound with distinct occupational use, there is low background exposure in the general population. A cross-sectional population-based survey in Bavaria/Germany showed detectable levels of aniline in 93.9 % of 1 004 persons (Kütting et al., 2009). According to previous data of this group (Weiss and Angerer, 2002), the excretion of aniline in the general population was 3.5 µg/l urine (median; upper 95th percentile: 7.9 µg/l).

5.2 Production and use information

According to ICIS (2008) global aniline production capacity was 4.98m tonnes/year in 2006, divided as follows:

Region	Aniline production (tonnes/year)
Western Europe	1.62 million
US	1.38 million
Asian-Pacific (excluding Japan)	1.15 million
Japan	474.000
Eastern Europe	316.500
Latin America	70.000
Asia/Middle East	64.000

The major starting chemical in the manufacturing of aniline is nitrobenzene. The oldest method for the reduction of nitrobenzene uses iron and acetic acid. Currently, most of the production is based on a modern method applying catalytic reduction of nitrobenzene (ICIS, 2008). After hydrogenation, the reaction mixture is separated to an organic phase containing aniline with dissolved water and an aqueous phase containing 4% aniline. The crude aniline is purified by distillation. Aniline is stripped from the aqueous phase and returned to the raw condensate. Alternative methods are based on the ammonolysis of phenol or chlorobenzene, which according to ICIS (2008) are only used by one Japanese company.

Among the different uses of aniline, the production of methylene diphenyl diisocyanate (MDI) accounted for over 81% of world aniline consumption in 2014 (IHS, 2015). Other large-scale applications of aniline include use as a chemical intermediate for rubber-processing chemicals, dyes, and pigments, plant protecting products and pharmaceuticals.

Further recent and quantitative data by TMR (2015) inform about the share of the current aniline consumption in different end-uses as follows:

- insulation: 46%
- rubber products: 12%
- consumer goods: 12%
- packaging: 7%
- automotive: 7%
- others: 16%

Until recently, most information sources reported the EU to be the largest consumer of aniline (ICIS, 2008; ECB, 2004). However, more recent data by (IHS, 2015) show a stronger Asian consumption, in particular in China, as follows:

Region	Aniline consumption (%)
China	39%
Western Europe	25%
USA	17 %
Japan	6%
South Korea	5%
Central Europe	3%
CIS/Baltic States	2%
India	2%
Brazil/other	1%

5.3 Occupational exposure

Occupational exposure mainly may occur during aniline manufacture, distribution and use, but also may be expected if formulations with residual aniline contents are handled, e.g. dyes, or as a result of decomposition, e.g. during rubber vulcanisation (ECB, 2004). Exposures are reported to be less than 1 ppm (TWA) in the manufacture of aniline; in the use of aniline TWA only occasionally exceed 1 ppm and are expected to be mostly below 0.5 ppm (HSE, 1997).

5.4 Routes of exposure and uptake

Relevant routes of exposure at the workplace are inhalation and skin contact. Under contemporary industrial conditions, skin contact is more relevant. Therefore, it is important that methods for biological monitoring are available, which can be recommended.

6 MONITORING EXPOSURE

Aniline can be monitored in the air of the workplace by applying the following methods (NIOSH 2011):

- OSHA PV2079
- NIOSH method 2002
- NIOSH method 2017

In all three methods aniline is sampled from the air in the workplace by adsorption onto a solid sorbent or absorption into solution, followed by extraction of aniline with an organic solvent. The aniline-containing extract can then be analysed by gas chromatography (GC), using flame ionisation detection (FID) or a nitrogen-specific detector (NSD), as shown in Table 4.

Table 4: Overview of sampling and analytical methods for monitoring aniline in the workplace.

n.d.: not determined

Method	Sorbent	Desorption solution	Analysis	Recovery (%)	LOQ	Concentration range	References
OSHA PV2079	Acid coated XAD-7 tube	Methanol ammonium hydroxide	GC-FID	91.4	0.03 mg/m ³	n.d.	OSHA 1994
NIOSH method 2002	Silica gel	Ethanol	GC-FID	98	0.01 mg/sample	0.1-3 mg/sample	NIOSH 1994a
NIOSH method 2017	Filter sulphuric acid treated+ silica gel	Ethanol	GC-FID	100	4 µg/sample	31-255 µg/sample	NIOSH 1998

OSHA PV2079 (OSHA 1994) is a partially evaluated method. The NIOSH 2002 method has been completely evaluated for aniline and states that use of a nitrogen-specific GC detector instead of a flame ionisation detector will greatly increase sensitivity. NIOSH method 2017 is partially evaluated and is considered to be a revision and combination of NIOSH method 2002 (NIOSH 1994a) and NIOSH method 2005 (NIOSH 1994b).

Biological monitoring of aniline exposures in the workplace can be carried out by measurement of

- methemoglobin (MHb) in blood;
- aniline in urine;
- haemoglobin adducts of aniline in blood.

Monitoring MHb in blood was applied before the introduction of methods based on the determination of the adducts of aniline released from haemoglobin, which are considered to be more specific (Drexler and Greim 2004; IPA 2013; Käfferlein et al. 2014; Lewalter and Gries 2001; Neumann 1988).

An overview of the available methods and their performance characteristics is presented in Table 5 below.

Table 5: Overview of the available methods for biomonitoring of occupational exposures to aniline.

Method	Application	Analysis	Standard deviation (rel)(Sw)*	Prognostic range(u)*	Recovery (%)	Detection limit	References
NIOSH Nr. 8317	Determination of aniline in urine	HPLC with electrochemical detection	n.d	1.4-1200 ng/mL	93-109	1.4 ng/mL	NIOSH 2003
MAK, August 1993	Determination of aniline in urine, plasma and erythrocytes	GC/MS	u:10.4 and 15.9% p:7.8 and 10.8% H:8.3 and 7.8%	u:21.8 and 33.3% p:26.2 and 19.7% H:17.4 and 16.3%	u:81-117 p:85-112 H:85-110	1.0 µg/L blood/urine or plasma	Lewalter and Biedermann 1994
MAK, May 2000	Determination of the adducts of aniline released from haemoglobin	GC/MS selective detection with negative chemical ionisation	4.5%	10.1 %	103%	1.0 ng/L blood	Lewalter and Gries 2001
Various methods	Met-Hb in blood	Spectrophotometric multicomponent analysis; a review is provided by Cruz-Landeira et al. (2002)					

*within series imprecision

u: urine ; p:plasma ; H:haemoglobin

In essence, there are no measuring difficulties at the proposed OEL and BLV levels.

7 HEALTH EFFECTS

Damage to the haematopoietic system is a result of the toxic effects on the erythrocytes that is caused by the methaemoglobin-forming activity of aniline. The spleen in particular can be damaged by the increased degradation of damaged erythrocytes and the resulting overload with cell debris, haemoglobin (Hb) and redox-active iron. Another aspect in animal studies is the increased extramedullary haematopoiesis in the spleen.

7.1 Toxicokinetics (*absorption, distribution, metabolism, excretion*)

7.1.1 Human data

Aniline is well absorbed following inhalation, oral and dermal exposure.

The results of earlier controlled studies with volunteers, in which uptake of aniline vapour through the skin were compared with aniline uptake via inhalation, were summarised by Lehnert and Henschler (1986) and Henschler (1992). At 25°C resting volunteers absorbed about 90 % of aniline vapour by inhalation and the absorption of aniline vapour from the skin and the respiratory tract were very similar. The rate of dermal uptake of aniline vapour was increased after a rise of temperature and humidity. Clothing (worker overall) reduced the dermal uptake from aniline in air so that the ratio of respiratory to dermal uptake increased from about 1:1 to 2.5:1 (Dutkiewicz and Piotrowski, 1961). Liquid aniline was also well absorbed through the skin. Based on the urinary excretion of the metabolite 4-aminophenol it was estimated that – depending on exposure time, temperature and moisture – up to 38 % of liquid aniline may be absorbed through the skin (Baranowska-Dutkiewicz, 1982). Impairment of the skin (e.g. erythema) facilitates the skin absorption of aniline (Korinth et al. 2008).

Quantitative data regarding oral absorption are not available. However, the increase in MHb levels following oral intake of aniline (see below section 7.2.1) shows that aniline is very well absorbed via the oral route.

A half-life of 3.5 h for aniline in humans has been reported, but without further details (Henschler, 1992). Nitrosobenzene, the product of aniline *N*-hydroxylation and subsequent oxidation, may react with sulfhydryl groups of glutathione and proteins, e.g. albumin and haemoglobin, and these conjugates can be found for much longer time periods (Lewalter and Korallus, 1985).

The metabolism of aniline and the excretion is qualitatively similar in humans and animals (see below). About 50 % of Europeans have a genetically caused lower activity of *N*-acetyltransferase (so called "slow acetylators"). This leads to a retardation of *N*-acetylation (ECB, 2004).

Quantitatively, only a small fraction of absorbed aniline is eliminated unchanged in urine and exhaled air (ACGIH 2001), and metabolism is the main elimination pathway. Volunteers inhaling aniline in concentrations of 5 to 300 mg/m³ (about 1.25 – 75 ppm) for 5 h exhaled less than 0.25% of the absorbed dose (Dutkiewicz, 1961). The remainder is likely metabolised by *N*-oxidation, or is excreted in urine as other metabolites (Parke, 1960; Bus and Popp, 1987; ACGIH, 2001).

Käfferlein et al. (2014) conducted a controlled human exposure study in volunteers. In a first pilot section of this study, 2 males and 2 females were exposed for 8 hours to 2 ppm airborne aniline. In this pilot experiment, exposure to aniline was carried out at 4 x 2-hour intervals, with 15-min breaks in between to facilitate blood sampling. The persons exercised for 4 x 20 min on a cycle ergometer to a previously determined aerobic/anaerobic threshold while being simultaneously exposed. This resulted in an increase of methaemoglobin levels up to 1.6 %, with a plateau after 6 hours. The maximal excretion of aniline in urine after exposure was 306 µg/l. The following main study included 19 non-smoking persons (10 males, 9 females) who were exposed to 2 ppm aniline for 6 hours. This period was broken into 3 x 2-hour intervals, with 15-min breaks for blood sampling. The persons exercised for 3 x 20 min on a cycle ergometer

during exposure in their previously determined individual workload (leading to a mean ventilation rate of approximately 30 l/min during exercise). The basal methaemoglobin level prior to exposure was 0.72 ± 0.19 %. Following exposure, the maximum methaemoglobin level in blood was 1.21 ± 0.29 % (range: 0.8–2.07 %), and aniline excretion in the urine was 168.0 ± 51.8 $\mu\text{g/l}$ (range: 79.5 ± 418.3 $\mu\text{g/l}$). After 24 hours, the mean level of methaemoglobin returned to the basal level (0.65 ± 0.18 %). No significant differences between males and females were noted. Details of the time course of methaemoglobin in blood during and after exposure are shown in Figure 1; the time course of aniline excretion in urine is shown in Figure 2. There was an elevation of the level of aniline-haemoglobin adducts, which depended on the acetylator status (NAT2; slow or fast acetylators). By contrast, the methaemoglobin levels and aniline excretion showed no significant effect of the acetylator status.

The study of Käfferlein et al. (2014) shows that a single exposure to aniline for 6–8 hours leads to methaemoglobin levels, which are far below the level of 4–5 %, which is regarded as being critical. The data also confirm that there will be no carry-over of elevated methaemoglobin to the next shift upon daily repetitive exposure. As the individual maximal methaemoglobin level under the given experimental conditions was 2.21 %, even a moderate physical activity will not lead to exceeding the critical level.

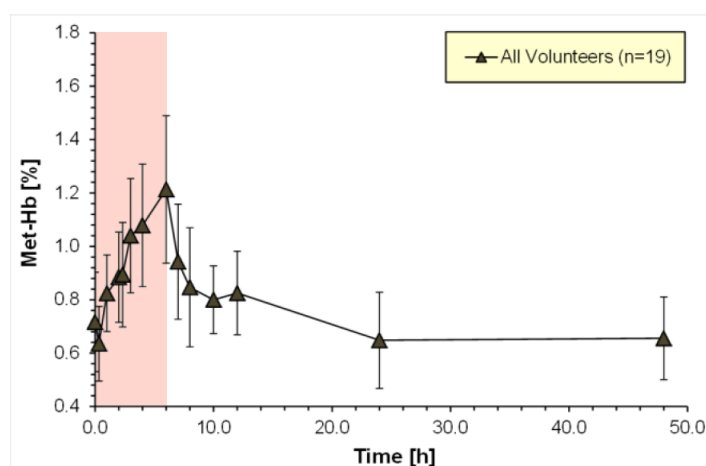


Figure 1. Time course of methaemoglobin (Met-Hb) levels in blood of 19 volunteers during and after experimental exposure to 2 ppm airborne aniline (from Käfferlein et al., 2014a).

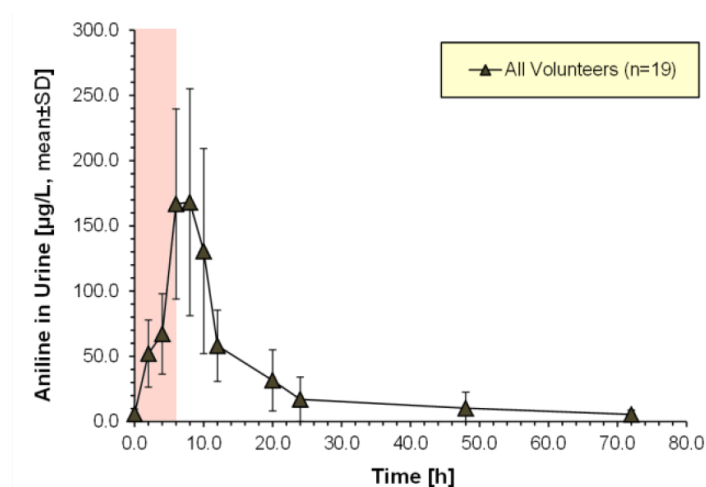


Figure 2. Time course of aniline excretion in the urine of 19 volunteers during and after experimental exposure to 2 ppm airborne aniline (from Käfferlein et al., 2014a).

In addition, the same group (Modick et al., 2016) reported that acetalilide and free aniline were excreted only in minor amounts, accounting for 0.14-0.36% of the dose.

7.1.2 Animal data

Aniline is well absorbed following all routes of administration. Oral absorption amounts to more than 90 % in rats and 56 %, 72 %, and 80 % for pigs, mice and sheep (ECB, 2004).

In the body, aniline is widely distributed, the highest concentrations being found in red blood cells, plasma, spleen, kidney, liver, bladder and the gastrointestinal tract. Due to their basicity, aniline and *N*-acetylaniline undergo enterogastric cycling (ECB, 2004). Aniline is able to cross the placenta. The concentration of aniline was slightly higher in foetal than in maternal blood of rats, while the half-life was 1.5 h in both foetal and maternal blood plasma (Maickel and Snodgrass, 1973).

Aniline is metabolised primarily in the liver by three metabolic pathways: *N*-acetylation, aromatic ring hydroxylation and *N*-hydroxylation. Small amounts of aniline are hydroxylated to 2- and 4-aminophenol which are conjugated and excreted in urine. The main pathway is *N*-acetylation followed by *p*-hydroxylation to *N*-acetyl-*p*-aminophenol (paracetamol/acetaminophen), which is mainly excreted in urine as sulphate in rats and as glucuronide in other species. In rats, sulphate conjugation becomes saturated at higher doses (250 mg/kg) and the proportion of glucuronide conjugates, of unconjugated metabolites and unchanged aniline increases. After a single dermal application of aniline in rats and mice, most of the dose is excreted as metabolites within 24 hours in urine (HSE, 1997).

N-Hydroxylation is a minor metabolic pathway but the most important step in the bioactivation of aniline. The product, phenylhydroxylamine (PHA), acts as substrate in a co-oxidation process in the erythrocytes by which nitrosobenzene and methaemoglobin (MHb) are formed. Since nitrosobenzene in erythrocytes may enzymatically be reduced back to phenylhydroxylamine, redox cycling is initiated with ongoing oxidation of haemoglobin to MHb. Although aminophenols may also initiate redox cycling, their formation from aniline and their *in vivo* potency are much lower than that of PHA so that PHA is the sole mediator of aniline-induced MHb-formation (Harrison and Jollow, 1987).

MHb is reduced back by erythrocyte NADH-dependent MHb-reductase. Under normal conditions, this is the only system that maintains Hb in its oxygen-carrying reduced state (and hence MHb-levels at or below 1 %) and is the rate-limiting enzyme controlling the toxicokinetics of MHb-reduction. Comparative investigations have shown species-specific differences with a five and ten times higher activity of MHb-reductase in erythrocytes from rats and mice than in human erythrocytes (ECB, 2004). On the other hand, the MHb-reductase activity in Beagle dogs is lower than in rodents and humans (Srivastava et al., 2002; Rockwood et al., 2003). Since Beagle dogs also lack arylamine *N*-acetyltransferases (Collins, 2001), they are much more susceptible to MHb-aemia than even slow-acetyllating humans (Pauluhn, 2005).

7.1.3 In vitro data

A comparative study on percutaneous penetration of a number of chemicals, including aniline, provided additional support for the assignment of a "skin" notation (Korinath et al 2012). The flux estimation for aniline was $752.2 \pm 213.5 \mu\text{g}/\text{cm}^2/\text{h}$ (mean \pm SEM).

7.1.4 Biological Monitoring

Nitrosobenzene, the metabolically active product of aniline *N*-hydroxylation and subsequent oxidation, can react with SH-groups of glutathione and proteins forming conjugates. By this reaction, covalent haemoglobin (Hb) adducts of aniline are formed which can be used for biological monitoring. These adducts are stable and detectable long after exposure has ceased and aniline and its metabolites have been eliminated. The

results of this determination are almost independent of sampling time provided that at least 3 consecutive days of aniline exposure have occurred prior to sampling. Elimination of these haemoglobin adducts occurs with the degradation of erythrocytes, which have a lifetime of about 3 months. Therefore, the determination of Hb-adducts allows for the assessment of an exposure over a period of several weeks whereas the determination of the MHB-level indicates short-term exposure (Sabbioni and Jones 2002).

Analytical methods for quantification of the haemoglobin adducts have been described (Kutzer et al 1997; Lewalter and Gries 2011; Lewalter and Korallus 1985;) and validated (DFG, 2000). In earlier publications on workers regularly exposed to aniline and of cases of acute aniline poisoning in workers it was stated that, when MHB levels were below 5 %, less than 100 µg aniline/l blood could be detected. It was also stated that MHB < 5 % can be expected when the concentration of unbound aniline in urine does not exceed 1 mg/l (Lehnert and Henschler 1986; Lewalter and Korallus 1985). This led to a recommendation of a biological limit (BAT value) by the MAK Commission of 100 µg aniline released from haemoglobin per liter blood and of 1 mg/ aniline / litre urine (Lehnert and Henschler 1986). An analytical method to determine aniline in urine based on gas chromatography-mass spectrometry has been described and validated by Weiss and Angerer (2002).

More recent publications on biological monitoring of aniline exposure by analysing haemoglobin adducts confirm that exposed and non-exposed subject can well be distinguished, including persons exposed to aniline by tobacco smoking (Korinth et al 2008; Richter et al 2001; Thier et al 2001). However, compared to the collectives of workers reported until the mid-1980s the occupational exposure to aniline in the recent studies was always very low, so that the biological limits recommended earlier could no longer be validated. For instance, haemoglobin adduct levels were reported for "exposed workers in the rubber industry" of 2.57 ± 1.18 , and for workers in a nitrobenzene reduction plant producing aniline of 5.18 ± 5.19 µg aniline / litre blood (mean ± SD; Korinth et al 2008; Thier et al 2001).

Independent of the route of administration, the characteristic toxic effect of aniline in humans and animals is methaemoglobinaemia. Metabolic activation of aniline is a prerequisite for MHB formation, aniline does not induce MHB formation in vitro (see section "toxicokinetics"). In methaemoglobin, the haem iron of haemoglobin is oxidised from the ferrous to the ferric state. In contrast to haemoglobin (Hb), MHB is unable to transport oxygen. Accumulation of MHB in erythrocytes and the corresponding decline in the level of oxygen-transporting Hb leads to toxic symptoms, the severity depending on the percentage of MHB. The first visible sign of MHB poisoning is cyanosis, which is followed by neurological and other symptoms of deficient oxygen delivery at higher concentrations. Other effects include protein, especially, Hb denaturation with the appearance of denatured protein deposits (Heinz bodies) in red blood cells. Heinz bodies may be observed after acute intoxications but mostly occur after repeated exposure to lower doses.

Although the formation of MHB represents the relevant endpoint of toxicity for OEL derivation (see Recommendation), determination of the MHB level, under the conditions of industrial practice, is not considered suitable for biological monitoring. After blood sampling the MHB reductase remains active in the erythrocytes, so that the time interval between blood sampling and MHB measurement becomes very critical (ACGIH 2001). Instead, ACGIH (2001) has recommended a BEI for the p-aminophenol excretion in urine (50 mg / g creatinine), after acid hydrolysis of the urinary conjugates. This was mainly based on the experimental human exposure studies by Dutkiewicz (1961) and supported by limited field studies at that time. For this parameter, there are no analytical difficulties, as outline by ACGIH (2001). However, there may be an interference in persons taking paracetamol (N-acetyl-p-aminophenol) as medicine, of which p-aminophenol is a urinary metabolite.

Relevant new data on biological monitoring became available with the human experimental exposure study described in Section 7.1.1.

7.2 Acute toxicity

7.2.1 Human data

Acute aniline poisoning in workers was frequent several decades ago. The effects are attributed to the formation of MHB. The victims suffered from cyanosis (which is why they were called "blue boys") and more severe symptoms at higher exposure (ECB 2004). Severe poisoning has been observed in combination with alcohol intake, which is reported to increase aniline toxicity (Henschler 1992).

According to earlier observations, 400-600 mg/m³ may be tolerated without much harm for up to one hour, whereas several hours of exposure to 100-250 mg/m³ produce slight symptoms. Concentrations of about 25000 mg/m³ or 0.35-1.43 g/kg bodyweight are reported to be lethal. Workers may develop some tolerance to symptoms but the cyanosis may persist (Smyth 1931).

Generally, an increase in MHB above the normal background level in blood (about 1.1 %) to 15 % will be without signs and symptoms. However, as known from CO-induced oxygen deficiency for sensitive risk groups (persons with latent restricted coronary or arterial function), much lower MHB-levels may be tolerable (Bolt et al 1985). Clinical cyanosis but no hypoxic symptoms except for a possible slight euphoria will develop at about 15-20 % MHB and more. Fatigue, anxiety, headache, weakness, dizziness, tachycardia, dyspnoea, and syncope will occur at 30-45 % MHB. Higher concentrations will cause a decreased level of consciousness and finally coma, heart failure and death at more than 60-70 % MHB (Henschler 1992; HSE 1997; NRC 2000).

Oral intake of 60 ml of aniline was fatal with death occurring the 4th day. Pathology revealed degenerative changes in myocardium, liver and kidney, lung oedema and oedema and haemorrhages in brain. Non-fatal but severe cases showed marked MHB-aemia with deep cyanosis, headache, collapse, coma, shock and generalised seizures (ECB 2004; Iwersen-Bermann and Schmoldt 2000).

The dose-response-relationship between oral aniline intake and MHB-formation was studied in 20 volunteers, which received a bolus dose of 5, 15 or 25 mg/day on three consecutive days. Some volunteers were given higher doses on subsequent days. All volunteers were healthy with no evidence of glucose-6-phosphat dehydrogenase deficiency in screening tests (Jenkins et al 1972). The mean maximum increase in MHB-formation occurred in less than 4 hours. After intake of 5 mg and 15 mg the increase of MHB was not significant (1.2% or 1.8%, respectively). Significant increases were seen at 25 mg (2.5 % MHB) and more. Doses of 35, 45 and 55 mg/person led to maximum MHB increases of 3.7 % (n=5); 7.1 % (n=5) and 5.2 % (n=2). At the highest dose of 65 mg, an MHB-level of 16 % was reached in the only volunteer exposed to this dose. As these data are pivotal for the derivation of an OEL, they are also included as Figure 1 in the 'Recommendation' section. No Heinz bodies were detected at any dose. With respect to MHB-formation after aniline exposure, humans appear much more sensitive than rats which were orally exposed to aniline in the same study (see below).

7.2.2 Animal data

Inhalation

A 4-h LC50 of 839 ppm (3.27 g/m³) was determined for rats, which were exposed nose-only to a mixture of aniline vapour and aerosol. A considerably lower 4-h LC50 of 478 ppm – indicating inhalation and dermal uptake – was determined in the same study in rats, which were exposed whole-body (Du Pont de Nemours and Co 1982).

As part of a subacute study (Kim and Carlson 1986, see below), rats were also exposed head-only once to 100 ppm aniline for up to 12 hours. Methaemoglobin levels as

estimated from the original graph were at about 10 % at 3 hours, 18 % at 6 hours and 23 % at 8-12 hours.

The time- and concentration-dependent aniline-induced MHB-formation was studied in male Beagle dogs (Pauluhn 2005). Four animals/group were exposed head-only to aniline vapour concentrations ranging from 15.8-493.6 mg/m³ (4-127 ppm) for up to 4 hours. MHB levels were at baseline level (< 0.8 %) at 15.8 mg/m³ (4 ppm). A significant increase of MHB occurred at 30.3 mg/m³ (7.8 ppm) and higher concentrations after at least 1 hour of exposure. At 243.4 mg/m³ (63 ppm), MHB-formation reached 10 % after 4 hours. At the same concentration, much higher MHB-levels occurred in one dog that was hyperventilating during exposure due to stress (about 40 % MHB) and in dogs that were exposed not nose-only but whole-body to aerosol-free aniline vapour (about 30 % MHB). Cyanosis in non-panting, "head only" exposed dogs was mild at 58.6 mg/m³ (15 ppm) but became more pronounced and was accompanied by salivation and paling of mucous membranes at 243 mg/m³ (63 ppm) and above. Haematological indices showed no consistent time- or concentration-related effects at any dose. Analysis of the entire concentration-response relationship indicates that after a 4-hour exposure a methaemoglobin concentration of 15 % is reached at 300 mg/m³ (77.3 ppm) and of 4 % at 160 mg/m³ (41 ppm). 23.6 mg/m³ (6.1 ppm) and 20.6 mg/m³ (5.3 ppm) are the threshold concentrations to cause a measurable increase (0.8 % MHB) in MHB-formation after 4 or 8 hours of exposure.

The formation of MHB after inhalation and oral administration (see below) was compared in Beagle dogs (Pauluhn 2002). Two groups of 4 animals each were exposed 4 hours head-only to 155 mg/m³ (40 ppm) or to 174 mg/m³ (45 ppm; corresponding to an exposure dose of 14.6 mg/kg). Dogs showed either no clinical signs (3/8) or a slight to moderate transient cyanosis of mucous membranes (4/8); animals were clinically unobtrusive shortly after cessation of exposure. Cyanosis persisted during the day in one dog, which showed panting and hyperventilation as signs of stress due to the exposure conditions. The MHB-level peaked within one hour after the end of exposure and reached a mean of 4.7 % at maximum (range 2.3-4.8 %) except for the panting and hyperventilating animal in which the MHB-level reached 24 %.

Oral

Oral LD50 ranging from 442 mg/kg to 930 mg/kg bw were obtained in studies with rats (ECB 2004).

MHB-formation after oral (gavage) administration of aniline to male rats was compared to the response in humans. In rats, 20 mg/kg bw led to a non-significantly increased MHB level of 3.3 %; doses of 40-300 mg/kg bw led to MHB levels between 12.3 and 18.1 % (no clear dose response). At 1000 mg/kg bw, the MHB level increased to about 48 % (Jenkins et al 1972).

MHB-formation after inhalation and oral administration was compared in Beagle dogs (Pauluhn 2002; see above). The animals received an oral dose of 15 mg/kg bw by gavage. Cyanosis was seen at the visible mucous membranes after administration but animals appeared normal next day. Marked differences were observed in the MHB-level after oral and inhalation exposure to the same doses (15 mg/kg bw) of aniline: The maximum MHB-level reached a mean of 26.4 % after oral exposure but was approximately 5-fold lower (mean: 4.7 % MHB) after inhalation exposure of dogs. Hyperventilation increased inhalation uptake of aniline and the MHB-level to nearly the same level that was observed after oral exposure. Similar although less pronounced differences were observed when the concentration of serum albumin adducts of aniline were measured.

As dogs, cats appear more sensitive than rats: MHB levels of > 80 % were observed 4 hours after administration of doses as low as 51 mg/kg bw (ECB 2004) and a dose of 5.8 mg/kg caused mean MHB level of 25.2 % (McLean et al 1969).

Dermal

Acute dermal LD50 of 1,540 and 1,290 mg/kg were determined for rabbits and guinea pigs, respectively. Cats appear to be more sensitive than these rodents, an LD50 of 254 mg/kg was reported for this species (ECB 2004).

Furthermore, a study with dogs clearly revealed that aniline vapour is taken up through the intact skin in high amounts and contributes to systemic aniline toxicity (Pauluhn 2002; see above).

7.2.3 In vitro data

There are classical early contributions to the toxicity of aromatic amines, including aniline, using in vitro methods. It was found that aniline itself does not produce methaemoglobin with erythrocytes in vitro. For this toxicity, the transformation to N-hydroxy-aniline is essential that is located in the liver (for review, see Uehleke 1973).

7.3 Specific Target Organ Toxicity/Repeated Exposure

7.3.1 Human data

In an occupational study of workers in a plant producing diphenylamine in which aniline was used as the raw material, aniline and hydrogen chloride were reported to be the only harmful chemicals to which the workers were exposed. The concentration of aniline was in the range of 1.3-2.75 mg/m³. A "definite" increase in Mhb content was claimed during the first year (no data presented) in the group of workers compared to the control; decreases in haemoglobin levels, erythrocyte count, and coagulation factors were also described. The validity of the effects claimed in this study cannot be assessed (Vasilenko et al 1972a, b).

7.3.2 Animal data

Studies with repeated administration have been carried out with rats and, to a very limited extent, with mice, guinea pigs and dogs.

Irrespective of the route of administration, the toxicity of aniline in rats manifests in effects on the red blood cells and the haematopoietic system with corresponding effects on the spleen, bone marrow, liver and kidney. Cyanosis with increased Mhb levels, erythrocyte lesions with formation of Heinz bodies and haemolytic anaemia are characteristic observations. Reticulocytosis and increased bone marrow and extramedullary erythropoiesis are compensatory reactions of red blood cell toxicity. The damaged erythrocytes are scavenged mostly in the spleen. Accumulation of haemosiderin in the spleen and sometimes also in liver and kidney, spleen congestion, dark colouration and increased spleen weight are observed after repeated administration. Splenitis, spleen hyperplasia and fibrosis are further signs after long-term exposure to aniline.

Except for a black discolouration of the spleen, no histological evidence of haematotoxicity was found in mice. However, a more detailed clinical and haematological examination has not been performed in this species.

Inhalation

In a subacute study, male Wistar rats (30/group) were exposed nose-only to 9.2, 32.4, 96.5, and 274.9 mg aniline/m³ (2.4; 8.4; 24.9; 70.9 ppm) for 6 h/day, 5 days/week (day 0-11), followed by a 2-week post-exposure observation period (up to day 28). Serial sacrifices were performed on days 0, 4, 11, 14, and 28. No mortality was observed during the study. Cyanosis was seen at concentrations ≥ 96.5 mg/m³ and did not progress during the exposure period. MHB-formation and associated erythrocytotoxicity were the main signs of toxicity. Changes included anemia, Heinz bodies, decreased haemoglobin and haematocrit, reticulocytosis, and effects on the spleen (splenomegaly, hemosiderin accumulation, increased hematopoietic cell proliferation), which were significant at 96.5 and 274.9 mg/m³. The NOAEC with respect to erythrocytotoxicity and associated effects was reported to be 32.4 mg/m³ (8 ppm). However, there was a borderline increase in splenic extramedullary hematopoiesis at this concentration which was judged by the author a homeostatic response rather than an unequivocal adverse effect. 9.2 mg/m³ (2.4 ppm) were not associated with any significant effect (Pauluhn, 2004).

In another subacute study, male CD rats were exposed head-only to 0, 17 ppm, 45 ppm, or 87 ppm (0; 64.7; 171.4 or 331.3 mg/m³) aniline vapour, 6 hours/day, 5 days/week, for 2 weeks (du Pont de Nemours and Co, 1982). MHB was significantly elevated at ≥ 45 ppm, but not at 17 ppm. This increase was accompanied by clinical symptoms as the animals exposed to 87 ppm were judged as slightly cyanotic. Haematotoxicity with concurrent spleen effects were seen at ≥ 45 ppm. Minimal or isolated splenic histopathological alterations were noted at 17 ppm (LOAEC).

Up to 15 male Sprague-Dawley rats/groups were exposed nose-only to 0, 10, 30, 50, or 150 ppm (0, 38, 115, 192, or 578 mg/m³) aniline for 8 hours/day for 5 days or for 12 hours/day for 4 days. One week after the initiation of exposure, the haematocrit was decreased by about 10 % at ≥ 30 ppm. MHB levels were increased above control levels at the beginning of the 2nd day of exposure to 50 and 150 ppm, with a tendency to cumulate. A slight increase in MHB (5 % MHB) but no cumulative effect was observed at 30 ppm (NOAEC: 10 ppm or 38 mg/m³) (Kim and Carlson, 1986).

In a subchronic study 9 male Wistar rats and 2 dogs were exposed for 26 weeks, 20 female albino mice and 10 guinea pigs animals were exposed for 20 weeks to 5 ppm (19 mg/m³) aniline vapour (whole body) for 6 hours/day, 5 days/week (Oberst et al., 1956). Rats were reported to lose weight during the last 3 weeks of exposure but no data were tabulated. Blood analysis indicated a marginal increase in MHB in rats only (0.6% in the 23rd week, no statistics or control levels given). Also, all rats showed a mild bluish skin colouration of their extremities, tail, eyes and nose during the last 4 weeks of exposure. There were no substance-related pathological alterations in any species. The validity of the study is limited because only one concentration was tested, histopathology and statistics were incomplete and autopsy was carried out only in a small number of animals. Based on the doubtful marginal increase of MHB and the absence of spleen toxicity, the concentration may be considered a NOAEC.

Oral

In a subacute study on the mode of action of aniline toxicity, aniline hydrochloride was fed with the diet to groups of 12 male F344 rats for one and four weeks (Mellert et al., 2004). All animals were sacrificed on day 28/29 after study begin. Dietary (weekly adjusted) nominal dose levels were 0, 10, 30, or 100 mg/kg · d but due to the instability of the test substance in the diet the actual corrected test substance intakes were 0, 6, 17 and 57 mg/kg · d of aniline hydrochloride (0, 4, 12 and 41 mg/kg · d aniline). Haemoglobin adducts of aniline were detectable at all dose levels after 1 and 4 weeks of aniline feeding. Heinz bodies were not observed in control animals and sporadically at the lowest dose, but were significantly increased at ≥ 12 mg/kg · d after 1 and 4 weeks. Haematological findings at ≥ 12 mg/kg · d indicated the presence of anaemia. At the highest dose, overt haemolytic anaemia was accompanied with an increase in serum transferrin and iron binding in blood, which reflected the perturbations in iron metabolism. Furthermore, a neutrophil leucocytosis at this dose indicated an inflammatory process in the spleen. With respect to organ pathology, minimal vascular

congestion of the spleen occurred in 2 animals after 1 week and in 4 animals after 4 weeks at 4 mg/kg · d. Additionally, spleen weight was increased after 1 and 4 weeks at ≥ 12 mg/kg · d. Effects were much more severe at 41 mg/kg · d and included focal perisplenitis with moderate vascular congestion and haemosiderin deposition in Kupffer cells of the liver. The LOAEL in this study was 4 mg/kg · d (no NOAEL). The authors concluded that the findings corroborate the contention that carcinogenic doses of aniline (see section carcinogenicity) lead to early haematological effects, inflammation reactions of the spleen and perturbations in iron metabolism.

In a chronic/carcinogenicity study with rats (CIIT, 1982, see below), haematological effects (reticulocytosis, decrease of erythrocyte counts, haemoglobin, haematocrit, increase in MCV), haemosiderosis and splenic haematopoiesis were observed at the lowest applied dose of 7 mg/kg · d (LOAEL).

Further studies with oral application by gavage, in drinking water or food revealed similar effects on blood and spleen in rats (Short et al., 1983; Jenkins et al., 1972; Khan et al., 1995, 1997; Gralla et al., 1979; CIIT, 1977; NCI, 1978). For corresponding effects, no NOAELs were derived in these studies and/or the LOAELs were higher than those in the two reported studies.

Dermal

There are no data available.

7.3.3 In vitro data

As outlined above, aniline itself does not produce methaemoglobin with erythrocytes in vitro. For this toxicity, the transformation to N-hydroxy-aniline is essential that proceeds in the liver (review see Uehleke 1973).

7.4 Irritancy and corrosivity

7.4.1 Human data

Aniline has a characteristic, somewhat pungent odour, which is detectable at 1 ppm (ACGIH 2001).

Although aniline has been used in a variety of industrial applications for many years, no data on local irritant effects of aniline on eyes, skin, or mucous membranes are available. Also, no such effects were described in clinical human studies.

7.4.2 Animal data

No data are available regarding respiratory irritation.

Skin

In standard tests on irritation, the application of undiluted aniline caused only slight erythema but no other effects on the skin of rabbits. However, in another test after a single application of 100-900 mg/kg bw to the skin of rats and rabbits signs of dermatitis were observed within 3-5 days, which resolved after 2-3 weeks. In a test, which was to study the acute dermal toxicity of aniline, rabbits developed subdermal haemorrhages and severe erythema after dermal exposure to undiluted aniline (ECB 2004; HSE 1997).

Eyes

After instillation of undiluted aniline into the eyes of rabbits, severe corneal opacity and severe conjunctival erythema and oedema were detected which were not reversible

within 8 days. In other tests, undiluted aniline produced lacrimation, inflammation of the conjunctiva and damage to the cornea but the effects were reversible within 24-48 hours. Comparable effects were observed after application of a saturated aqueous solution of aniline (ECB 2004).

In an acute inhalation toxicity study with rats, mild to severe corneal damage with corneal clouding was observed after the 4-hour nose-only exposure to 681-896 ppm aniline vapour and aerosol. The eye effects persisted for 2 weeks (ECB 2004).

7.4.3 In vitro data

No data are available.

7.5 Sensitisation

7.5.1 Human data

Skin sensitisation was observed in a maximisation test with 25 healthy volunteers. For the induction, undiluted aniline was applied five times to irritated skin (pre-treatment with sodium lauryl sulphate, SLS). The challenge was carried out with the highest non-irritating concentration of aniline (10 %), again on SLS-pretreated skin. Seven of 25 volunteers showed a positive sensitisation reaction, which were judged by the author as a mild reaction rate (ECB 2004; HSE 1997).

Positive reactions in patch tests have also been reported in monitoring surveys and in studies with patients suffering from eczematous dermatitis. Here, the positive reactions are often associated with a group allergy to other aromatic amines, which are substituted at the para-position (para-group compound cross reactivity) (ECB 2004).

Data regarding the potential for aniline to produce respiratory sensitisation are not available.

7.5.2 Animal data

The skin sensitisation potential of aniline was investigated in a study using three different protocols with 10 guinea pigs in each treatment and 4 in each control group (Goodwin et al 1981). Pre-tests ensured that the induction induced moderate irritation and that the challenge reaction was carried out with the maximum non-irritant concentration. No skin sensitisation could be detected in a modified Draize test. A positive reaction in 1 of 10 animals was seen in the Magnusson-Kligman test. The strongest response was observed in a Single Injection Adjuvant test (SIAT). Following repeated challenges, positive reactions were reported for 5 of the 10 guinea pigs.

Data regarding the potential for aniline to produce respiratory sensitisation are not available.

7.5.3 In vitro data

No data are available.

7.6 Genotoxicity

7.6.1 Human data

In cultures prepared from peripheral blood samples of workers exposed to aniline for 4-9 years, an increased incidence of chromosomal aberrations (fragments, gaps, breakages) were observed compared to healthy controls. No data were provided regarding exposure or co-exposure to other chemicals. The validity of these findings cannot be assessed (HSE 1997).

Further data are not available.

7.6.2 Animal data

In a host-mediated assay, an ether extract of 24-h urine samples of rats which had been given 300 mg/kg aniline orally had a mutagenic effect in *S. typhimurium* TA98 in the presence of S9 mix (ECB 2004).

Aniline feeding or injection had no mutagenic effect in several sex-linked recessive lethal assays in *Drosophila melanogaster* (Bomhard and Herbold 2005).

Studies on DNA strand breaks in liver, kidney, bone marrow and spleen of mice or rats treated with aniline by i.p. injection gave both positive and negative results. I.p. administration of 61-420 mg/kg aniline led to a dose-dependent increase in SCE in bone marrow cells of mice (Bomhard and Herbold 2005).

Covalent binding of radioactivity from ¹⁴C-radiolabelled aniline to DNA was observed after single or repeated pre-administration of unlabelled aniline to rats. The highest binding was observed in kidneys, followed by large intestine and spleen and the effect was increased by pre-dosing. Substantial binding to DNA in the liver of rats or in organs of mice was not detected in this study (McCarthy et al 1985).

An induction of micronuclei in the bone marrow of mice and rats was observed in a number of studies, mostly at high doses that are known to cause marked MHB-formation and acute general toxicity. Positive results were generally obtained in mice in studies with i.p. application, whereas both positive and negative results were described in studies with mice and rats after oral administration of aniline. An increased number of micronuclei in peripheral blood was observed in a subchronic feeding study with mice (Bomhard and Herbold 2005).

Under conditions which were clearly positive in a micronucleus test, a slight increase in chromosomal aberrations in bone marrow cells of rats was observed in one study after oral administration of aniline (ECB 2004); however, in another study with i.p. administration of aniline, no such increase could be observed in mice (Bomhard and Herbold 2005).

4-Aminophenol, a metabolite of aniline, induced micronuclei bone marrow cells, liver cells, and splenocytes of mice (ECB 2004).

A dominant-lethal assay conducted according to recent guidelines was performed with male rats, which received up to 200 mg/kg bw/d (which slightly increased mortality) orally for 5 days and were subsequently mated to untreated females for 10 weeks (CTL 1998). No evidence for a dominant-lethal effect was observed except for an overall slight but significant increase in the number of early deaths and a decrease in the number of live implants in the 3rd week in the highest dose-group. The result is considered inconclusive (ECB 2004).

In essence, mutagenicity/genotoxicity tests conducted with aniline were mostly negative. Positive results were generally confined to high doses that were accompanied with signs of toxicity. However, it must be noted that distinct metabolites of aniline displayed genotoxicity, as explained in more detail above.

7.6.3 In vitro

Aniline was not mutagenic in standard bacterial gene mutation test using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 in the absence or presence of metabolic activation by S9 mix from Aroclor-induced rat or Syrian hamster livers. Also, no mutagenicity was observed in tests with *Escherichia coli* WP-2 uvrA and in yeasts. A mutagenic effect was observed in the strain TA98 in the presence of S9 mix and the co-mutagen norharman; a highly potent direct mutagen aminophenyl-norharman was identified as the reaction product of aniline and norharman (ECB 2004).

In assays using mammalian cells, aniline did not induce mutations at the HPRT locus in V79 cells in the absence of S9 mix. In the presence of S9 mix, a mutagenic response was observed at high concentrations, which exceeded the maximum concentration recommended by current test guidelines (Bomhard and Herbold 2005). Aniline showed mutagenic and clastogenic effects in several mouse lymphoma assays both in the absence and in the presence of exogenous metabolic activation system. Generally, the effects were weak or confined to high concentrations, which reached or were close to marked cytotoxicity (Bomhard and Herbold 2005).

Tests on chromosomal aberrations in Chinese hamster cells showed controversial results. A positive response was obtained, mostly at high concentrations, in five tests; in two of them, the positive response was restricted to the assay with S9 mix, while in two other tests the effect was seen in the absence of S9 mix. Aniline did not induce micronuclei in Syrian hamster embryonic cells; in Chinese hamster lung cells, an increase in the presence of S9 mix was observed but the effect was not dose-dependent (Bomhard and Herbold 2005).

Aniline induced a slight increase in sister chromatid exchanges (SCE) in cell lines from Chinese hamster, in rat liver epithelial cells and in human fibroblasts. SCE were also observed in concanavalin A induced human T-lymphocytes from whole blood cultures, but not in pure lymphocyte cultures. Therefore, it was concluded that erythrocytes contribute to the transformation of aniline into genotoxic intermediates. Two metabolites of aniline, o-aminophenol and N-phenylhydroxylamine, were much more effective in inducing SCE in human fibroblasts at 0.1 mM and 0.05 mM, respectively, than aniline at a far higher concentration (10 mM) (ECB 2004; NRC 2000).

DNA strand breaks were found in mouse lymphoma cells in the presence of S9 mix, but only at an exceedingly high concentration of aniline (2 g/l). Aniline did not induce unscheduled DNA synthesis (UDS) in primary cultures of rat, mouse, hamster and human hepatocytes (Bomhard and Herbold 2005; ECB 2004).

7.7 Carcinogenicity

7.7.1 Human data

In two cohort studies with 4622 and 1223 men, which were employed in the British dyestuff industry for at least 6 months, the incidence of bladder cancer was higher than expected in employees who had been exposed to several chemicals including aniline (but not the known carcinogens 2-naphthylamine and benzidine) (Case et al 1954; Case and Pearson 1954). In a further study in an industrial rubber plant, co-exposure to chemicals including aniline, o-toluidine and hydroquinone was associated with a significant increase of bladder cancer in the group of 708 workers who were definitively exposed and in that of 288 who were possibly exposed (NIOSH Alert 1990; Ruder et al 1992; Ward et al 1991). Overall, the workers in all of these studies were exposed to a number of different substances and the carcinogenic potential of aniline cannot be assessed from these findings.

7.7.2 Animal data

Carcinogenicity studies were carried out with oral administration of aniline hydrochloride in rodents. Most evidence is available from two feeding studies with rats.

In one study with F344 rats (50 animals/sex and treatment group; 25 animals/sex and control group), animals were fed diet with 0, 0.3 % or 0.6 % aniline hydrochloride (equivalent to aniline doses of 0, 174 or 360 mg/kg bw/d) for 103 weeks followed by a post-treatment period of 5 weeks. Survival was not affected by treatment; body weight gain was slightly reduced in females and in high-dose males. There were no splenic tumours in control males and females. The incidences of several types of mesenchymal tumours, primarily of the spleen, were increased in male treated rats: haemangiosarcomas of the spleen were observed in 19/50 males at the low dose and in 20/46 at the high dose, fibrosarcomas in 3/50 at the low and 7/46 at the high dose. The combined incidence of fibrosarcomas and sarcomas of the spleen was also statistically significantly higher in male rats as was the combined incidence of fibrosarcomas and sarcomas of multiple body organs in male rats. In female rats, the number of animals with fibrosarcomas or sarcomas of either the spleen alone or multiple organs of the body cavity was significantly associated with treatment. Although this result was not supported by the Fischer exact tests, it was considered indicative of a compound-related carcinogenic effect because of the rarity of these tumours (observed incidences: 0/24 in the control group, 1/50 in the low-dose and 7/50 in the high-dose females). Non-neoplastic effects included splenic lesions (capsular fibrosis, fatty changes, hyperplasia, increased erythropoiesis, haemosiderosis) and increased haemosiderosis in the renal tubular epithelium in both sexes at both doses levels (NCI 1978). The splenic tumour incidence was confirmed in re-examination, which also showed that the occurrence of non-neoplastic splenic lesions was strongly correlated with tumour incidence. It was concluded that sarcomas arise from pre-existing fibrotic areas and splenic fibrosis and hyperplasia are likely to present pre-neoplastic lesions (Weinberger et al 1985).

In another study, male and female F344 rats (130 animals/sex and group) received aniline hydrochloride for 104 weeks in food at body doses of 0, 10, 30 and 100 mg/kg · d (equivalent to body doses of aniline of 0, 7, 22 and 72 mg/kg · d). Survival was reduced in high-dose males, body weight was not affected by treatment. The incidence of splenic tumours in males of the high-dose group was much higher than in the control and the other two dose groups. Most of these tumours were stromal sarcomas (21), but there were also haemangiosarcomas (6) and several types of other splenic sarcomas (8). One stromal sarcoma was observed in the mid-dose group. No splenic tumours were seen in control animals of both sexes and in low- and mid-dose females, one splenic haemangiosarcoma was observed in a high-dose female. The incidence of other tumours showed no significant increase. Non-neoplastic findings in the spleen of animals were fatty metamorphosis (high-dose males), stromal hyperplasia (high-dose, both sexes) and chronic capsulitis of the spleen, mostly at high-dose animals but less frequently also at the low- and mid-dose. Furthermore, haemosiderin deposition and extramedullary haematopoiesis were more severe in high-dose males, splenic atrophy occurred more frequently in high-dose males and females. In males and in high-dose females, the severity and frequency of splenic congestion were increased and several blood parameters (haematocrit, haemoglobin concentration and erythrocyte count) were decreased (CIIT 1982).

In the same NCI-study as mentioned above, male and female B6C3F1 mice received 0, 0.6 % or 1.2 % of aniline hydrochloride in the diet for 103 weeks followed by a post-treatment period of 5 weeks. Survival was not reduced by treatment, body weight gain was reported to be slightly reduced in both groups of treated males. No treatment-related tumours were observed in any of the groups. The only non-neoplastic lesion related to treatment was a chronic inflammation of the bile duct in both groups of treated males (NCI 1978).

The results of a further study (Hagiwara et al 1980) which was not reported in great detail were summarised by HSE (1997). In this study, groups of male Wistar rats received drinking water with 300 or 1200 ppm aniline for 80 weeks with or without co-exposure to 0.05 % norharman in the diet. No increase in tumour incidence was observed in any treatment group. Because of the lack of data and the short duration of exposure, the study was considered inadequate for the assessment of aniline carcinogenicity (HSE 1997).

Further studies of uncertain validity on the carcinogenic effects of aniline were summarised by ECB (2004). In these studies, data on test substance purity were not given and testing procedures and documented results were not comparable to current standard test protocols. In an early study on Osborne-Mendel rats (White et al 1948) fed with diet containing 0.033% of aniline hydrochloride for 420-1032 days (average 654), cirrhosis and hepatomas in the liver and haemorrhage, fibrosis and sarcomas in the spleen were reported. No data were given on impurities by other substances or the distributions of lesions in between the sexes. In an early study by Druckrey (1950) rats received 22 mg aniline hydrochloride/day (120-220 mg aniline hydrochloride/kg bw/d) with the drinking water for up to 750 days. No tumour response was observed. In another study, no proliferative changes of the bladder epithelium were observed in rats fed with a diet containing 5-7.5 mg aniline/animal for up to 256 days (Ekman and Strömbeck 1949). In Syrian hamsters, weekly subcutaneous injections of 1.9 mmol/kg b.w. did not induce proliferative lesions of the urinary bladder when the experiment was terminated after 87 weeks (Hecht et al 1983). Following subcutaneous injection of aniline at doses of 1 mg/day or of 10 mg/day to rabbits (6 or 60 mg/week), a single urinary bladder tumour was observed in one low-dose rabbit, which died after four weeks of treatment (Perlmann and Staehler 1932).

Mode of action of spleen toxicity and carcinogenicity

As described above, aniline has experimentally induced spleen tumours in rats. Aniline is categorised by SCOEL as a Group C carcinogen with a practical threshold, because carcinogenic doses caused early effects on haematological parameters, inflammatory reactions in the spleen and perturbations of iron metabolism as a result of haemolytic anaemia (Kan et al 1993, 1995, 1997). Recently, this has been further substantiated.

Ma et al (2008) exposed male Sprague-Dawley rats subchronically to aniline (0.5 mmol/kg/day *via* drinking water for 30 days). This aniline treatment led to a significant increase in splenic oxidative DNA damage, measured as 8-hydroxy-deoxyguanosine in spleen.

A second study (Wang et al 2010) evaluated the potential contribution of haem oxygenase-1 (HO-1), which catalyses haem degradation and releases free iron. Male Sprague-Dawley rats were given 1 mmol/kg bw/day aniline in water by gavage for 1, 4, or 7 days, and respective controls received water only. Aniline exposure led to significant increases in HO-1 mRNA expression in the spleen (2- and 2.4-fold at days 4 and 7, respectively) with corresponding increases in protein expression, as confirmed by ELISA and Western blot analysis. Furthermore, immune-histochemical assessment of spleen showed stronger immune-staining for HO-1 in the spleens of rats treated for 7 days, confined mainly to the red pulp areas. The increase in HO-1 expression was associated with increases in total iron (2.4- and 2.7-fold), free iron (1.9- and 3.5-fold) and ferritin levels (1.9- and 2.1-fold) at 4 and 7 days of aniline exposure. The data suggested that HO-1 up-regulation in aniline-induced splenic toxicity could be a pro-oxidant mechanism, mediated through iron release.

In a third study, Ma et al (2011) exposed male Sprague-Dawley rats to aniline (0.5 mmol/kg bw/day) *via* drinking water for 30 days, while controls received drinking water only. The DNA base excision repair (BER) activity of the glycosylases NEIL1 and 2 was assayed using a bubble structure substrate containing 5-hydroxyuridine (preferred substrates for the NEIL1 and NEIL2) and by quantitating the cleavage products. Aniline treatment led to a 1.25-fold increase in the NEIL1/2-associated base excision repair activity in the nuclear extracts of spleen compared to the controls. Real-time PCR analysis for NEIL1 and NEIL2 mRNA expression in the spleen revealed 2.7- and 3.9-fold increases, respectively, in the aniline-treated rats compared to controls. Likewise, Western blot analysis showed that protein expression of NEIL1 and NEIL2 in the nuclear extract of spleens from aniline-treated rats was 2.0- and 3.8-fold higher than controls, respectively. The aniline treatment also led to stronger immuno-reactivity for NEIL1 and NEIL2 in the spleens, confined to the red pulp areas. The results were interpreted to show that aniline-induced oxidative stress is associated with an induction of NEIL1/2.

A fourth study (Ma et al 2013) examined whether the repair enzymes NTH1 and APE1 contribute to the repair of oxidative DNA lesions in the spleen following aniline treatment.

This was identical to that in the preceding studies. The treatment led to significant increase in NTH1- and APE1-mediated BER activity in the nuclear extracts of spleen of aniline-treated rats compared to the controls. NTH1 and APE1 mRNA expression in the spleen showed 2.9- and 3.2-fold increases, respectively. This was confirmed by Western blot analysis. The increased repair activity of NTH1 and APE1 was discussed as an important mechanism for the removal of oxidative DNA lesions.

In essence, new data are fully in line with the view that the (experimental) carcinogenic effect of aniline is linked to a mode of action that is associated with a threshold: oxidative stress is involved in the spleen, and in response to oxidative stress DNA repair pathways are operative.

Thereby, the categorisation in SCOEL group C of carcinogens is further supported.

7.8 Reproductive toxicity

7.8.1 Human data

In an insufficiently documented study, menstrual disturbances, ovarian dysfunction and spontaneous abortion were mentioned to occur in women exposed to aniline and other chemicals. Due to incomplete documentation and missing exposure data, no conclusions can be drawn from this study (ECB 2004). Further data are not available.

7.8.2 Animal data

Fertility

Fertility studies are not available.

No treatment-related effects were observed on testes weight and histopathology at interim and terminal sacrifice in a chronic/carcinogenicity study in male rats at aniline doses up to 72 mg/kg ·bw/d. In females, ovary weight was not reduced by aniline exposure at interim sacrifice at 26, 52, and 78 weeks but was lower at termination of the study in the high-dose group (CIIT 1982). No dose-dependent effects on histology of reproductive organs were observed in rats and mice in another chronic/carcinogenicity study (NCI 1978).

Developmental toxicity

Aniline hydrochloride was evaluated for teratogenicity and postnatal effects in F344 rats. Pregnant dams were given the test substance by gavage at doses of 0, 10, 30 and 100 mg/kg bw/d (0, 7.2, 21.6, 71.8 mg aniline/kg bw/d) during gestational day 7-20 or from day 7 to parturition. Maternal toxicity manifested as a statistically significant decrease in absolute weight gain (high dose), a dose-dependent increase in spleen weight (significant at all doses) and in other signs characteristic of aniline toxicity (increased Mhb formation, decreased erythrocyte count) at the highest dose. No embryo/foetotoxic effects and no teratogenicity were observed at any dose. Foetal examination revealed a significant but marginal increase in liver weight and a marginally increased erythrocyte volume (MCV) in the high-dose group. A dose-dependent but non-significant increase in the number of litters in which one or more postnatal deaths occurred was seen in all treated groups (total litters effected: 3/16, 4/15, 5/16) compared to control (2/15). After cessation of aniline exposure at parturition, maternal effects persisted at least for the period of nursing. Pups examined post-natally had increased MCV on day 0 (high dose). On the 25th post partum day, a decreased body weight (high dose), increased liver weight (low and mid, but not high dose) and a non-significant increase in spleen weight were observed. No signs of toxicity were seen in pups on day 60. No behavioural changes were detected in several tests (Price et al 1985).

High doses of aniline hydrochloride (≥ 260 mg/kg, single i.p. injection) may cause foetal cardiovascular malformations and cleft palate in rats. The effects are attributed to the Mhb-induced maternal hypoxia (Matsumoto 2003).

In a screening study with mice treated by gavage with 560 mg aniline/kg · d on gestation day 6-12, maternal toxicity including death of dams and a reduced birth weight and weight gain of offspring were observed but no effects on the number of live pups per litter or on the percentage survival during the first 2 weeks (Hardin et al 1987).

7.9 Mode of action and adverse outcome pathway considerations

As outlined in chapter 7.7., mechanistic data are fully in line with the view that the (experimental) carcinogenic effect of aniline is linked to a mode of action that is associated with a threshold: oxidative stress is involved in the spleen, and in response to oxidative stress DNA repair pathways are operative.

This firmly supports the categorisation of aniline in SCOEL group C of carcinogens.

7.10 Lack of specific scientific information

A pre-existing information gap on toxicokinetics and methaemoglobin formation in experimentally exposed humans has been filled by the recent study of Käfferlein et al (2014). At present, there is no lack of scientific information to derive an OEL.

8 GROUPS AT EXTRA RISK

As outlined in chapter 7.1.1, the volunteer study of Käfferlein et al. (2014) showed that the methaemoglobin levels induced by aniline showed no significant effect of the individual NAT-2 acetylator status. Hence, a group at extra risk cannot be identified.

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