Monocyclic aromatic amino and nitro compounds:

toxicity, genotoxicity and carcinogenicity, classification in a carcinogen category

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1 Introduction

It was long believed that monocyclic amino compounds (arylamines) are generally not carcinogenic and that for carcinogenicity arylamines had to have at least two benzene rings. Only after the hepatocarcinogenic properties of 2,4,6-trimethylaniline had been reported (Morris 1965) were the monocyclic aromatic amino and nitro compounds included in the NCI bioassay programme for testing substances for carcinogenicity (Weisburger and Fiala 1981). In the *List of MAK and BAT Values* numerous monocyclic aromatic amino and nitro compounds are classified in the carcinogen categories 1 to 3. Now that the two new categories, 4 and 5, have been created for the classification of carcinogens, it is necessary—especially for substances in category 3—to decide which compounds can be reclassified according to the new criteria in one of the new categories (Neumann *et al*. 1998, DFG 2004).

In a comparative review of the appropriate data, the present document aims to establish whether the monocyclic aromatic amino and nitro compounds which are currently classified in the *List of MAK and BAT Values* may be considered as a group for the purposes of classification in carcinogen categories and whether classification on the basis of analogy may be justified. Because it is considered that these substances share common mechanisms of action and that therefore any differences in effect are quantitative rather than qualitative, it seems unsatisfactory that individual substances of this group are found classified in the Carcinogen categories 1 to 3, mostly because of differences in the amount of data available (Table 1). And it has been suggested that the carcinogenic effects and the activity of these substances could be a result mainly of their acute and chronic toxicity and less of their genotoxicity, which could justify their classification in the new category 4.

Tables 1 to 4 present all the monocyclic aromatic amino and nitro compounds which are found to date in the *List of MAK and BAT Values*, both those which are classified in one of the carcinogen categories, those with a MAK value, and those for which insufficient data were available and which are therefore listed in Section IIb of the *List of MAK and BAT Values*. The substances are not presented in the order of their current classification in carcinogen categories but in six groups formed on the basis of structural con-

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siderations. Amino and nitro derivatives are considered together as *N*-substituted aryl compounds. The data have mostly been taken from the MAK documentation for the individual substances; the literature sources are cited there. These data have been supplemented with data published especially during the last 10 years and with information from the databases of the European Chemicals Bureau of the EU. An exhaustive review of the data has not been attempted and often only the most relevant data have been included. The six groups of substances:

mono-*N*-substituted benzene derivatives
(1) aniline and substances which can be metabolized to yield aniline derivatives
(2) *o*-toluidine and its derivatives
(3) *p*-toluidine and its derivatives
poly-*N*-substituted benzene derivatives
(4) phenylenediamine and substances which can be metabolized to yield phenylenediamine
(5) di-substituted and poly-substituted toluene derivatives and analogous benzene derivatives
(6) *N*-substituted aminophenols and nitrophenols and substances which can be metabolized to yield phenols

The structural formulae of the monocyclic aromatic amino and nitro compounds included in the *List of MAK and BAT Values* are shown in Figure 1.

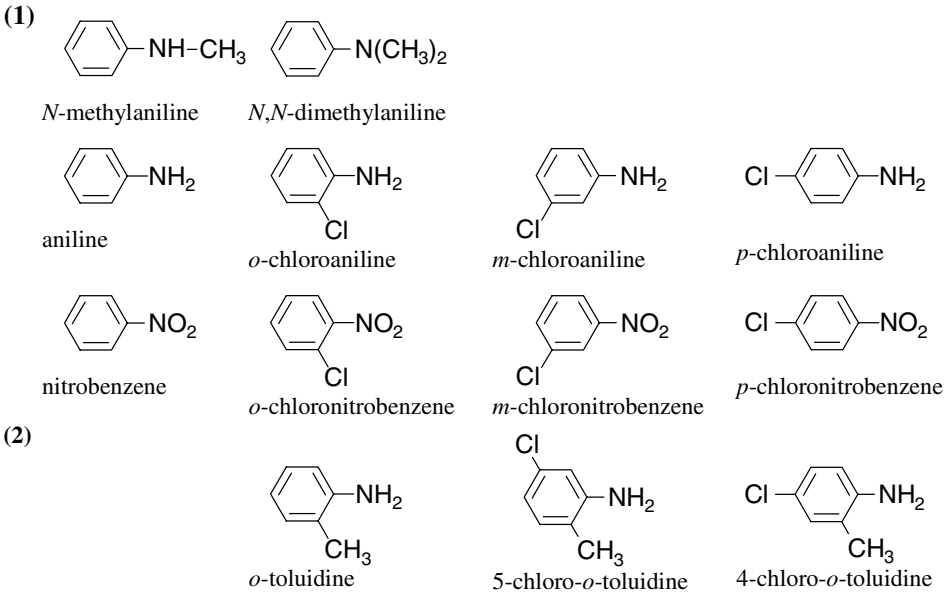
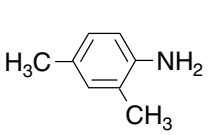
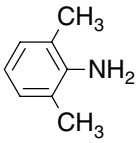


Figure 1. Structural formulae of the monocyclic aromatic amino and nitro compounds in the *List of MAK and BAT Values* 2004

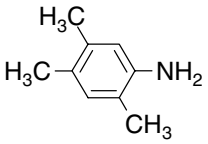
(2)



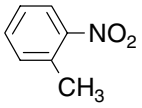
2,4-xylidine
(2,4-dimethylaniline)



2,6-xylidine
(2,6-dimethylaniline)

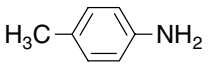


2,4,5-trimethylaniline

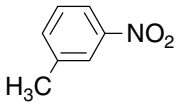


2-nitrotoluene

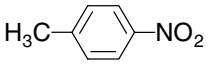
(3)



p-toluidine

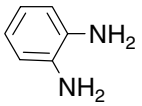


3-nitrotoluene

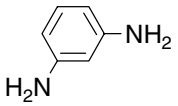


4-nitrotoluene

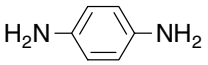
(4)



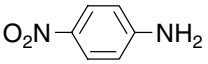
o-phenylenediamine
(1,2-diaminobenzene)



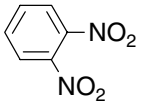
m-phenylenediamine
(1,3-diaminobenzene)



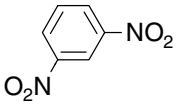
p-phenylenediamine
(1,4-diaminobenzene)



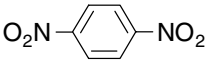
4-nitroaniline



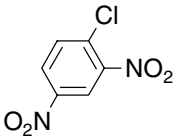
o-dinitrobenzene
(1,2-dinitrobenzene)



m-dinitrobenzene
(1,3-dinitrobenzene)



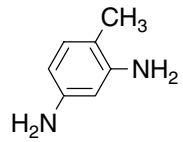
p-dinitrobenzene
(1,4-dinitrobenzene)



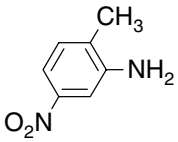
1-chloro-2,4-dinitrobenzene

Figure 1 (continued). Structural formulae of the monocyclic aromatic amino and nitro compounds in the *List of MAK and BAT Values* 2004

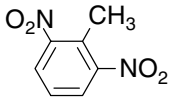
(5)



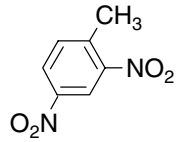
toluene-2,4-diamine



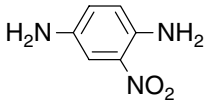
5-nitro-*o*-toluidine
(2-amino-4-nitrotoluene)



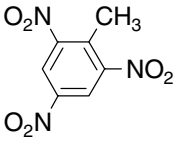
2,6-dinitrotoluene



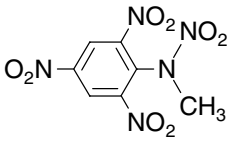
2,4-dinitrotoluene



2-nitro-*p*-phenylene-
diamine

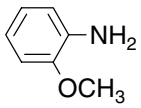


2,4,6-trinitrotoluene

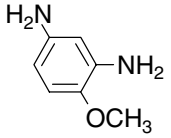


N-methyl-*N*,2,4,6-
tetranitroaniline

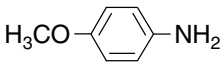
(6)



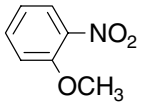
o-anisidine
(2-methoxyaniline)



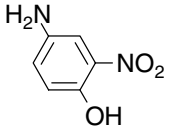
2,4-diaminoanisole



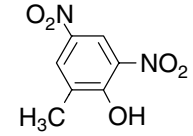
p-anisidine
(4-methoxyaniline)



2-nitroanisole



2-nitro-4-aminophenol



4,6-dinitro-o-cresol

Figure 1 (continued). Structural formulae of the monocyclic aromatic amino and nitro compounds in the *List of MAK and BAT Values* 2004

2 Classification

The amount of data available for the individual substances is very varied and results in marked differences in classification (Table 1). When appropriate tests have been carried out, tumours have generally been observed; sometimes the incidences were in the range of the significance limits; the sex and species differences are mostly inexplicable.

3 Toxicity

Practically all the listed monocyclic aromatic amino and nitro compounds induce acute methaemoglobin (metHb) formation in experimental animals (Table 2). *p*-Phenylenediamine and 2,4-diaminoanisole seem to be exceptions; however, 2,4-diaminoanisole has been reported to cause anaemia. In some cases adaptation has been described after repeated uptake of the substance, that is, initial increases in the level of metHb disappear during the course of treatment. Usually the animals become anaemic. Heinz bodies are frequently reported but do not always seem to correlate with the metHb formation. Haemosiderosis is almost always observed and put down to the increased turnover of erythrocytes. The distribution of the deposits in the organism can differ widely.

Expressed in terms of the LD_{50} for the rat (Table 2), the acute toxicity of the listed monocyclic aromatic amino and nitro compounds ranges from moderate to low. The low values range from 300 to 400 mg/kg body weight, the high up to over 1000 mg/kg body weight. Exceptions are the dinitrobenzenes, *p*-phenylenediamine and 4,6-dinitro-*o*-cresol for which values below 100 mg/kg body weight have been reported. In the case of the dinitrobenzenes, the high toxicity is a result of the severe haematotoxicity: in tests of 24 monocyclic aromatic amino and nitro compounds, *p*-dinitrobenzene caused most metHb formation in the cat (Bodansky 1951). For *p*-phenylenediamine, the development of oedema and neuromyopathy is indicative of effects specific for the rat (Tainter *et al.* 1926). Likewise the highly toxic, not yet classified substance 4,6-dinitro-*o*-cresol (IIb in the *List of MAK and BAT Values*) operates by a different mechanism as a respiratory chain uncoupler (DFG 1976).

As a parameter for bioavailability of biologically active metabolites, the available haemoglobin binding indices (HBI) are listed in Table 2. With practically all the compounds such adduct formation can be demonstrated; given the simultaneous metHb formation, this is only to be expected (Sabbioni 1994). The lowest and highest values differ by a factor of 100. In summary,

- the comparable HBIs of aniline, *N*-methylaniline and *N*,*N*-dimethylaniline support the conclusion that the toxicity of these compounds is also comparable (see below);
- the values for *o*-chloroaniline and *p*-chloroaniline are very different;
- the HBIs for the three nitrotoluene isomers are the same;
- the value for dinitrobenzene is conspicuously high;
- the value for 2,4-diaminotoluene is conspicuously low.

Table 1. Monocyclic aromatic amino and nitro compounds: classification, MAK values and tumour locations

Substance [CAS number]	Category [1]	MAK value	Tumour location [2]		MAK documentation [3] References
			rat	mouse	
(1) aniline and substances which can be metabolized to yield aniline derivatives					
aniline [62-53-3]	**3B**	**2 ml/m^3**	spleen sarcomas, haemangiosarcomas, fibrosarcomas, osteosarcomas	no tumours	Volume 6
***N*-methylaniline** [100-61-8]	–	**0.5 ml/m^3**	no tumours (inadequately studied)	no tumours (inadequately studied)	Volume 6
***N*,*N*-dimethylaniline** [121-69-7]	**3B**	**5 ml/m^3**	sarcomas in spleen and thymus, osteosarcomas	forestomach papillomas	Volume 3
***o*-chloroaniline** [95-51-2]	–	**–**	not studied	not studied	Volume 3
***m*-chloroaniline** [108-42-9]	–	–	not studied	not studied	Volume 3
***p*-chloroaniline** [106-47-8]	**2**	–	spleen sarcomas	liver adenomas, carcinomas and haemangiosarcomas, spleen haemangio-sarcomas	Volume 3, Chhabra *et al*. 1991
nitrobenzene [98-95-3]	**3B**	–	liver adenomas and carcinomas	lung adenomas	Volume 19, ECB 2000b
***o*-chloronitrobenzene** [88-73-3]	**3B**	**–**	multiple tumours (inadequately studied)	liver carcinomas (inadequately studied)	Volume 4, Nair *et al*. 1986
***m*-chloronitrobenzene** [121-73-3]	–	–	not studied	not studied	Volume 4
***p*-chloronitrobenzene** [100-00-5]	**3B**	–	no tumours (inadequately studied)	vascular tumours (inadequately studied)	Volume 4
(2) *o*-toluidine and its derivatives					
***o*-toluidine** [95-53-4]	**2**	–	fibrosarcomas, angiosarcomas, osteosarcomas, haemangiosarcomas mesotheliomas, multiple tumours e.g. in bladder, liver, mammary gland	haemangiosarcomas, liver adenomas and carcinomas	Volume 3, ECB 2000e

Table 1. continued

Substance [CAS number]	Category [1]	MAK value	Tumour location [2]		MAK documentation [3] References
			rat	mouse	
5-chloro-*o*-toluidine [95-79-4]	**3B**	–	(adrenal glands)	haemangiosarcomas, liver	Volume 6
4-chloro-*o*-toluidine [95-69-2]	**1**	–	(hepatomas)	haemangiosarcomas, spleen	Volume 6
2,4-xylidine (2,4-dimethylaniline) [95-68-1]	**2**	–	no tumours (inadequately studied)	lung (inadequately studied)	Volume 19, BUA 1995
2,6-xylidine (2,6-dimethylaniline) [87-62-7]	**2**	–	adenomas, carcinomas and sarcomas of the nasal cavity, fibromas, and fibrosarcomas of the subcutis, liver nodules, hepatomas	not studied	Volume 19, BUA 1995, ECB 2000f
xylidine isomers: 2,3-xylidine, 2,5-xylidine, 3,4-xylidine, 3,5-xylidine) [87-59-2, 95-78-3, 95-64-7, 108-69-0]	**3A**	–	fibromas (2,5-xylidine; inadequately studied)	vascular tumours, hepatomas (2,5-xylidine; inadequately studied)	Volume 19, BUA 1995
2,4,5-trimethylaniline [137-17-7]	**2**	–	liver, lung, multiple tumours	liver, multiple tumours	Volume 4
2-nitrotoluene [88-72-2]	**2**	–	mesotheliomas, fibromas, fibrosarcomas, liver	haemangiosarcomas, fibromas, liver	Volume 8, Greim 2002
(3) *p*-toluidine and its derivatives					
***p*-toluidine** [106-49-0]	**3B**	–	no tumours (oral administration); liver, local subcutaneous sarcomas (s.c.)	liver adenomas and carcinomas (oral administration)	Volume 3, ECB 2000g
3-nitrotoluene [99-08-1]	**–**	**5 ml/m^3**	not studied	not studied	ECB 2000h
4-nitrotoluene [99-99-0]	–	**5 ml/m^3**	clitoris	(lung)	Bayer AG 2003, NTP 2002

Table 1. continued

Substance [CAS number]	Category [1]	MAK value	Tumour location [2]		MAK documentation [3] References
			rat	mouse	
(4) phenylenediamine and substances which can be metabolized to yield phenylenediamine					
***o*-phenylenediamine** (1,2-diaminobenzene) [95-54-5]	**3B**	**–**	liver carcinomas (oral administration)	liver carcinomas (oral administration); local subcutaneous sarcomas (s.c. injection)	Volume 13, BUA 1993, ECB 2000i
***m*-phenylenediamine** (1,3-diaminobenzene) [108-45-2]	**3B**	–	no tumours (oral administration); local subcutaneous sarcomas (s.c. injection)	no tumours (oral, dermal administration)	Volume 6, BUA 1993, ECB 2000j
***p*-phenylenediamine** (1,4-diaminobenzene) [106-50-3]	**3B**	**0.1 mg/m³**	no tumours (oral administration)	no tumours (oral, dermal administration)	Volume 6, BUA 1993, ECB 2000k
4-nitroaniline [100-01-6]	**3A**	–	no tumours	(haemangiosarcomas)	Greim 1999, Nair *et al.* 1990
dinitrobenzene (all isomers) [25154-54-5]	**3B**	–	not studied	not studied	Volume 1
1-chloro-2,4-dinitrobenzene [97-00-7]	–	**–**	no tumours (inadequately studied)	no tumours (inadequately studied)	BG Chemie 1992; ECB 2000n
(5) di-substituted and poly-substituted toluene derivatives and analogous benzene derivatives					
toluene-2,4-diamine [95-80-7]	**2**	–	liver, multiple tumours	liver	Volume 6
5-nitro-*o*-toluidine (2-amino-4-nitrotoluene) [99-55-8]	**2**	–	(liver)	liver, haemangiosarcomas	Volume 6
2,6-dinitrotoluene [606-20-2]	**2**	**–**	liver, multiple tumours	not studied	Volume 6
2,4-dinitrotoluene [121-14-2]	**2**	**–**	fibromas, fibroadenomas, mammary gland, liver	kidney	Volume 6
2-nitro-*p*-phenylenediamine [5307-14-2]	**3B**	–	no tumours	liver	Volume 4
2,4,6-trinitrotoluene [118-96-7]	**3B**	**0.011 ml/m³**	(urinary bladder)	not studied	Volume 1, Furedi *et al.* 1984

Table 1. continued

Substance [CAS number]	Category [1]	MAK value	Tumour location [2]		MAK documentation [3] References
			rat	mouse	
***N*-methyl-*N*,2,4,6-tetranitroaniline** [479-45-8]	**3B**	–	(stomach)	not studied	Volume 11
(6) *N*-substituted aminophenols and nitrophenols and substances which can be metabolized to yield phenols					
***o*-anisidine** (2-methoxyaniline) [90-04-0]	**2**	–	urinary bladder, kidney, thyroid gland	urinary bladder	Volume 10
2,4-diaminoanisole [615-05-4]	**2**	–	thyroid gland, Zymbal gland, clitoris	thyroid gland	Volume 6
***p*-anisidine** (4-methoxyaniline) [104-94-9]	**3B**	–	urinary bladder, preputial gland, liver	no tumours	Greim 2001
2-nitro-4-aminophenol [119-34-6]	**3B**	–	urinary bladder, fibroadenomas	lung (inadequately studied)	Volume 4
2-nitroanisole [91-23-6]	**2**	–	leukaemia, urinary bladder, kidney, colon, lower urinary tract	liver adenomas and carcinomas	Volume 9, ECB 2000p
4,6-dinitro-*o*-cresol [534-52-1]	–	–	no tumours	not studied	Volume 19

[1] Carcinogen categories
[2] especially the main target organs are listed; low incidences are indicated with brackets
[3] volume numbers refer to MAK documentation in present series, *Occupational Toxicants,* Volumes 1–20
s.c.: subcutaneous

Not all the results can be explained satisfactorily but the data suggest additional arguments for the comparative assessment of the substances.

In an evaluation of the acute and chronic toxicity of these substances, the neurotoxic effects should be included. These have often been described but, apart from a role of oxygen deficiency, practically no quantitative data or mechanistic suggestions have been published.

For assessment of the carcinogenic properties, the frequently observed but probably not always recorded development of fibrosis is of significance. Its appearance is regularly associated with damage in the typical target organs spleen, liver and kidneys and it could be causally involved as an important parameter in the development of tumours (NCI 1978). The mechanism of action is not fully understood. The study of the primary reactions which result in toxicity should be continued, for example, at the molecular level, in order to identify appropriate parameters for dose-dependency.

Table 2. Monocyclic aromatic amino and nitro compounds: toxicity

Substance	Effects of single exposures	Effects of repeated exposures	LD_{50} rat (mg/kg body weight, *p.o.*)	HBI[1]	MAK documentation,[2] References
(1) aniline and substances which can be metabolized to yield aniline derivatives					
aniline	spleen, haemosiderosis, metHb	anaemia, Heinz bodies liver, kidneys	440	22	Volume 6, Albrecht and Neumann 1985
***N*-methylaniline**	metHb, Heinz bodies	anaemia, spleen, liver, kidneys	no data	16	Volume 6, Birner and Neumann 1988
***N*,*N*-dimethylaniline**	metHb	anaemia, haemosiderosis, spleen, liver	1300	11	Volume 3, Birner and Neumann 1988
***o*-chloroaniline**	metHb, Heinz bodies	anaemia, kidney, haemosiderosis, spleen, liver	116	0.5	Volume 3, ECB 2000a, NTP 1998, Sabbioni 1994
***m*-chloroaniline**	metHb, Heinz bodies	anaemia, haemosiderosis, spleen, liver, kidney	1104	12.5	Volume 3, NTP 1998, Sabbioni 1994
***p*-chloroaniline**	metHb, liver, kidneys	anaemia, spleen, Heinz bodies, haemosiderosis, liver, kidney	300–400	569	Volume 3, Chhabra *et al.* 1990, 1991, NTP 1998, Birner and Neumann 1988
nitrobenzene	metHb, liver, kidneys	anaemia, haemosiderosis, spleen, liver (necrosis), kidney, testis	640	79	Volume 19, Albrecht and Neumann 1985
***o*-chloronitrobenzene**	metHb, Heinz bodies	anaemia, haemosiderosis, spleen, kidney, liver (necrosis)	180–500	no data	Volume 4, Travlos *et al.* 1996
***m*-chloronitrobenzene**	metHb	metHb, Heinz bodies, testis	470	no data	Volume 4
***p*-chloronitrobenzene**	metHb	anaemia, haemosiderosis, Heinz bodies, spleen, liver, kidney, testis	400–1000	"high"	Volume 4, Travlos *et al.* 1996

Table 2. continued

Substance	Effects of single exposures	Effects of repeated exposures	LD_{50} rat (mg/kg body weight, *p.o.*)	HBI [1]	MAK documentation, [2] References
(2) *o*-toluidine and its derivatives					
***o*-toluidine**	metHb	anaemia, haemosiderosis, spleen, urinary bladder, kidney	600–1300	4	Volume 3, Birner and Neumann 1988
5-chloro-*o*-toluidine	metHb	liver, kidney	about 1000	28	Volume 6, BIA 2003, Birner and Neumann
4-chloro-*o*-toluidine	metHb, Heinz bodies	anaemia, Heinz bodies, spleen, liver, urinary bladder	700–1000	28	Volume 6, Birner and Neumann 1988
2,4-xylidine (2,4-dimethylaniline)	metHb, liver	liver (increased endoplasmic reticulum, necrosis), spleen, kidney	470	2.3	Volume 19, Birner and Neumann 1988
2,6-xylidine (2,6-dimethylaniline)	metHb	spleen, haemosiderosis fibrosis, liver (enzyme induction), kidney	1230	1.1	Volume 19, ECB 2000f, Sabbioni 1994, Vernot *et al.* 1977
xylidine (2,3-xylidine, 2,5-xylidine, 3,4-xylidine, 3,5-xylidine)	metHb (3,5-xylidine)	fibromas, liver (2,5-xylidine; inadequately studied)	930 (2,3-) 1300 (2,5-) 810 (3,4-) 710 (3,5-)	 7.3 (2,5-) 0.7 (3,4-) 14.0 (3,5-)	Volume 19, Sabbioni 1994, Vernot *et al.* 1977
2,4,5-trimethyl-aniline	metHb	spleen, liver, lung	1500	0.7 (0.2)	Volume 4, Birner and Neumann 1988
2-nitrotoluene	metHb, liver, olfactory system	haemosiderosis, fibrosis; spleen, liver, kidney, testis	890–2500	5	Volume 8, 2002, Dunnik *et al.* 1994
(3) *p*-toluidine and its derivatives					
***p*-toluidine**	metHb	anaemia, liver (enzyme induction)	650–1200	2	Volume 3, Birner and Neumann 1988

Table 2. continued

Substance	Effects of single exposures	Effects of repeated exposures	LD_{50} rat (mg/kg body weight, *p.o.*)	HBI [1]	MAK documentation,[2] References
3-nitrotoluene	(liver)	haemosiderosis, anaemia, fibrosis, spleen, liver, kidney, testis	1070–2200	4	Dunnik *et al*. 1994, ECB 2000 h
4-nitrotoluene	metHb, (liver)	haemosiderosis, fibrosis; spleen, liver, kidney, testis	2144–4700	4	Bayer AG 2003, Dunnik *et al*. 1994
(4) phenylenediamine and substances which can be metabolized to yield phenylenediamine					
***o*-phenylenediamine** (1,2-diaminobenzene)	metHb (liver, kidney, spleen)	no data	500–1300	no data	Volume 13, BUA 1993
***m*-phenylenediamine** (1,3-diaminobenzene)	metHb	liver, kidney	540–780	0.7	Volume 6, BUA 1993, Neumann *et al*. 1993
***p*-phenylenediamine** (1,4-diaminobenzene)	(no metHb reported)	oedema, liver, kidney	56–114	no data	Volume 6, BUA 1993
4-nitroaniline	metHb, liver, kidney	spleen, haemosiderosis, liver, kidney	750–3000	no data	Greim 1999, Nair *et al*. 1986
dinitrobenzene (all isomers)	metHb, Heinz bodies (*p*>*m*>*o*)	anaemia, spleen, haemosiderosis, liver	30–250 (LD_{low})	69 *m*-DNB	Volume 1, Neumann *et al*. 1993
1-chloro-2,4-dinitrobenzene	metHb, Heinz bodies	(liver, spleen; inadequately studied)	640–1070	no data	BG Chemie 1992, ECB 2000n
(5) di-substituted and poly-substituted toluene derivatives and analogous benzene derivatives					
toluene-2,4-diamine	(metHb)	liver (cirrhosis)	170–300	< 0.02	Volume 6, Neumann *et al*. 1993
5-nitro-*o*-toluidine (2-amino-4-nitrotoluene)	metHb, Heinz bodies	anaemia, liver, kidney	574	1.0	Volume 6, Zwirner-Baier *et al*. 1994

Table 2. continued

Substance	Effects of single exposures	Effects of repeated exposures	LD_{50} rat (mg/kg body weight, *p.o.*)	HBI [1]	MAK documentation,[2] References
2,6-dinitrotoluene	metHb, Heinz bodies	anaemia, liver (necrosis)	535–800	1.2	Volume 6, Zwirner-Baier *et al*. 1994
2,4-dinitrotoluene	metHb (no Heinz bodies)	anaemia, Heinz bodies liver	500–1000	0.7	Volume 6, Zwirner-Baier *et al*. 1994
2-nitro-*p*-phenylenediamine	no data	liver	350–3000	no data	Volume 4
2,4,6-trinitrotoluene	metHb, Heinz bodies	spleen, liver, haemosiderosis	600–1800	6	Volume 1, Zwirner-Baier *et al*. 1994
***N*-methyl-*N*,2,4,6-tetranitroaniline**	metHb	anaemia, spleen, liver, kidney	no data	no data	Volume 11
(6) *N*-substituted aminophenols and nitrophenols and substances which can be metabolized to yield phenols					
***o*-anisidine** (2-methoxyaniline)	metHb, Heinz bodies	anaemia, spleen	2000	no data	Volume 10
2,4-diaminoanisole	(no metHb reported)	(anaemia), liver (hyperplasia)	500	no data	Volume 6
***p*-anisidine** (4-methoxyaniline)		metHb, Heinz bodies, anaemia, spleen, liver, urinary bladder	1400	no data	Greim 2001
2-nitro-4-aminophenol	no data	no data	3000–9000	no data	Volume 4
2-nitroanisole	gastrointestinal tract, kidney	anaemia, spleen, metHb, urinary bladder, liver, kidney	740–1000	no data	Volume 9
4,6-dinitro-*o*-cresol	(uncoupler)	metHb, Heinz bodies, liver, kidney, heart	25–85	no data	Volume 19

[1] HBI: haemoglobin binding index: binding [mmol/mol Hb] per dose [mmol/kg body weight]
[2] volume numbers refer to MAK documentation in present series, *Occupational Toxicants,* Volumes 1–20
metHb: methaemoglobin

4 Evidence of non-genotoxic effects

That the genotoxic effects of the aromatic amines cannot on their own account for the carcinogenicity of these substances and that the acute and chronic toxic effects could play an important role, especially in tumour promotion, has been suggested often in recent years (Bitsch *et al*. 1997). Therefore the non-genotoxic effects of these substances should be given more attention. There is considerable evidence of effects which are usually thought to be receptor-mediated, for example, the enzyme induction by many of these substances. The role of proliferation-promoting effects and fibrosis formation has already been mentioned. These develop as a result of the toxic effects in the typical target organs, spleen, liver and kidney. In the long-term studies with nitrobenzene, for example, all the tissues in which tumours developed had been altered by chronic toxic processes and showed increased proliferation rates or inflammation. As early as 1984, Goodman *et al.* (1984) demonstrated an association between the development of fibrosis and sarcomas. Aniline, *o*-toluidine and *p*-chloroaniline induced with similar potency fibrosis in the spleen parenchyma or capsule. The fibrosis-inducing properties of monocyclic aromatic amino and nitro compounds could be counted among the tumour-promoting properties of these substances.

It is not clear whether the fibrosis develops as a result of unspecific cytotoxicity—e.g. overloading of the spleen by the increased erythrocyte elimination—or whether a role is played by specific biochemical mechanisms—e.g. impairment of mitochondrial respiration and inhibition or acceleration of apoptosis.

With only few of the substances have initiation-promotion studies been carried out to test for tumour-promoting activity. In such initiation-promotion studies, 2,4-dinitrotoluene, for example, acted only as a promoter, 2,6-dinitrotoluene as initiator and promoter (Leonard *et al.* 1983, Popp and Leonard 1983).

There are also results available from *in vitro* tests for promoting properties. At a pH of 6.7 in a test for morphological transformation of Syrian hamster embryo (SHE) cells, toluene-2,4-diamine, 2,4-dinitrotoluene and *o*-anisidine had transforming activity, but toluene-2,6-diamine and *p*-phenylenediamine did not (Kerckaert *et al.* 1998). In another study, none of four dinitrotoluene isomers yielded positive results in a test for morphological cell transformation. Likewise the metabolites toluene-2,4-diamine, 5-nitro-*o*-toluidine and 3-nitro-*o*-toluidine yielded negative results in this study. 2,4-Dinitrotoluene and 2,6-dinitrotoluene inhibited intercellular communication, but at toxic concentrations (Holen *et al*. 1990).