European Commission

SCOEL/REC/301

o-Toluidine, 2-methylaniline

Recommendation from the
Scientific Committee on Occupational Exposure Limits

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Directorate-General for Employment, Social Affairs and Inclusion
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2017

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RECOMMENDATION FROM THE SCIENTIFIC COMMITTEE ON OCCUPATIONAL EXPOSURE LIMITS FOR *o*-TOLUIDINE, 2-METHYLANILINE

8-hour TWA:	not assigned
STEL:	not assigned
BLV:	not assigned
BGV:	0.2 µg/l
Additional categorisation:	SCOEL carcinogenicity group A (genotoxic carcinogen)
Notation:	'skin'

The present Recommendation was adopted by SCOEL on 2017-02-08

RECOMMENDATION EXECUTIVE SUMMARY

Outcome Considerations

ortho-Toluidine (*o*-toluidine) is a high-production-volume chemical used in the manufacture of rubber chemicals, herbicide intermediates, dye intermediates, and some drugs like the local anesthetic prilocaine. *o*-Toluidine has been listed as a human carcinogen (Group 1)(IARC 2010, 2012) based on sufficient evidence of carcinogenicity from studies in both experimental animals and humans. In humans occupationally exposed to *o*-toluidine the urinary bladder is the major tumor site.

Occupational exposure to *o*-toluidine can occur by inhalation or skin contact (IARC 2010) during its production, or during its use in the production of chemical intermediates. Measured *o*-toluidine in urine and/or as hemoglobin adducts in occupationally exposed workers provide evidence that *o*-toluidine is absorbed following inhalation exposure (Korinth et al. 2007, 2006, Ward et al. 1996, Jones et al. 2005). Exposure to *o*-toluidine can be demonstrated by detection of *o*-toluidine and its N-acetyl-metabolites in urine or of hemoglobin adducts of *o*-toluidine in blood (Brown et al. 1995, Ward et al. 1996, Teass et al. 1993). It can be assumed that urinary levels reflect recent exposure, while, because of the 120-day half-life of human red blood cells, hemoglobin adducts reflect cumulative exposure (Cheever et al. 1992; NTP, 2014).

Once absorbed, *o*-toluidine is rapidly distributed, metabolized, and excreted (Brock et al. 1990, Cheever et al. 1980, Son et al. 1980).

Six occupational historical cohort studies reporting urinary bladder cancer risk among *o*-toluidine-exposed workers were evaluated (NTP, 2014), including three cohort mortality studies conducted among workers in the dyestuffs industry (Case and Pearson 1954, Pira et al. 2010, Rubino et al. 1982, Piolatto et al. 1991, Ott and Langner 1983), two incidence/mortality studies in the rubber chemicals industry (Ward et al. 1991, 1996, Prince et al. 2000, Carreón et al. 2010, 2014, Hanley et al. 2012), and one incidence study in a plant manufacturing 4-chloro-*o*-toluidine (Stasik, 1988). Analysis of these studies revealed they do not provide a basis for human risk assessment, mainly because of inadeqautely defined exposure.

Also in experimental animals *o*-toluidine caused urinary bladder neoplasms (Weisburger et al., 1978; NCI, 1979; Hecht et al. 1982, 1983; NTP, 2014). Especially the urinary bladder cancer findings in female rats show a clear dose response behaviour (NCI, 1979; NTP, 2014). The incidences of urinary bladder cancer are lower in male rats and not statistically significantly increased as compared to control. Nevertheless, they are considered to be exposure related because these types of tumors are rare and they were observed in all three carcinogenicity studies (NTP, 2014).

The mechanisms underlying the carcinogenicity of *o*-toluidine likely include several modes of action such as metabolic activation resulting in formation of reactive metabolites that bind to DNA and proteins, mutagenicity, oxidative DNA damage, chromosomal damage, and cytotoxicity (NTP, 2014). SCOEL concludes that *o*-toluidine causes DNA and chromosomal damage and induces mutations and is therefore considered as a carcinogen with a genotoxic mode of action.

Derived Limit Values

Given that *o*-toluidine is considered to be a carcinogen with a genotoxic mode of action a health based recommended occupational exposure limit (HBROEL) can not be defined. Instead risk numbers were defined. Given the absence of adequate human data and animal inhalation studies, risk number were estimated using the results from the study in *o*-toluidine hydrochloride was administered to rats in feed for 101 to 104 weeks (NCI,

1979). Especially the data on formation of the transitional-cell carcinomas of the urinary bladder in female rats reported in this NCI study appear suitable for dose reponse modelling, resulting in a BenchMark Dose causing 10% tumor incidence above background level (BMD_{10}) of 42.2 mg/kg bw per day.

This BMD_{10} value is based on a rat study with dietary life time exposure.

A BMD_{10} of 42.2 mg/kg bw per day can be further estimated to correspond to an inhaled dose of about 840 mg/m^3 as an 8-hour TWA at occupational exposure during 40 out of 75 years, 48 out of 52 weeks and 5 days/week (assuming a body weight of 70 kg, an inhaled amount of 10 m^3 for 8 h working day, and equal absorption via inhalation and the oral route, i.e. 42.2 mg/kg bw per day * 70 kg / 10 m^3 * 75/40 * 52/48 * 7/5).

Allometric scaling of this dose level of 840 mg/m^3 from rat to human using a default value of times 0.25 provides a POD for 10% increase in tumor risk of 210 mg/m^3

Using this value the following risk numbers were derived:

A tumor risk of 1 : 10 at the BMD_{10} of 210 mg/m^3 (48 ppm)

A tumor risk of 1:1000 at 2.10 mg/m³ (0.48 ppm)

A tumor risk of 1 : 10 000 at 0.210 mg/m^3 (0.048 ppm)

A tumor risk of 1 : 10^6 at 2.10 microg/m^3 (0.00048 ppm)

Using this value it can also be calculated that at the ACGIH TLV-TWA of 2 ppm (8.8 mg/m^3) the tumor incidence is estimated to be 1: 239.

Skin notation

o-Toluidine is easily absorbed through the skin (Korinth et al. 2007). Because dermal exposure and uptake may impose a significant contribution to systemic exposure a skin-notation for *o*-toluidine, as already existing in many countries, is recommended.

Biological Monitoring

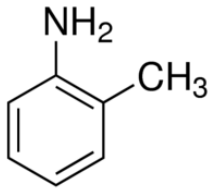
Exposure to *o*-toluidine can be measured also by biomonitoring. Both urinary *o*-toluidine and toluidine haemoglobin adduct analyses have been used to monitor occupational exposure to *o*-toluidine but the measurement of urinary excretion of total (free and conjugated) *o*-toluidine after hydrolysis is the most commonly used method. There are studies reporting that *o*-toluidine concentrations in workplace air associated with elevated urinary levels of different groups of industry workers (Brown et al. 1995, Korinth et al., 2007). No correlation equations between air levels and urinary o-toluidine levels has been, however, established. Since *o*-toluidine is a genotoxic carcinogen, no health-based BLV can be set for it. However, the data on the *o*-toluidine levels in the urine of general population can be used to set a BGV for urinary *o*-toluidine. On the basis of the studies by Kütting et al. (2009) and Weiss and Angerer (2005) the 95th percentile of urinary *o*-toluidine (free and conjugated) among the non-smoking population is approximately 0.2 µg/l. This value of 0.2 µg/l is set as a BGV for *o*-toluidine measured from post-shift urinary samples in the end of the working week. Since smoking increases urinary *o*-toluidine levels, this value applies only for non-smokers/can be applied only after abstaining from smoking.

RECOMMENDATION FROM THE SCIENTIFIC COMMITTEE ON OCCUPATIONAL EXPOSURE LIMITS FOR *o*-TOLUIDINE, 2-METHYLANILINE

RECOMMENDATION REPORT

1. CHEMICAL AGENT IDENTIFICATION AND PHYSICO-CHEMICAL PROPERTIES

Name:	*o*-toluidine
Synonyms:	1-amino-2-methylbenzene, 2-aminotoluene, *o*-methylaniline
Molecular formula:	C_7H_9N
Structural formula:	



EC No.:	EC No: 202-429-0
CAS No.:	95-53-4
Molecular weight:	107.15 g/mol
Conversion factors: (20 °C, 101.3kPa)	1 ppm = 4.4 mg/m^3 1 mg/m^3 = 0.23 ppm

2. EU HARMONISED CLASSIFICATION AND LABELLING

Information about the EU harmonised classification and labelling for *o*-toluidine is provided by ECHA (2016a), as summarised in Table 1.

Table 1: Classification according to Regulation (EC) No 1272/2008, Annex VI, Table 3.1 "List of harmonised classification and labelling of hazardous substances" (ECHA, 2016a)

Classification		Labelling			Spec. Conc. Limits, M-factors
Hazard Class & Category Code(s)*	**Hazard statement code(s)***	**Hazard statement code(s)***	**Suppl. Hazard statement code(s)**	**Pictograms, Signal Word Code(s)**	
Acute Tox. 3	H301	H301		GHS06 GHS09 GHS08 Dgr**	
Eye Irrit. 2	H319	H319			
Acute Tox. 3	H331	H331			
Carc. 1B	H350	H350			
Aquatic Acute 1	H400	H400			

* *Acute Tox.3, H301* *Toxic if swallowed*
Eye Irrit. 2, H319 *Causes serious eye irritation*
Acute Tox. 3, *H331* *Toxic if inhaled*
Carc. 1B, H350 *May cause cancer*
Aquatic Acute 1, H400 *Very toxic to aquatic life*

** *Signal word code 'Dgr' for 'Danger*

3. CHEMICAL AGENT AND SCOPE OF LEGISLATION

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

o-Toluidine is also a carcinogen or mutagen for humans in accordance with Article 2(a) and (b) of Directive 2004/37/EC and falls within the scope of this legislation.

4. EXISTING OCCUPATIONAL EXPOSURE LIMITS

At EU level, no *OEL* has been adopted yet for *o*-toluidine. However, OEL's do exist in various EU Member States as well as outside the EU. These OEL's are presented in Table 2 as examples and the list should not be considered as exhaustive.

No *BLV* (Biological Limit Valua) has been adopted yet for *o*-toluidine at EU level. However in Germany DFG did set a *BAR* (Biologischer Arbeitsstoff-Referenzwert)[1] of *0.2 µg o-toluidine (after hydrolysis)/L urine.* The derivation of this biological reference value is based on the studies of Kütting et al. (2009), Schettgen et al. (2001) and Weiss and Angerer (2005). Sampling takes place at the end of the exposure or shift (DFG 2012).

Overall, the 8 hrs TWA values adopted in EU Member States and other countries overseas can be grouped into 3 clusters in the following ranges/levels:

- 0.5-0.9 mg/m³ (0.1-0.2 ppm)
- 4.5-8.8 mg/m³ (1-2 ppm)
- 22 mg/m³ (5 ppm)

Half of the EU MS's shown in Table 2 use 0.1-0.2 ppm (0.5-0.9 mg/m^3), where half uses 1-2 ppm (4.5-8.8 mg/m³). From the non-EU Member States only Norway and Switzerland use 0.1-0.2 ppm (0.5-0.9 mg/m^3) where most other countries use 1-2 ppm (4.5-8.8 mg/m³). Only the USA OSHA PEL is 22 mg/m³ (5 ppm). The differences may be due to the use of a different key study, a different point of departure (if the same study is used) and/or the use of different assessment factors to extrapolate from the point of departure (POD) to the OEL. Also, socio-economic feasibility aspects may be underlying. Importantly, NIOSH did not propose a quantitative value for the REL (recommended exposure limit). Instead, NIOSH recommends *o*-toluidine to be controlled and handled as a potential human carcinogen and therefore exposure should be kept to the lowest feasible level (US NIOSH 2016).

Cherrie (2011a and b) reported an estimated yearly incidence of cancers in the EU (for the year 2010) due to previous occupational exposure to *o*-toluidine to be 22, and the estimated number of deaths to amount to 10. The authors also concluded that if no action is taken 490 cancers and 150 cancer deaths are projected to occur over the next 60 years as the exposure levels will probably continue to decrease yearly (Cherrie 2011a). The methodology used is described in a report from the same series (Cherrie 2011b).

[1] BAR ("Biologische Arbeitsstoff-Referenzwerte") describe the background level of a substance which is present concurrently at a particular time in a reference population of persons of working age who are not occupationally exposed to this substance. The BAR are based on the 95th percentile without regarding effects on health. It must be taken into account that the reference level of the background exposure can be influenced by such factors as age, sex, social status, residential environment, life style and geographical region. The reference level for a substance or its metabolite in biological material is derived with the help of the measured level in a random sample from a defined population group. Occupational exposure can be assessed by comparing biomonitoring values in occupationally exposed persons with the BAR.

Table 2: Overview of existing OELs for *o*-toluidine

EU	**TWA*** **(8 hrs)**		**STEL#** **(15 min)**		**Remarks**	**References**
	ppm	mg/m³	ppm	mg/m³		
Austria	0.1	0.5	0.4	2	TRK@	AU GKV (2011)
Belgium	2	8.9	-	-	8 hrs TGG (TWA)	BE KB (2014)
Denmark	2	9				DK DWEA (2016)
Finland	2	-	4	-	STEL = 15 min. average	FI MSAH (2012)
France	2	9			VME = TWA 8 hrs	FR INRS (2012)
Germany	-	-	-	-	Only biological value	DFG (2012)
Hungary	-	0.5	-	-	$4x10^{-5}$ risk value	HU MHSFA (2000)
Ireland	0.2	0.9	-	-		IE HSA (2011)
Spain	0.2	0.89	-	-	**	ES INSHT (2011)
United Kingdom	0.2	0.89	-	-	TWA	UK HSE (2011)
Non-EU						
Australia	2	8.8	-	-	TWAEV	AU SWA (2011)
Canada (Ontario)	2	-	-	-	TWA	CA OML (2013)
Canada (Québec)	2	8.8	-	-		CA IRSST (2010)
New Zealand	0.2	0.89	-	-		NZ HS (2013)
Norway	1	4.5	-	-		NO NLIA (2011)
Switzerland	0.1	0.5	-	-		CH SUVA (2016)
USA (OSHA)	5	22	-	-	PEL	US OSHA (2006)
USA (ACGIH)	2	8.8	-	-	TLV-TWA	US ACGIH (2012)
USA (NIOSH)	-	-	-	-	No REL value as potential occupational carcinogen	US NIOSH (2016)

* Occupational Exposure Limit (e.g. MAK, TRK, TLV, PEL, REL)
\# Short Term OEL (e.g. STEL)
@ Technische RichtKonzentration = Based on technical feasibility.
$ Recommended Exposure Level: Exposure to be minimized to the lowest feasible level
** Agente químico al que se aplica el Valor Límite Biológico de los inductors de la metahemoglobina [biological limit value for induction of methaemoglobinaemia applies]

5. OCCURRENCE, USE AND OCCUPATIONAL EXPOSURE

5.1 Occurrence

o-Toluidine occurs in certain vegetables, in tobacco leaves and in the aroma of black tea. It is an intermediate in the biodegradation of *o*-nitrotoluene, and thus detected at former munition sites. The effluent concentration from wastewater treatment plants from manufacturing and processing companies was below the detection limits at three sampling sites. Toluidine (isomers not specified, but *o*-toluidine likely to be present) was detected as a component of coal oil. *o*-Toluidine is formed during pyrolysis (OECD 2004).

The chemical has been detected in tattoo inks and in baby slips (Danish EPA 2012, RAPEX 2016) as well as in permanent hair dyes and commercial henna samples (Akyüz and Ata 2008; Johansson et al. 2015). It is unknown whether as an impurity or breakdown product of an azo-colorant (Johansson et al. 2015).

Minimal release to the environment of this substance is likely to occur from industrial use, being used as an intermediate in further manufacturing of other substances in closed systems (ECHA 2016b). *o*-Toluidine was found in the rivers Rhine and Elbe in Germany in 1997 and 1998, with the highest *o*-toluidine concentrations being 0.1 µg/l (OECD 2004).

Monitoring data (survey 1981-1983) indicate that the general population may be exposed to *o*-toluidine via ingestion of food and dermal contact with this compound or other products containing 2-aminotoluene (HSDB). *o*-Toluidine has been found as human metabolite of aptocaine and prilocaine, both local anaesthetics (HSDB).

5.2 Production and use

Production of *o*-toluidine started in the middle of the 19th century in the dye industry (Richter 2015). *o*-Toluidine is commercially manufactured by reduction of *o*-nitrotoluene. In 2001, the worldwide production volume of *o*-toluidine was estimated to be 59 000 tonnes by 11 producers (OECD 2004). In 2011, *o*-toluidine was manufactured by at least 15 companies worldwide (SRI 2012). *o*-Toluidine is currently manufactured and/or imported in the EEA (European Economic Area) in 10 000 – 100 000 tonnes per year. According to information submitted by REACH registrants, there are currently 5 registrants/suppliers in the EU, i.e. 4 in Germany and 1 in Sweden (ECHA 2016b).

While production and use have changed over time and at the same time from one country to the other, *o*-toluidine in general has the following site-limited uses as a curing agent in epoxy resins and an intermediate in producing azo dyes and pigments, acid-fast dyestuffs, triarylmethane dyes, sulphur dyes, indigo compounds, photographic dyes and synthetic rubber and rubber vulcanising chemicals. By far the largest use is as an intermediate in the manufacture of herbicides (Galleria Chemica, OECD 2004, NTP 2011, IARC 2012, ECHA 2016b). There are about 10 metallic mordants for industrial use with *o*-toluidine levels of < 0.1 % (OECD 2004). As recently as 2000 its major use was in the production of dyes and pigments, although in Europa this use has decreased because of regulatory restrictions (Cherry 2011). Currently in the EU, this substance has a general industrial use as feedstock in the manufacture of other chemicals or internediates). It is used as laboratory chemical as well (ECHA 2016b, Cherry 2011a).

No direct consumer use is known for *o*-toluidine (OECD 2004). *o*-Toluidine is banned from cosmetics by the EU Cosmetics Regulation. The use of azo dyes that release *o*-toluidine during degradation is not permitted for textiles and other consumer articles in the EU. Thus, an exposure of consumers and of the environment due to releases from consumer products appears to be negligible (OECD 2004). Major uses of *o*-toluidine in the past that have been stopped or diminished in the US, are uses in the manufacture of dyes and of 4-chloro-*o*-toluidine. Uses in the manufacture of dye intermediates, herbicide precursors and prilocaine are reported to continue currently in the US (NTP 2014).

5.3 Occupational exposure

Number of sites

Based on the number or registrants/suppliers under REACH, there are probably 5 production sites in the EU (ECHA 2016b). Worldwide there are many more, i.e. 4 in the US, 11 in China and 1 in Japan (MolBase 2016). Another source mentioned that no US producers were identified in 2014 (NTP 2014). The reason for this discrepancy is unclear.

It is unknown how many sites there are in the EU where *o*-toluidine is used as feedstock for synthesis of other chemicals or intermediates, as laboratory chemical or for education. According to the number of people that are exposed (Table 3), the number of sites could well be in the hundreds as number of people exposed per site could vary from a few to tens or maybe more than hundred.

Despite the ban (Cosmetics Regulation) *o*-toluidine is found in hair dye products as well as in blood of users (professional hairdressers and private users) in Sweden, together with its Hb-adduct (Johansson et al. 2015).

Number of people exposed

The estimated number of people exposed in the EU is 5500, of which 2900 are in manufacture of chemicals, chemical products and man-made fibres or manufacture of rubber products (Cherry 2011). See table 3 for overview per sector. *o*-Toluidine and its Hb-adduct were found in blood of users (professional hairdressers and private users) in Sweden, despite the ban on its use in those products (Johansson et al. 2015, Cosmetics Regulation). As most hair dye products are produced by multinationals and sold in many countries throughout the EU, the actual number of workers exposed is probably considerably higher.

Table 3: Number of workers exposed to *o*-toluidine in the EU (classified by NACE code[#])

NACE (Rev 1.1)	**Industry sector**	**Historical exposure classification**	**People exposed**
24	Manufacture of chemicals, chemical products and man-made fibres	Medium	2855
251	Manufacture of rubber products	Medium	250
73	Research and development	Background	131
75	Public administration and defence	Background	460
80	Education	Background	1305
85	Health and social work	Background	564
TOTAL			**5565**

[#] Numbers and industry code definitions from Cherry 2011. NACE = Nomenclature statistique des activités économiques dans la Communauté européenne [Statistical classification of economic activities in the European Community]

Levels of exposure

Biological monitoring data demonstrate worldwide exposure to *o*-toluidine, occupational and non-occupational, both in smokers and non-smokers. Occupational exposure levels generally decreased with time as can be seen in Table 4. A monitoring exercise by NIOSH in the US in March 1990 demonstrated that airborne *o*-toluidine levels in a rubber chemicals department had dropped below 1 ppm ≡≈ 5 mg/m^3 (Ward et al. 1991). The data in this NIOSH cohort were used as well as extended later by several other authors. From 1995 on, company-implemented extensive engineering controls caused a significant reduction in breathing zone exposure concentrations in most of the departments in a rubber chemical manufacturing plant (Hanley et al. 2012). Certainly after 1995, measured airborne levels were well below the existing OELs, i.e. PEL 5 ppm and TLV 2 ppm (Carreon et al. 2014). The geometric mean levels of *o*-toluidine for samples collected by NIOSH were also lower than the most stringent exposure limit among European nations of 0.1 ppm set by Austria and Switzerland. The excess bladder cancer observed at the plant, even for the lower exposed groups, suggests however that occupational exposure limits for *o*-toluidine may need to be re-examined, and that further measures, such as dermal exposure control/reduction, may be required (Carreon et al. 2014). A future evaluation of the bladder cancer incidence among workers employed after 1995 would be necessary to identify if current exposure levels at the plant are adequate to prevent excess bladder cancer. Aromatic amines like *o*-toluidine are easily absorbed through the skin (Korinth et al. 2007). In their study, it was found that the use of gloves efficiently reduced systemic exposure due to decreased dermal penetration of *o*-toluidine. Furthermore, use of skin care creams reduced the percutaneous uptake of aromatic amines. Notably and in contrast, the use of skin barrier creams enhanced it (Korinth et al. 2007). This phenomenon is known as 'penetration enhancement' (several references in Korinth et al. 2007).

Although banned from cosmetic products, *o*-toluidine was measured in fixative and 'perming lotion + fixative' in a hairdressers study (Johansson et al. 2015). Hairdressers (all non-smoking women) were studied with respect to levels of *o*-toluidine Hb-adducts in blood. For comparison of the levels of adducts, people with personal use of hair dye products and a group of controls were included. For professional hairdressers, the *o*-toluidine Hb-adduct concentrations correlated with increasing frequency for all types of hair treatment. This increase was significant (p-value 0.020) for the 'treatment' of 'hair waiving' (Johansson et al. 2015). To what extent the measured *o*-toluidine is already present as impurity in the hair dye product or formed as an artefact due to desamination of 2,5-diaminotoluene remains to be established (Schettgen et al. 2011). The correlation between the frequency of use and the *o*-toluidine Hb-adduct concentrations though strongly suggests that these hairdressers are in one or another way exposed to *o*-toluidine. In a separate study in Turkey, levels of *o*-toluidine up to 1.5 mg/g commercial hair dye products were found (Akyüz and Ata 2008).

Table 4 includes biomonitoring findings of *o*-toluidine in urine. It is noted that during preparation of samples containing 2,5-diaminotoluene (a hair dye that can be excreted as well) *o*-toluidine could well be formed by desamination (Richter 2015).

Evaluation of hemoglobin (Hb) adducts that reflect longer-term exposures, showed mean *o*-toluidine Hb-adduct levels to be 10 times higher in exposed versus unexposed workers at a rubber chemical manufacturing plant and > 100 times higher than the means in unexposed populations previously studied (various references in Hanley et al. 2012).

Table 4: Historical exposure overview (airborne, urine and blood Hb-adducts)

Activity/Department	**Sampling**	**Airborne mean or low-high [mg/m³]**	**Urinary *o*-toluidine[$] [µg/l]**	**Blood Hb-adduct pg/g Hb**	**Reference**
Chemical plant distillation and extraction in USSR	Around 1960 probably	5.7-6.5			Khlebnikova et al. 1970
Dye production plant US	1940s	< 0.5			Ott and Langner 1983
Production of thioindigo US	1940s		300–1700		Ott and Langner 1983
Production anti-oxidant and accelerator for tire manufacturing US	±1989		18.5 preshift 103.7 postshift		Stettler et al. 1992
Rubber chemical workers US	1990		18 preshift 104 postshift		Teass et al. 1993
Rubber chemicals department US	±1995	0.516±0.513	15.4 preshift 98.7 post-shift	3515 control 40 830 exposed	Ward et al. 1996
Workers in TNT factory China	±2004			386 control 675 exposed	Jones et al. 2005
Vulcanising hydraulic rubber articles Germany	±2005	0.027-0.094	55-243		Korinth et al. 2006
Rubber industry Germany	±2006	0.0001-0.524	38.6 (mean) post-shift	2 900	Korinth et al. 2007
Chemical plants US	?		14-20 preshift 80-130 post-shift		Ruder et al 1992 Teass et al. 1993 Ward et al. 1996 Richter 2015
Hairdressers Turkey	±2007	In product < 1500 mg/g			Akyüz and Ata 2008
Hairdressers Sweden	±2014	In product 0.12-0.17 ng/g[#]		7-22	Johansson et al. 2015

[$] Not specified. Could be *o*-toluidine only, but mostly it is total of *o*-toluidine + conjugates as far as released by acid or base hydrolysis of the urine sample. Important is that significant elevated levels where reported in exposed workers.

5.4 Routes of exposure and uptake

The routes of potential human exposure to *o*-toluidine are inhalation, ingestion, via the skin and via eye contact (NIOSH Pocket Guide 2016).

As *o*-toluidine is a volatile compound, main exposure of workers is via inhalation. A skin-notation exists in many countries as dermal exposure and uptake may impose a significant contribution to systemic exposure (OSHA).

6. MONITORING EXPOSURE

o-Toluidine can be monitored in the air of the workplace by applying the following methods (NIOSH 2011; MAK 2016):

- OSHA (1988). Method 73
- DFG (1992). Method 2003
- NIOSH (1994). Method 2002
- NIOSH (1998). Method 2017

In all four methods *o*-toluidine is sampled from the air in the workplace by adsorption onto a solid sorbent or absorption into solution, followed by extraction of *o*-toluidine with an organic solvent. The *o*-toluidine-containing extract can then be analysed by gas chromatography (GC), using a flame ionisation detector (FID), a nitrogen-specific detector (NSD) or an electron capture detector (EC) as shown in Table 5. NIOSH Method 2002 (NIOSH 1994) and OSHA Method 73 (OSHA 1988) are fully evaluated methods while NIOSH Method 2017 (NIOSH 1998) is partially evaluated.

Table 5: Overview of sampling and analytical methods for monitoring airborne *o*-toluidine in the workplace[#]

Method	Sorbent	Desorption solution	Analysis	Recov. (%)	Limit	Concentration range	Ref's
OSHA Method 73	Filter*	n.a.	GC-EC	99.3	LOQ: 0.22 ppb 0.97 µg/m^3 (100 L sample)	n.a.[***]	OSHA (1988)
DFG Method 2003	Silica gel	Methanol	GC-FID	>90%	LOQ Absol: 0.25 ng/sample Rel: 0.01 mg/m^3	n.a.	DFG (1992)
NIOSH Method 2002	Silica gel	Ethanol	GC-FID or GC/NSD	n.a.	LOD: 0.01 mg/sample	0.1-3 mg/sample	NIOSH (1994)
NIOSH Method 2017	Silica gel + filter*	Ethanol	GC-FID or GC/NSD	n.a.	3 µg/sample	30-252 µg/sample	NIOSH (1998)

LOQ: limit of quantification; GC: gas chromatography; EC: electron capture; ECD: electro-chemical detection; FID: flame ionization detection; NSD: nitrogen-specific detector

* glass fiber filter, sulphuric acid treated

** n.a.: not available

Biomonitoring of *o*-toluidine exposures in the workplace (see Table 6) can be carried out by the measurement of

- *o*-Toluidine in urine, plasma and/or erythrocytes (usually total *o*-toluidine after hydrolysis of conjugates)
- Hemoglobin adducts of *o*-toluidine in blood.

Table 6: Overview of the available methods for biomonitoring of occupational exposures to *o*-toluidine[#]

Method	Application	Analysis	Standard deviation (rel)(S_w)*	Prognostic range(u)*	Recovery (%)	Limit	Ref.
DFG 1993	*o*-Toluidine and its conjugates in urine, plasma and erythrocytes (haemoglobin)	GC/EC or GC/MS	At 5 + 50 µg/L: Urine: 10.4 and 8.6% Plasma: 8.2 and 8.4% Heamoglobin: 6.3 and 7.3%	At 5 + 50 µg/L: Urine: 21.8 and 18.0 % Plasma: 17.2 and 17.6% Heamoglobin: 13.1 and 15.3%	Urine: 85-112 Plasma: 90-109 Heamoglobin: 92-116	Detection limit 1 µg/L urine, blood or plasma solution	DFG (1993)
DFG 2000	Adducts released from haemoglobin	GC/MS with NCI	3.6 %	8.1% (10 ng/L blood and n=10)	122	Detection limit 1 ng/L blood	DFG (2000)
NIOSH 8317	*o*-Toluidine in urine	HPLC/ ECD	23% (average RSD)	Range: 1.4 – 1200 ng/ml	86-101 (at range 102-4.2 ng/mL)	LOD 0.6 ng/mL	NIOSH (2003)

*GC: gas chromatography; EC: electron capture; ECD: electro-chemical detection; MS: mass selective detection; NCI: negative chemical ionisation*

* *Within-series imprecision*

** *n.a.: not available*

Several studies propose minor modifications of the basic methods mentioned above. An overview of different concepts of *o*-toluidine biomonitoring methods applied to date is shown in Table 7.

Table 7: Overview of *o*-toluidine biomonitoring methods applied to date[1]

Method applied	Biomarker	Detection limits	References
HPLC - fluorescence detection	Hb and albumin protein adducts in rat blood	5 pmol/mg Hb	Cheever et al. 1992
HPLC/ECD (urine)	*o*-Toluidine and N-acetyl-*o*-toluidine in urine	0.6 µg/L in urine	Teass et al. 1993
HPLC and (after acid hydrolysis) GC with ^{14}C, fluorescence and/or ECD	Hb adducts in blood	Not determined	Teass et al. 1993
GC/MS	Free *o*-toluidine + acid-labile conjugates in urine	50 ng/L	Weiss and Angerer 2005
	Free *o*-toluidine + acid-labile conjugates in urine	4 ng/L (LOQ 12 ng/L)	Riedel et al. 2006

[1] *HPLC: high-performance liquid chromatography; ECD:eletrochemical detection; EC: electron capture detection; LC: liquid chromatography; SPME: solid-phase microextraction; GC: gas chromatography; MS: mass selective detection*

7. HEALTH EFFECTS

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

7.1.1. Human data

The primary routes of exposure to *o*-toluidine in humans are the respiratory tract and skin (IARC 2000). Detection of *o*-toluidine in urine and/or as hemoglobin adducts in occupationally exposed workers (Korinth et al. 2007, 2006, Ward et al. 1996, Jones et al. 2005) indicates that *o*-toluidine is absorbed following inhalation. This is corroborated by the fact that *o*-toluidine concentrations in workplace air correlated significantly with concentrations in the urine of different groups of rubber industry workers (Brown et al. 1995).

Exposed workers in the rubber chemicals industry had approximately 25 times higher post-shift urinary levels of *o*-toluidine compared to non-exposed workers and the post-shift urinary exposure levels among exposed workers were 6-fold higher than the pre-shift levels (Stettler et al. 1992). Furthermore, the fact that pre-shift urinary levels of exposed workers were higher than those of non-exposed workers indicates that *o*-toluidine is not completely eliminated between work shifts (Brown et al. 1995, Stettler et al. 1992). Also studies with rubber industry workers showed dermal absorption of *o*-toluidine (Korinth et al. 2006, Korinth et al. 2007). These studies demonstrated relative internal exposure to be depended more on the skin condition of the workers than on the concentrations of *o*-toluidine in the air. In workers with skin lesions (burns, erythema, or eczema) internal exposure was significantly higher than in workers with healthy skin.

In a first study Korinth et al. (Korinth et al. 2006) reported on the relevance of dermal absorption of o-toluidine based on astudy in four workers with different skin status (healthy, erythematous, burned skin and dishydrotic eczema). The results revealed dermal absorption of *o*-toluidine through damaged epidermal barrier is significantly higher (1.5 to 2 fold) than through healthy skin.

In another study Korinth et al. (Korinth et al. 2007) examined 51 workers occupationally exposed to *o*-toluidine. The workplace conditions, the use of personal protective equipment and risk factors for the skin were evaluated by means of a questionnaire. The results obtained revealed that hemoglobin adducts of *o*-toluidine was significantly higher in workers with erythema than in workers with a healthy skin (mean values amounting to 417.9 ng/liter vs 118.3 ng/liter). Wearing gloves significantly reduced the internal exposure. Furthermore, frequent use of skin barrier creams was shown to enhance dermal absorption of *o*-toluidine.

No tissue distribution studies were identified for humans and no studies of *o*-toluidine metabolism in humans were identified. It is expected that metabolic pathways are similar to those reported in experimental animals.

It is also important to note the genetic polymorphism for N-acetyltransferases resulting in increased susceptibility to aromatic amine-inuced cancer for slow-acetylators (Golka et al. 2002).

7.1.2. Animal data

In male rats *o*-toluidine is well absorbed following oral exposure (IARC 2000, 2010). Upon absorption, *o*-toluidine is rapidly distributed, metabolized, and excreted (Brock et al. 1990, Cheever et al. 1980, Son et al. 1980). Distribution data from rats exposed by subcutaneous (s.c.) injection or oral gavage revealed 48 hours later the presence of radiolabel *o*-toluidine and its metabolites in urine (83.9% of the dose), faeces (3.3%) and exhaled air (1.4%) as well as the presence of low levels of radioactivity (<0.05 % of the dose in total) in the liver, kidney, lung, spleen, colon, and urinary bladder with the highest concentrations (0.12-0.34% of the dose) occurring in the liver (Son et al. 1980).

Brock et al. (1990) reported tissue distribution of *o*-toluidine in male Crl:CD® BR rats 72 hours after receiving a single 500 mg/kg oral dose of radiolabeled *o*-[ring-U-^{14}C]toluidine. The highest concentrations were found in whole blood, spleen, kidneys, and liver.

The primary route of excretion in rats is the urine regardless of exposure route (Son et al. 1980, Cheever et al. 1980).

Metabolism studies were reported for male rats exposed to *o*-toluidine by oral or subcutaneous injection (Kulkarni et al. 1983, Son et al. 1980). The major metabolic pathways were N-acetylation and hydroxylation at the para position (to the amine group), followed by glucuronide or sulfate conjugation. Minor metabolites resulted from hydroxylation at the ortho position (to the amine group), oxidation of the methyl group, and oxidation of the amine group. A subsequent study by Kulkarni et al. (1983) identified N-hydroxy-*o*-toluidine in rat urine and the authors noted that this metabolite might play a role in urinary bladder carcinogenicity.

Cheever et al. (1980) reported unmetabolized *o*-toluidine and 4-amino-meta-cresol formation upon oral administration to rats suggesting that ring hydroxylation was a major metabolic pathway.

In addition to the metabolites mentioned, Gupta et al. (1987) predicted the existence of several metabolites based on data from other aromatic amines. These included N-acetoxy-*o*-toluidine, N-acetyl-N-hydroxy-*o*-toluidine, N-acetoxy-N-acetyl-*o*-toluidine, 2-hydroxy-6-methyl-acetanilide, and *o*-azotoluene. N-acetoxy-*o*-toluidine could undergo non-enzymatic breakdown to form several reactive electrophilic species (i.e., nitrenium ions, nitrene, and free radicals) that could bind to macromolecules. Another pathway involves oxidation of unconjugated phenolic metabolites to form reactive quinone imines (English et al. 2012, Skipper et al. 2010). These metabolic pathways are especially of interest when considering the possible mode of action underlying the genotoxicity and carcinogenicity of *o*-toluidine.

The NTP evaluation (NTP 2014) indicates that in rats *o*-toluidine is metabolised to N-hydroxy-*o*-toluidine followed by further oxidation of the N-hydroxy metabolite to nitrosotoluene in the blood, which induces methemoglobinemia.

The species dependent differences in metabolism of aromatic amines may be related to species dependent differences in toxicity. Especially the ability of each species to catalyse N-acetylation of aromatic amines, may be of importance given that detoxification by N-acetylation reduces the risk for urinary bladder cancer (NTP, 2014, Golka et al. 2002).

7.1.3. In vitro data

Several *in vitro* studies reported that *o*-toluidine (administered as a pure compound or dissolved in phosphate buffer and 5% ethanol) was readily absorbed through excised human skin mounted on diffusion cells (Lüersen et al. 2006, Wellner et al. 2008). About 15% of the applied dose penetrated over 7 hours and 50% within 24 hours (Lüersen et al. 2006). Korinth et al. (2008) reported an in vitro study using diffusion cells and excised human skin showing that skin barrier creams enhanced dermal absorption of *o*-toluidine.

7.1.4. Toxicokinetic modelling

No toxicokinetic models have been identified.

7.1.5. Biological monitoring

As described in chapter 6, there are established biomonitoring methods available on *o*-toluidine. The most commonly used method to monitor internal exposure to *o*-toluidine is to measure total (free and conjugated) *o*-toluidine in urine. Also *o*-toluidine haemoglobin adducts formed by the reaction of nitrosoderivatives of *o*-toluidine with terminal thiols of cysteine in haemoglobin can be used as a biomarker of exposure. Since *o*-toluidine is relatively rapidly metabolised and excreted into the urine, urinary *o*-toluidine reflects the exposure within the past 24 h whereas haemoglobin adducts give information on the exposure during the past four months.

There is some information available on the background levels of *o*-toluidine haemoglobin adducts within the general (non-occupationally exposed) population. In the study by Weiss and Angerer (2005) the median level of haemoglobin adducts among 200 representatives of the general population was 22.6 ng/l and the 95th percentile was 82 ng/l (range <LOD-5929 ng/l). No significant difference between smokers and non-smokers were seen. Other studies on haemoglobin adducts are mainly from the 1990's and show variable levels of Hb-adducts among small populations of smokers or non-smokers. The mean levels in these studies vary typically between 2 ng/l (0.03 ng/g Hb) and 50 ng/l (330 pg/g Hb)(Falter et al., 1994, Branner et al., 1998, Bryant et al., 1988, Stillwell et al. 1987).

A more comprehensive database is available on urinary *o*-toluidine levels in the general population. Although the data are mainly from Germany, these data allow also the identification of 95th percentiles for the general population.

Weiss and Angerer (2005) evaluated the exposure to arylamines among 200 adult occupationally non-exposed volunteers (average age 38.5 y, range 22-73 y), half of which were living in urban area and the rest in a remote area. No difference in urinary *o*-toluidine levels were seen between urban or remote areas. Smokers showed higher urinary *o*-toluidine excretion than non-smokers or passive smokers. The median levels and 95th percentiles for total urinary *o*-toluidine were as follows: non-smokers (n=115) 0.085 and 0.263 µg/l, passive smokers (n=37) 0.087 and 0.158 µg/l, smokers (n=45) 0.206 and 0.541 µg/l.

Kütting et al. (2009) evaluated urinary *o*-toluidine levels among 1004 German volunteers from the Bavarian region. Age of the volunteers varied between 3-84 years (median 42 years). The median level of urinary *o*-toluidine among this population was 0.03 µg/l (range 0.03-172.93) and 95th percentile was 0.23 µg/l. The levels were below the level of quantification in 17.7% of the participants. Smokers (n=145) showed significantly higher excretion of *o*-toluidine than non-smokers (n=856); the 95th percentile for smokers was 0.41 and for non-smokers 0.19 µg/l. Based on this, separate reference values for smokers and non-smokers were proposed by the authors. No difference in urinary levels was seen between men and women. Neither were any correlations with the use of hair dyes or e.g. use of dark-dyed textiles observed. Thus, apart from smoking other environmental sources of exposure to *o*-toluidine remained unclear.

Other recent studies on the background levels of *o*-toluidine among the general population include the study by Schettgen et al., in which a median urinary level of 0.1 µg/l and a 95th percentile of 0.28 µg/l were identified among the 40 representatives of the general population without any known occupational exposure to *o*-toluidine (Schettgen et al., 2001). Lindner et al. (2011) determined the exposure to several tobacco smoke constituents (including o-toluidine) among 1148 smokers and 395 non-smokers in three European countries (Germany, Switzerland, UK). In smokers, the mean daily urinary excretion of o-toluidine was 0.179 µg (SD 0.491 µg) whereas in non-smokers the urinary excretion remained at the level of 0.0635 µg (SD 0.128 µg). These can be calculated to correspond to mean urinary excretion levels of 0.1 and 0.04 µg/l, respectively.

Data on occupationally exposed workers are presented in Chapter 6. Elevated post-shift levels have been observed in chemical industry and in rubber manufacturing in recent studies by Korinth et al. (2006, 2007), Ruder et al. (1992), Teass et al. (1993), and Ward et al. (1996). Korinth et al (2007) showed a significant correlation between o-toluidine concentrations in air and in urine, and between concentrations in air and the level of hemoglobin adducts, while also the correlation between urinary values and hemoglobin adducts was significant. Urinary levels of exposed workers varied between values below detection limit – 292 µg/l whereas in non-exposed workers levels remained below 11.9 µg/l.

Brown et al. (1995) analysed pre- and post-shift samples collected from potentially exposed and unexposed workers and reported elevated concentrations of up to 65 µg/l of o-toluidine in urine from exposed workers, whereas in non-exposed workers the levels remained below 2.6 µg/l. Also pre-shift values of o-toluidine exposed workers were elevated when compared to the pre- or post-shift values of non-exposed workers. They concluded that the method could be useful for determination of o-toluidine exposures in individuals acutely or chronically exposed to high levels. In a further study they have also showed a statistically significant correlation between urinary o-toluidine levels and Hb-adduct levels were observed in occupationally exposed workers (Ward et al. 1996).

Elevated pre-shift urinary values, observed in many of these studies (e.g. Brown et al. 1995) suggest delayed urinary clearance of *o*-toluidine after occupational exposure and/or accumulation over the working week. This suggests that optimal timing for biomonitoring would be post-shift in the end of the working week.

Although several studies have reported increased o-toluidine urinary levels in relation to elevated workplace air levels, no correlation equations between air and urinary levels have been established. In addition, since *o*-toluidine is a genotoxic carcinogen, no health-based BLV can be set. However, data on *o*-toluidine levels in the urine of the general population can be used to set a BGV for urinary *o*-toluidine. On the basis of the studies by Kütting et al. (2009) and Weiss and Angerer (2005) the 95th percentile of urinary *o*-toluidine (free and conjugated) among the non-smoking population is approximately 0.2 µg/l. For smokers 95th percentiles of 0.4- 0.5 µg/l were observed in these studies.

7.2. Acute toxicity

7.2.1. Human data

No data on acute human toxicity of *o*-toluidine have been identified.

In a survey article, it is reported that concentrations of 40 ppm (176 mg/m^3) toluidine (isomer not specified) in the atmosphere for more than 60 minutes caused severe toxic effects in workers, 10 ppm (44 mg/m^3) lead to symptoms of illness and concentration in the atmosphere greater than 5 ppm (22 mg/m^3) indicate unsatisfactory conditions (no further details included) (Goldblatt, 1955).

7.2.2. Animal data

Inhalation

Based on the results of an acute toxicity study by inhalation, in male rats exposed head-only to *o*-toluidine-vapor/aerosol for 4 hours, the calculated LC50 value was 862 ppm (3827 mg/m^3)(DuPont Chem. 1981 as referred to in OECD 2004).

Oral exposure

Acute oral toxicity of *o*-toluidine was characterized in male Sprague Dawley rats showing an oral LD50 of 900 mg/kg bw (Jacobsen 1972). The oral LD50 of *o*-toluidine as also reported to be 2951 mg/kg in Osborne-Mendel rats (Lindstrom et al., 1969), and 15 mg/kg in an unspecified strain of mice (IARC, 1982). Smyth et al. (1962) reported an oral LD50 for *o*-toluidine of 0.94 ml/kg bw equal to about 940 mg/kg bw.

Dermal exposure

Smyth et al. (1962) reported an LD50 upon skin exposure of rabbits to *o*-toluidine of 3.25 ml/kg bw equal to about 3250 mg/kg bw.

7.2.3. In vitro data

In vitro data on acute toxicity of *o*-toluidine were not available.

7.3. Specific Target Organ Toxicity/Repeated Exposure

7.3.1. Human data

o-Toluidine has been listed as a human carcinogen (Group 1)(IARC, 2010, 2012) based on sufficient evidence of carcinogenicity from studies in both experimental animals and humans. In humans occupationally exposed to *o*-toluidine the urinary bladder is the major tumor site. These data are described in more detail in section 7.7.1.

Prince et al. (2000) evaluated the NIOSH cohort evaluated for the incidence of bladder cancer (see section 7.7.1 for further details) for the incidence of ischemic heart disease (IHD) among these workers in the "rubber chemicals'' manufacturing department of a Western New York plant. The authors concluded that IHD mortality among workers in the rubber chemical department was elevated, particularly among those under 50 years of age and that, in addition to the rotating shift pattern for employees assigned to two chemical production departments, also chemical exposures present in the rubber chemical department may be a potential occupational risk factor for IHD. A specifc link to exposure to *o*-toluidine was not reported.

7.3.2. Animal data

7.3.2.1. Inhalation

7.3.2.2. Oral exposure

Short et al. (1983) reported the subacute toxicity of *o*-toluidine in male Fischer 344 rats exposed orally for 5, 10 or 20 days to 225 mg/kg bw per day (amounting to 25% of the estimated oral LD50). At this single dose level 10 out of 30 rats died and effects reported in the remaining animals included reduced body weigth at 5 and 10 days, and increased spleen weight at all time points. Histopathologic evaluation revealed splenic congestion, increased hematopoiesis and hemosiderosis, and bone marrow hyperplasia in treated animals.

Several carcinogenicity studies for *o*-toluidine adminstered by the oral route in rats and mice have been published. These studies are described in more detail in section 7.7.2.

Dermal exposure

A carcinogenicity study with subcutaneous injection of *o*-toluidine in rats (Pliss, 2004)(as referred to in NTP, 2014) is also availble and described in some more detail in section 7.7.2.

7.3.3. In vitro data

No data have been identified.

7.4. Irritancy and corrosivity

7.4.1. Human data

No human data on irritancy have been identified.

7.4.2. Animal data

7.4.2.1. Skin

Smyth et al. (1962) reported irritation on uncovered rabbit belly upon exposure to *o*-toluidine.

No signs of irritation were reported during the 8-day observation period after undiluted *o*-toluidine was applied to the inner surface of one ear of each of two rabbits for 24 hours under semi-occlusive conditions (Thyssen, 1979).
In a further study, undiluted *o*-toluidine was applied for 24 hours to the intact and to the scarified skin of the flank of rabbits under occlusive conditions. Slight to moderate erythema and moderate edema of the intact skin were observed for 72 hours. At 72 hours these animals exhibited scaling, which was still observed on day 8. Necrosis was observed in 1/6 animals (intact skin) and in 6/6 animals (scarified skin) until termination of the study. Overall, the authors evaluated *o*-toluidine as moderately irritating (BASF, 1979).

7.4.2.2. Eyes

Smyth et al. (1962) reported severe corneal injury in rabbits exposed to *o*-toluidine graded at level 8 on a 10-grade ordinal series and is based upon the degree of corneal necrosis that results from instillation of various volumes and concentrations of the chemical.

In a further study with 6 rabbits, which was performed according to Fed. Reg. 38 No 187,1500.42, 1973 *o*-toluidine was judged to be highly irritating due to slight corneal opacity of the total corneal area, slight to moderate conjunctival edema and redness and the observed discharge up to the termination of the study (BASF, 1979).

7.4.3. In vitro data

No data have been identified.

7.5. Sensitisation

7.5.1. Human data

No data have been identified.

7.5.2. Animal data

No data have been identified.

7.5.3. In vitro data

No data have been identified.

7.6. Genotoxicity

7.6.1. Human data

Böhm et al. (2011) reported *o*-toluidine-releasing DNA adducts in 11 urinary bladder tumor samples (urothelial carcinomas) taken from 12 cancer patients and in 13 urinary bladder epithelial samples and 10 bladder submucosal tissue samples taken from 46 sudden death victims at autopsy. NTP indicated that the major limitation of the study is that it is does not provide definite evidence of covalent DNA binding. DNA-releasing adduct levels in bladder tumors were significantly higher than in non-cancerous bladder tissue but did not differ between smokers and non-smokers.

7.6.2. Animal data

The in vivo genotoxic effects of *o*-toluidine have been investigated in experimental animals evaluating various endpoints including DNA adducts, DNA damage, single-strand breaks, clastogenic effects, altered sperm morphology, and DNA synthesis inhibition (NTP, 2014). The results from these studies indicate that *o*-toluidine is able to form DNA adducts and to damage DNA and chromosomes in vivo. In a bone marrow sister chromatid exchange assay, high doses of *o*-toluidine produced positive results in mice (McFee et al., 1989). A positive result has also been reported for the induction of DNA single strand breaks in mice (Cesarone et al., 1982).

Formation of *o*-toluidine DNA adducts has been reported in rats in vivo in liver and nasal mucosa (Brock et al., 1990, Duan et al., 2008).

In vivo genotoxic effects were reported in target organs where carcinogenic effects occurred (liver in mice and urinary bladder in rats and mice)(NTP, 2014). In addition, *o*-toluidine induced DNA strand breaks in the kidney, colon, and stomach in rats, and lung, stomach, and brain in mice. Micronucleus (MN) formation was detected in rat peripheral blood cells (NTP, 2014).

7.6.3. In vitro

The in vitro genoxicity of *o*-toluidine has been extensively investigated and reviewed (Danford 1991, IARC 1987, 2000, 2010, 2012). Two international collaborative studies on large numbers of carcinogens investigated *o*-toluidine (Ashby et al. 1985, de Serres and Ashby 1981). Overall, the data show that *o*-toluidine is positive for genotoxicity in bacterial system as well as in mammalian in vitro models.

Results form bacterial test systems reveal that *o*-toluidine, which shows weakly positive results in a standard test, requires specific assay conditions to demonstrate positive effects (NTP, 2014). *o*-Toluidine has been tested for reverse and forward mutations and prophage induction in *Salmonella typhimurium*, for reverse mutations, differential toxicity, growth inhibition, and prophage induction in *Escherichia coli* and for DNA damage (rec assay) in *Bacillus subtilis*. *o*-Toluidine did not cause mutations in *S. typhimurium* or *E. coli* without metabolic activation (NTP, 2014). *o*-Toluidine tested positive in a few studies in *S. typhimurium* strains TA100, TA98, and TA1538 with metabolic activation and some modifications to the standard protocol such as for example use of higher concentrations of S9 and/or specific preparations of S9 (NTP, 2014).

The results from these studies were also evaluated by NTP (NTP, 2014). NTP (NTP, 2014) concluded that the mutagenicity of *o*-toluidine in the Ames test is consistent with findings on other arylamines, showing low mutagenic activity that might be explained by deficiencies in metabolizing enzymes (e.g., sulfotransferases and acetyltransferases) in the test strains. Other possible explanations for the low mutagenicity include a lack of sufficient quantities of the enzymes required for bioactivation or that reactive intermediates formed might not be able to cross the bacterial cell wall.

o-Toluidine also tested positive in mammalian in vitro assays with human as well as rodent cells. Most of these studies were reviewed by Danford (1991), IARC (2010) and NTP (2014). Genetic effects in human as well as in non-human mammalian cells included DNA damage, unscheduled DNA synthesis (UDS), gene mutation, sister chromatid exchange (SCE), chromosomal aberrations, aneuploidy and/or micronucleus formation. Metabolic activation was required for positive effects in the UDS assay but not for SCE or micronucleus formation (NTP, 2014).

In addition, *o*-toluidine induced cell transformation in rodent cells, an endpoint that reflect an early event in the neoplastic process indicating a need for a more detailed assessment of the carcinogenic potential of *o*-toluidine (Maire et al. 2012; Pant et al. 2012).

Moreover, *o*-toluidine metabolites were tested for genotoxicity in in vitro assays. N-oxidized *o*-toluidine metabolites including N-hydroxy-*o*-toluidine and *o*-nitrosotoluene were mutagenic in the Ames test with *S. typhymurium* strains TA98, TA100, TA1535, and/or TA1538 in the presence of S9 (Gupta et al. 1987, Hecht et al. 1979). This observation is consistent with N-hydroxylation being a bioactivation step.

Overall, it can be concluded that *o*-toluidine can bind to DNA, cause DNA and chromosomal damage, and induce mutations.

7.7. Carcinogenicity

7.7.1. Human data

Six occupational historical cohort studies reporting urinary bladder cancer risks among *o*-toluidine-exposed workers were available (NTP, 2014), including three cohort mortality studies conducted among workers in the dyestuff industry (Case and Pearson 1954, Pira et al. 2010, Rubino et al. 1982, Piolatto et al. 1991, Ott and Langner 1983), two incidence/mortality studies in the rubber chemical industry (Ward et al. 1991, 1996, Prince et al. 2000, Carreón et al. 2010, 2014, Hanley et al. 2012), and one incidence study in a plant manufacturing 4-chloro-*ortho*-toluidine (Stasik, 1988). Each of the studies conducted external or internal analyses on urinary bladder cancer risk, and four of the studies reported also some or all other cancer sites.

Case and Pearson (1954) presented the results of a standardized cohort mortality study of dyestuff workers in the United Kingdom conducted among male workers manufacturing magenta. The study considered the role of aniline in the manufacturing of auramine and magenta (fuchsine) as possible causative agents for tumours of the urinary bladder. Exposure to *o*-toluidine was inferred based on knowledge of the industrial process in the United Kingdom during that time period. The survey demonstrated that contact of workers for more than six months with aromatic amines causes increased bladder tumor frequency.

A second standardized cohort mortality study was based on a cohort of Italian dye stuff workers. Rubino et al. (1982) published results of an initial mortality analysis up to 1976 in which exposure was assessed via individual work history. The authors reported a marked excess of bladder cancer when comparing cause-specific mortality of workers in a dyestuff factory in Northern Italy to national figures. Mortality due to bladder cancer was much higher in workers in benzidine and naphtylamine manufacturing processes. Excess bladder cancer was also high among workers in manufacture of fuchsin. The authors concluded that there was evidence that *o*-toluidine and 4,4'-methylene bis(2-methylaniline) were implicated in the excess mortality.

Piolatto et al. (1991) reported a follow up study up to 1989 (Piolatto et al. 1991). In this analysis, they added 8 years of follow-up to the same cohort. In relation to job category, manufacture of beta-naphthylamine or benzidine was associated to the highest risk, followed by fuchsin or safranine T manufacture and by use or intermittent exposure to naphthylamine or benzidine. A possible link with *o*-toluidine exposure was not mentioned.

Pira et al. (2010) reported an update on the bladder cancer risk in this cohort of Italian dyestuff workers who were heavily exposed to aromatic amines up to 2003. Overall, 56 deaths from bladder cancer were observed. The standardized mortality ratio for bladder cancer increased with younger age at first exposure and increasing duration of exposure.

Ott and Langner (1983) reported a standardized mortality study among male workers engaged in the manufacture of thio- and bromindigo dyes in the United States. Exposure to *o*-toluidine occurred only during the manufacture of thioindigo dyes but was not adequately quantified. The mortality of 342 employees assigned to three aromatic amine-based dye production areas was examined in relation to duration of employment and interval since entry into these areas. No deaths due to bladder cancer were observed, and no statistically significant increases in mortality by work area or duration of exposure within work area were found, based on comparison with the mortality of the U.S. white male population.

Ward et al. (1991) reported a retrospective cohort study of the incidence of bladder cancer at a chemical plant in western New York State. The study was conducted by NIOSH, and included rubber chemical workers exposed to *o*-toluidine and aniline. Incidence rates of bladder cancer among workers at the plant were compared with those of the population of New York State (excluding New York City). The study reported an

increased bladder cancer risk in a cohort of 1749 workers potentially exposed to *o*-toluidine and aniline at a chemical manufacturing plant. Increased risk of bladder cancer was strongly associated with increased duration of employment in the department where *o*-toluidine and aniline were used. The authors concluded that it is more likely that *o*-toluidine is responsible for the observed excess number of cases of bladder cancer, because *o*-toluidine is a more potent animal carcinogen than aniline and is known to produce bladder tumors in rats, although aniline may also have played a role.

In a follow up study Ward et al. (1996) reported an environmental and biological monitoring survey conducted to evaluate exposures to aniline and *o*-toluidine in the rubber chemical department of their earlier study. Personal air sampling for aniline and *o*-toluidine was performed and urine samples were collected before and after work shifts. Personal air sample measurements showed that airborne concentrations of aniline and *o*-toluidine were well within the limits allowed in the workplace by OSHA. However, urinary aniline and *o*-toluidine levels were substantially higher among exposed workers than among unexposed control subjects. The most striking differential was observed for post-shift urinary *o*-toluidine levels, amounting to 2.8 +/- 1.4 micrograms/L in unexposed subjects and 98.7 +/- 119.4 micrograms/L in exposed subjects. Average aniline-Hb and *o*-toluidine-Hb adduct levels were also significantly higher among exposed workers than among unexposed control subjects. The authors concluded that these data support the conclusion that occupational exposure to *o*-toluidine is the most likely causal agent of the bladder cancer excess observed among workers in the rubber chemicals department of the plant under study, although a role for exposure to aniline and 4-ABP could not be ruled out.

Markowitz and Levin (2004) reported additional data to the NIOSH cohort study published in 1991 (Ward et al. 1991), mentioning 19 additional cases of bladder cancer among workers in this cohort, yielding a total of 34 cases of bladder cancer in the cohort to the date of analysis. The authors concluded that the number of bladder cancers diagnosed in the most recent period has increased and that the timing of onset of exposure to *o*-toluidine of numerous cases of bladder cancer after 1968, and especially 1975, suggested that potentially confounding occupational exposures other than *o*-toluidine were not the cause for the observed excess bladder cancer.

Urinary bladder cancer cases and deaths identified in the 1991 study (Ward et al., 1991) were re-analyzed by Carreón et al. (2010). They argued that additional information showed that workers in certain departments had been misclassified regarding *o*-toluidine exposure, and conducted a reanalysis of the data using updated exposure categories. Among workers classified as definitely exposed, increasing risks were observed for longer duration of employment and time since first employment in the exposed departments. The authors concluded that their findings were comparable to the results reported earlier by NIOSH, and confirm that workers in this plant have an increased risk of bladder cancer.

An updated, detailed exposure survey of ambient levels of *o*-toluidine and co-exposures in this retrospective NIOSH study on cancer incidence and mortality of workers employed at a rubber chemical manufacturing plant was subsequently reported by Hanley et al. (2012). In this study three new exposure assessment schemes were presented. First, the original exposure-department groups were updated reclassifying some departments. Second, four exposure categories were defined based on department-job combinations. Further, an approximate rank of the "relative" exposure level was assigned to each job title department combination over time to calculate average and cumulative exposure scores for each cohort member based on the personal work history.

Carreón et al. (2014) reported a further reanalysis of this cohort, refining the exposure classification using department-job combinations and by approximate exposure ranks as outlined by Hanley et al. (2012). This re-analysis showed excess bladder cancer compared to the New York State population, with higher elevations among workers

exposed at an estimated moderate/high level and in the highest cumulative rank quartile. Based on this reanalysis bladder cancer incidences remained elevated in this cohort and significantly associated with estimated cumulative exposure. The authors indicated that their results were consistent with earlier findings in this and other cohorts. Given the other concurrent chemical exposures, the authors concluded that *o*-toluidine is most likely responsible for the bladder cancer incidence elevation and recommend a further re-examination of occupational exposure limits.

In another study in the rubber chemical industry standardized cancer mortality and incidence among a cohort of 2160 male production workers from a chemical factory in north Wales was analyzed. An initial follow-up of this study was reported by Sorahan and Pope (1993), with follow-ups to 1992 (incidence) and 1996 (mortality) by Sorahan et al. (2000), and a follow-up to 2005 by Sorahan (2008). Analysis by duration of employment was conducted for this cohort but no quantitative data on *o*-toluidine exposure were reported, making the study inadequate for quantitative risk assessment. Also the fact that the cohort was exposed to other chemicals in addition to *o*-toluidine (2-mercaptobenzothiazole (MBT), aniline, phenyl-β-naphthylamine (PBN)), the small numbers in the exposed subcohorts, relatively crude measures of exposure assessment for the four chemicals under study, and presence of unconsidered potential chemical confounders render to study unsuitable for quantitative interpretation.
Richardson et al. (2007) reported a population-based case-control study based on a Canadian cancer registry study of male urinary bladder cancer in which job-exposure matrices of exposure to a range of occupational agents were applied. The study investigated the risk of bladder cancer in association with exposure to over 12 000 occupatinal chemical agents. The final analysis resulted in a subset of 29 chemicals for which increasing significant dose-reponse relationships were observed. While 4-chloro-*o*-toluidine exhibited a significant dose–response relationship (P=0.01), no excess risk was observed for *o*-toluidine.

Castro-Jiménez and Orozco-Vargas (2011) reported a hospital-based case-control study in Colombia determining the risk factors for childhood acute lymphoblastic leukemia (ALL) and, in particular, the role of the parental occupational exposure to carcinogenic and probably carcinogenic hydrocarbons before the child's conception. A statistically significant increase in ALL was observed among children whose parents were both estimated to have had potential occupational exposure to *o*-toluidine, although there is potential confounding by exposure to other hydrocarbons that can be associated with an increase in ALL risk. Therefore, the data presented are not adequate to evaluate the relationship between exposure to *o*-toluidine and childhood ALL.

Stasik (1988) reported a standardized urinary bladder cancer incidence analysis among male workers in Germany engaged in the manufacture of the azo dye and chlordimeform pesticide intermediate 4-chloro-*o*-toluidine (4-COT), in which *o*-toluidine is used as a precursor. The standardized incidence rate for urothelial carcinomas in the 4-COT subcohort was 73 times higher than expected and was comparable with the results obtained for polycyclic arylamines, which have been identified as human carcinogenic agents. The authors concluded that the results suggest an association between occupational exposure to 4-COT and carcinomas of the urinary bladder observed among production workers. The authors reprted that there were four monocyclic amines at the plant, includng N-acetyl-*o*-toluidine, 6-chloro-*o*-toluidine, *o*-toluidine and 4-chloro-*o*-toluidine but that exposure to 4-COT in the plant was predominant. Therefore they concluded that it was suggested that the observed clinical malignancy is connected with 4-COT. Exposure levels were not quantified.

Vigliani and Barsotti (1961) reported on occurence of bladder tumors in six Italian dye stuff factories. A link with aromatic amine exposure was established especialy for benzidine and alpha- en beta-naphtylamine, but data for *o*-touidine were resticted to mixture exposure.
Khlebnikova et al. (1970) reported on problems of industrial hygiene and health status of workers engaged in the production of *o*-toluidine. Evaluation of this study in previous

reports on *o*-toluide concluded that the data did not allow establishment of risk estimates for exposure to *o*-toluidine (NTP, 2014).

Zavon et al. (1973) reported increased incidence of bladder cancer in workers involved in the manufacture of benzidine. Some of the study subjects had co-exposure to *o*-toluidine but the authors concluded that the common chemical to which all workers were exposed was benzidine which was considered the most likely carcinogen. Only benzidine concentrations at different workplaces were quantified.

Finally also Conso and Portal (1982) reported on a possible role for industrial exposure to *o*-toluidine and *o*-aminoazotoluene in the incidence of tumours of the bladder.

7.7.2. Animal data

Rats

Several carcinogenicity studies for *o*-toluidine in rats have been published. These include feeding studies in male rats of different strains (F-344 or Charles River)(Weisburger et al., 1978, Hecht et al. 1982, NTP 1996), in male and female F344 rats (NCI, 1979, Goodman et al., 1984), and a study with subcutaneous injection in rats (Pliss, 2004)(as referred to in NTP, 2014).

Weisburger et al. (1978) reported tumor induction upon dietary administration of a series of twenty-one aromatic amines or derivatives including *o*-toluidine to Charles River rats and male and female HaM/ICR mice. *o*-Toluidine, administered at 8,000 or 16,000 mg/kg diet for 3 months or at 4,000 or 8,000 mg/kg diet for 15 months, induced tumors in one or more tissues in all three of these animal models. In male rats, it induced numerous subcutaneous fibromas and fibrosarcomas. A few transitional cell carcinomas of the bladder were also seen, as well as an increase in multiple tumors.

Hecht et al., (1982) administered *o*-toluidine hydrochloride and one of its metabolites, *o*-nitrosotoluene, at levels of 0.028 mol/kg diet to groups of 30 male F-344 rats for 72 weeks. Both compounds induced tumors of the bladder and the liver, whereas nitrosotoluene induced significantly more tumors of the bladder (16/30 rats) and liver (20/30) than *o*-toluidine hydrochloride (bladder, 4/30; liver, 3/30). Both compounds also induced peritoneal tumors and fibroma of the skin and spleen with comparable incidences, while *o*-toluidine hydrochloride induced more mammary tumors (13/30) than *o*-nitrosotoluene (3/30). The *o*-toluidine metabolite *o*-nitrosotoluene was more potent in inducing especially bladder and liver tumors than *o*-toludine indicating the importance of oxidation in the induction of bladder and liver cancer by *o*-toluidine.

Elwell (1996) reported the results of a comparative toxicity and carcinogenicity study performed by the NTP on *o*-nitrotoluene and *o*-toluidine hydrochloride administered in feed to male F344/N rats. *o*-Toluidine hydrochloride was administered in feed at 5,000 ppm to male F344/N rats for 13 or 26 weeks. *o*-Toluidine hydrochloride caused mesothelial hyperplasia and mesothelioma, an accumulation of hemosiderin pigment in renal tubule epithelium, and increased incidences of hematopoiesis, hemosiderin accumulation, and capsular fibrosis in the spleen.

In a 2-year bioassays *o*-toluidine hydrochloride was administered to groups of 50 male and 50 female rats and mice in feed at concentrations of 3,000 or 6,000 ppm for rats and 1,000 or 3,000 ppm for mice for 101 to 104 weeks (NCI, 1979). When compared to controls, rats given *o*-toluidine hydrochloride in feed showed increased incidences of benign and malignant tumors (sarcomas) of the spleen in males and females, mesotheliomas of the abdominal cavity or scrotum in males (control, 0%; 3,000 ppm, 24%; 6,000 ppm, 12%), and transitional-cell carcinomas of the urinary bladder in females (0%, 20%, 47%). *o*-Toluidine hydrochloride exposure also resulted in increased incidences of fibromas of the subcutaneous tissue in males (0%, 56%, 55%) and fibroadenomas or adenomas of the mammary gland in females (36%, 40%, 71%).

Especially the data on formation of the transitional-cell carcinomas of the urinary bladder in female rats reported in this NCI study appear suitable for dose reponse modelling. Table 8 presents the data in some more detail and Table 9 presents the outcomes of the dose reponse modelling.

Table 8: Incidences of transitional-cell carcinoma of the urinary bladder in female rats orally exposed to *o*-toluidine for 2 years (NCI, 1979)

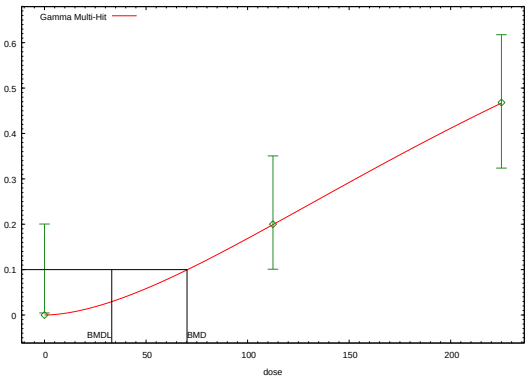
Dose level *o*-toluidine hydrochloride ppm in diet	Dose level *o*-toluidine hydrochloride mg/kg bw per day[1)]	Dose level *o*-toluidine mg/kg bw per day[2)]	No of animals	No of animals with transitional-cell carcinoma
0	0	0	20	0
3000	150	112.5	45	9
6000	300	225	47	22

1) Calculated using a conversion factor of 1 ppm = 0.05 mg/kg bw per day
2) Calculated taking into account that 107.16 (=75 %) of the molecular weight of *o*-toluidine hydrochloride is *o*-toluidine.

Table 9: Results from a BMD analysis of the data on incidences of transitional-cell carcinoma of the urinary bladder in female rats exposed to *o*-toluidine for 2 years (NCI, 1979) representing the benchmark dose (BMD_{10}), the 95 % benchmark dose lower confidence limit of the $BMDL_{10}$ ($BMDL_{10}$) for a BMR of 10% extra risk with characteristics of the model fit. The selected model (BMD/BMDL < 3, lowest AIC) is given in bold

Model Type	**Risk Type**	**BMR**	**Restricted model**	**No of parameters**	**Model accepted**	**p-value (goodness of fit)**	**AIC**	**BMD mg/kg bw per day**	**BMDL mg/kg bw per day**	**BMD/ BMDL**
Gamma	Extra	0.1	yes	2	yes	1.00	114.00	70.2	33.1	2.12
Logistic	Extra	0.1	na	2	yes	0.15	116.06	96.1	75.3	1.28
LogLogistic	Extra	0.1	yes	2	yes	1.00	114.00	72.0	30.0	2.40
Probit	Extra	0.1	na	2	yes	0.21	115.57	90.9	70.9	1.28
LogProbit	Extra	0.1	yes	2	yes	1.00	114.00	75.4	55.7	1.36
Weibull	Extra	0.1	yes	2	yes	1.00	114.00	68.2	33.1	2.06
Multistage Cancer	Extra	0.1	na	2	yes	1.00	114.00	64.5	33.1	1.95
Multistage	Extra	0.1	yes	2	yes	1.00	114.00	64.5	33.1	1.95
Quantal Linear	**Extra**	**0.1**	**na**	**1**	**no**	**0.67**	**112.79**	**42.2**	**31.7**	**1.33**

Gamma Multi-Hit Model, with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



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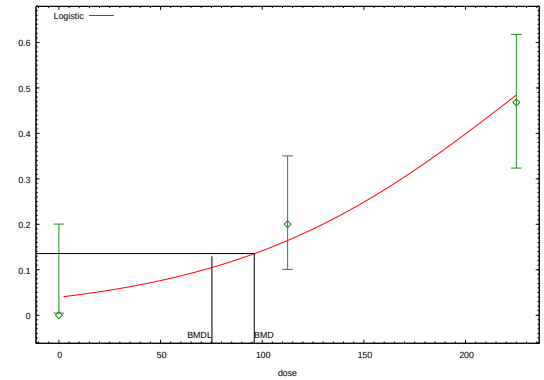
Logistic Model, with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



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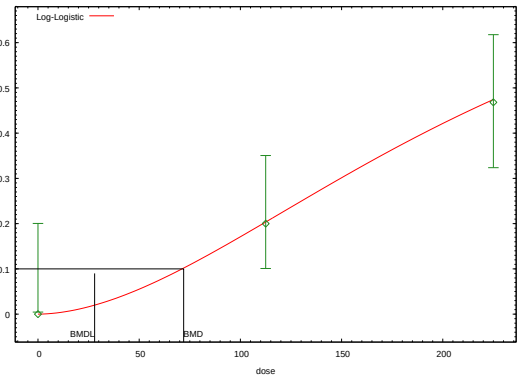
Log-Logistic Model, with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



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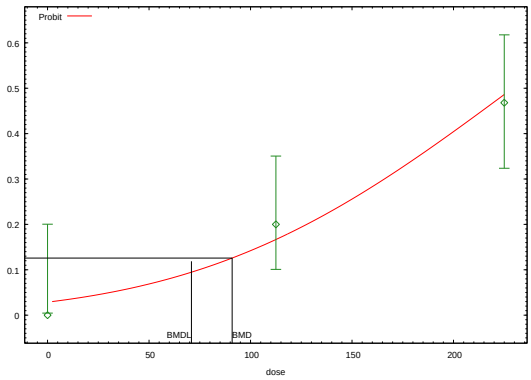
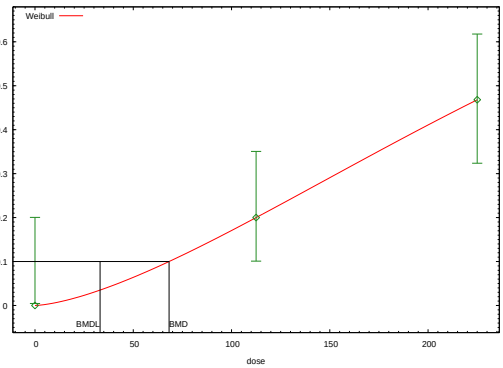
Probit Model, with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



13:58 04/20 2016

Weibull Model, with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL



14:01 04/20 2016

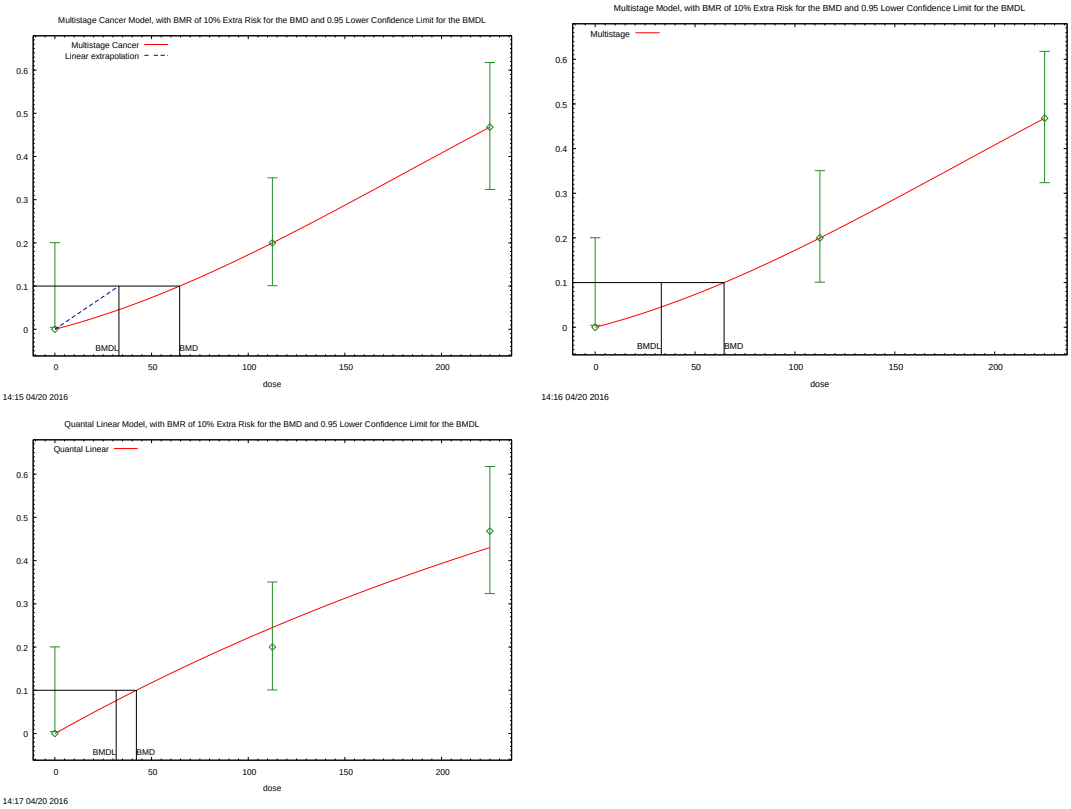


Figure 1: Graphs of the BMD analysis using the different models to fit the data on incidences of transitional-cell carcinoma of the urinary bladder in female rats orally exposed to *o*-toluidine for 2 years (Table 8) (NCI, 1979)

From these data the fit with the Quantal-linear model gives the best fit with a BMD_{10} of of 42.2 mg/kg bw per day.

Goodman et al. (1984) reported on the induction of splenic fibrosis and sarcomas in F344 rats fed diets containing *o*-toluidine HCl and five other substances. The rats, were given the compounds from 6 weeks of age up to 2 years at the estimated maximum tolerated dose (6000 mg/kg diet estimated to amount to an expsoure of 300 mg/kg bw per day) and one-half that dose (3000 mg/kg diet estimated to amount to an expsoure of 150 mg/kg bw per day). In all six bioassays, dose-dependent incidences of splenic sarcomas and fibrosis were seen, with the highest incidences in male rats.

Pliss (2004)(as referenced in NTP 2014) reported a two-year carcinogenicity study with subcutaneous injection of *o*-toluidine in mice and rats for the entire study duration. Tumors developed in 40% of the rats including subcutaneous fat tumors, mammary fibroadenomas, leukemia, renal tumors and hepatic sarcoma.

Mice

Three feed studies in different strains of female and male mice (Albino CD-1 [HaM/ICR] and B6C3F_1) are available (NCI 1979, Weisburger et al. 1978; NTP, 1996).

Weisburger et al., (1978) reported tumor induction upon dietary administration of a series of twenty-one aromatic amines or derivatives including *o*-toluidine to Charles River rats and male and female HaM/ICR mice. *o*-Toluidine, administered at 16,000 or 32,000 mg/kg diet for 5 months or at 4,000 or 8,000 or 16,000 mg/kg for 13 months, induced tumors in one or more tissues in all three of these animal models. In both male and female mice significant incidences of hemangiosarcomas and hemangiomas occurred in abdominal viscera.

The NCI study (1979) also tested *o*-toluidine for possible carcinogenicity in B6C3F1 mice. Groups of 50 mice of each sex were administered *o*-toluidine hydrochloride at 1,000 or 3,000 ppm in feed, for 101 to 104 weeks. Matched controls consisted of 20 untreated mice of each sex. In mice given *o*-toluidine hydrochloride, there were increased incidences of hemangiosarcomas in males (5%, 4%, 24%) and of hepatocellular carcinomas or adenomas in females (0%, 8%, 26%) (NCI, 1979). Although especially the data on the hepatocellular carcinomas reveal a clear dose response, dose reponse modelling was not performed given that the dose levels and data indicate that BMD10 values to be obtained would be higher than those obtained for the data on transitional-cell carcinoma of the urinary bladder in female rats .

Pliss (2004)(as referred to in NTP, 2014) reported a two-year carcinogenicity study with subcutaneous injection of *o*-toluidine in mice and rats for the entire study duration. Tumors were induced in 19% of mice including lung and kidney adenomas and leukemias.

Hamsters

Hecht et al. (1983) reported a study on the carcinogenicity of various aromatic amines including *o*-toluidine in Syrian golden hamsters. Total doses of 99 mmol/kg of *o*-toluidine, administered by subcutaneous injection for 52 weeks failed to induce tumors up to observation at 87 weeks.

Dogs

In a study reported by Pliss (2004)(as referred to in NTP, 2014) five dogs were exposed to *o*-toluidine, first in the food and later by gavage, for up to 9 years. There were no untreated controls and two dogs died of non-cancer related deaths within three years of the study onset. In two dogs bladder tumors developed.

Conclusion

NTP (NTP, 2014) evaluated these animal studies and concluded that four feeding studies in rats (Weisburger et al., 1978, NCI, 1979, Hecht et al., 1982, NTP, 1996), two feeding studies in mice (Weisburger et al., 1978, NCI, 1979), two subcutaneous studies, one in hamsters (Hecht et al., 1983) and one in rats (Pliss, 2004)(as referred to in NTP, 2014) could inform the carcinogenicity assessment.

NTP concluded that the collective data from the four feeding studies in experimental animals provide strong evidence that *o*-toluidine exposure causes urinary bladder neoplasms in female rats, with weaker evidence observed for male rats. In female F344 rats, dietary exposure to *o*-toluidine caused a dose-related statistically significant increased incidence of urinary bladder cancer in both exposure groups. Esepecially in the NCI sudy (NCI, 1979) there appeared to be a dose-related progression, especially in female rats, from transitional-cell hyperplasia to transitional-cell carcinoma of the urinary

bladder.

Analysing these data by dose reponse modelling revealed a BMD10 of 42.2 mg/kg bw per day which can be further estimated to correspond to an inhaled dose of about 414 mg/m^3 as an 8-hour TWA at occupational exposure of 5 days/week (assuming a body weight of 70 kg, an inhaled amount of 10 m^3 for 8 h working day, and equal absorption via inhalation and the oral route)." (i.e. 42.2 mg/kg bw/day * 70 kg / 10 m^3 *7/5).

7.8. Reproductive toxicity

No data available.

7.8.1. Human data

No data available.

7.8.2. Animal data

7.8.1.1. Fertility

No data available.

7.8.1.2. Developmental toxicity

No data available.

7.8.1.3. Inhalation

No data available.

7.8.1.4. Oral

No data available.

7.8.1.5. Dermal

No data available.

7.8.1.6. Other routes

No data available.

7.8.3. In vitro data

7.9. Mode of action and adverse outcome pathway considerations

No specific considerations.

7.10. Lack of specific scientific information

No specific considerations.

8. CANCER RISK ASSESSMENT

The Point of Departure (POD) for defining risk number was based on the results from a BMD analysis of the data on incidences of transitional-cell carcinoma of the urinary bladder in female rats exposed to *o*-toluidine via the diet for 2 years (NCI, 1979). In this BMD analysis the fit with the Quantal-linear model gives the best fit with a BMD_{10} of of 42.2 mg/kg bw per day.

This BMD_{10} value is based on a rat study with dietary life time exposure.

A BMD_{10} of 42.2 mg/kg bw per day can be further estimated to correspond to an inhaled dose of about 840 mg/m^3 as an 8-hour TWA at occupational exposure during 40 out of 75 years, 48 out of 52 weeks and 5 days/week (assuming a body weight of 70 kg, an inhaled amount of 10 m^3 for 8 h working day, and equal absorption via inhalation and the oral route, i.e. 42.2 mg/kg bw per day * 70 kg / 10 m^3 * 75/40 * 52/48 * 7/5).

Allometric scaling of this dose level of 840 mg/m^3 from rat to human using a default value of times 0.25 provides a POD for 10% increase in tumor risk of 210 mg/m^3

Using this value the following risk numbers were derived:

A tumor risk of 1 : 10 at the BMD_{10} of 210 mg/m^3 (48 ppm)

A tumor risk of 1:1000 at 2.10 mg/m^3 (0.48 ppm)

A tumor risk of 1 : 10 000 at 0.210 mg/m^3 (0.048 ppm)

A tumor risk of 1 : 10^6 at 2.10 microg/m^3 (0.00048 ppm)

Using this value it can also be calculated that at the ACGIH TLV-TWA of 2 ppm (8.8 mg/m^3) the tumor incidence is estimated to be 1: 239.

9. GROUPS AT EXTRA RISK

Susceptibility to aromatic amine-induced bladder cancer has been associated with the genetic polymorphism for N-acetyltransferases resulting in increased susceptibility to aromatic amine-inuced cancer for slow-acetylators (Golka et al. 2002).

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