The MAK Collection for Occupational Health and Safety

Addendum to Aniline

Assessment Values in Biological Material – Translation of the German version from 2016

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Addendum to Aniline

BAT Value Documentation

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Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated the BAT value (biological tolerance value) for aniline, considering aniline in urine to characterise the internal exposure and the methaemoglobin formation as critical effect. Available publications as well as unpublished study reports are described in detail.
In a new chamber study, 19 individuals were exposed to the present MAK-value of 2 ml aniline/m³ for 6 hours with defined physical activity. The background level of methaemoglobin was 0.7 ± 0.2%. At the end of exposure, an increase of up to 1.2 ± 0.3% (range 0.8–2.1%) was observed. The mean excretion of aniline in urine after hydrolysis at the end of exposure was 168 ± 52 µg/l (range 79.5–418 µg/l).
The BAT-value relates to an air concentration of 2 ml/m³ and a methaemoglobin level of 5%. Considering this study, a BAT-value of 500 µg aniline (after hydrolysis)/l urine was evaluated. This value is a maximum concentration because of acute toxic effects of methaemoglobin. Sampling time is at the end of exposure or the end of the working shift.
For the hemoglobin adduct of aniline, a BLW ("Biologischer Leitwert") of 100 µg aniline (released from aniline-haemoglobin conjugate)/l blood was evaluated based on occupational-medical experiences in field studies.

Keywords

aniline; aminobenzene; phenylamine; occupational exposure; biological tolerance value; BAT value; BLW; toxicity

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Addendum to Aniline

BAT (2015)	**500 µg aniline (after hydrolysis)/l urine**[1)] Sampling time: end of exposure or end of shift
BLW (2015)	**100 µg aniline (released from haemoglobin conjugate)/l blood** Sampling time: after exposure for at least 3 months
MAK value (1981)	**2 ml/m³ (ppm) ≙ 7.7 mg/m³** [2)]
Absorption through the skin (1981)	H
Carcinogenicity (2006)	Carcinogen Category 4

1 Re-evaluation

1.1 Carcinogenicity

In a long-term study, aniline produced tumours of the spleen in rats (Greim 2007, translated), but it is not considered by the Commission to be primarily genotoxic, and is classified in carcinogen category 4. This allows the establishment of a MAK and a BAT value (biological tolerance value).

In the meantime, this assessment has been confirmed by mechanistic studies.

The methaemoglobinaemia caused by aniline leads to an increased degradation of erythrocytes in the spleen, combined with an increased accumulation of iron. The triggered oxidative stress results in secondary genotoxicity, which explains the tumour formation occurring at high doses (Ma et al. 2008, 2011, 2013; Wang et al. 2010). However, this also means that such a tumour formation is not to be expected when the methaemoglobin concentration is assessed as not adverse. With methaemoglobin levels of 4% to 5%, therefore, no tumour formation is to be expected in humans.

1.2 Exposure and effects

Recent research results make a re-evaluation of the BAT values for aniline from 1986 (see BAT Documentation 1986, translated) possible. From a controlled exposure

1) The substance can be present as vapour and as aerosol at the same time.

2) derivation of the BAT value as ceiling value because of acute toxicity

study, correlation data between the MAK value of 2 ml/m³ and the effect parameter "methaemoglobin" as well as the two exposure parameters "aniline in urine" and "aniline haemoglobin adduct" are now available (Käfferlein et al. 2014 a). This study consists of two parts – a pilot study and a main study:

In the **pilot study**, 4 persons (2 men, 2 women, non-smokers, slow acetylators) were exposed in an exposure chamber for 8 hours to aniline at the level of the current MAK value of 2 ml/m³. Exposure took place over four periods of 2 hours each with 15-minute intervals for blood sampling. During the exposure periods, volunteers undertook 4 · 20 minutes physical activity on a bicycle ergometer up to the individual aerobic/anaerobic threshold, which had previously been determined. This exposure increased the methaemoglobin level up to 1.56%, a plateau being attained after 6 hours. The highest individual urinary excretion of total aniline after this exposure was 306 µg/l.

In the **main study**, 19 persons (10 men and 9 women, all non-smokers, 15 slow acetylators and 4 fast acetylators) were exposed to 2 ml aniline/m³ in the chamber for 6 hours each. Exposure was again over three periods of 2 hours each with 15-minute intervals for blood sampling. Again, during this time, 3 · 20 minutes physical activity on a bicycle ergometer up to a ventilation rate of 30 l/minute were to be performed.

1.2.1 Effect marker in blood – methaemoglobin (Met-Hb)

The methaemoglobin level was 0.72% ± 0.19% before exposure. At the end of exposure, the maximum methaemoglobin levels were 1.21% ± 0.29% (range: 0.80%–2.07% methaemoglobin), as given in Figure 1. After 24 hours, the methaemoglobin level was back to the value obtained before exposure (0.65% ± 0.18%). No difference was found between slow and fast acetylators.

1.2.2 Effect marker in urine – total aniline

Figure 2 shows the results of the total urinary aniline excretion. Immediately after the exposure, the excretion reached a mean level of 168 ± 51.8 µg/l urine (range: 79.5–418.3 µg/l). No significant differences between men and women were found.

1.2.3 Effect marker in blood – aniline-haemoglobin adduct

As further marker for aniline exposure, the haemoglobin adduct of aniline was determined before and after a chamber exposure to 2 ml aniline/m³. Figure 3 shows the course (mean value and standard deviation). The range of individual peak concentrations was 0.74 µg/l–18.04 µg/l blood. There was no difference between the adduct levels of men and women. In slow acetylators, the aniline-Hb adduct levels, with an average of 6.08 µg/l blood, were higher by about 30% than those in fast acetylators with 4.69 µg/l.

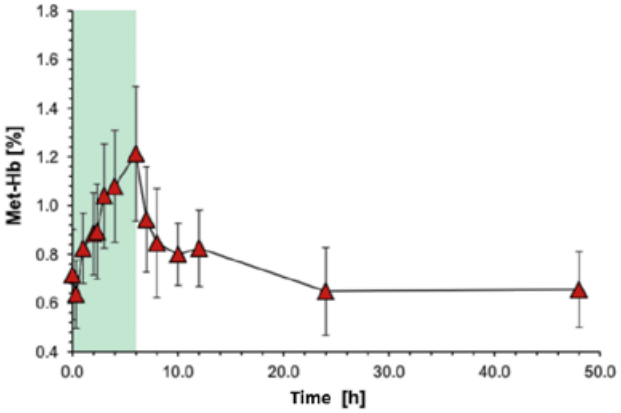

Figure 1 Kinetics of the methaemoglobin formation in 19 persons before, during and after a chamber exposure to 2 ml aniline/m³ for 6 hours (main study, Käfferlein et al. 2014 b)

1.3 Evaluation of the BAT values and the BLW ("Biologischer Leitwert")

New studies relevant for evaluation have been published since the last addendum to the BAT value for aniline in 1994 (see BAT Documentation 1994, translated).

On the basis of occupational-medical results, a BAT value of 1 mg/l urine (related to free aniline) and 100 µg haemoglobin adduct/l blood, related to an air concentration of 2 ml aniline/m³ and a methaemoglobin level of 5% has applied for aniline since 1986. A re-evaluation of the BAT values on the basis of available correlation data between the MAK value of 2 ml aniline/m³ and the parameters "methaemoglobin", "aniline in urine" and "aniline-haemoglobin adduct" can now be carried out.

In the chamber study described in Section 10.2 with an exposure to 2 ml aniline/m³ (inhalation; dermal contact was excluded as far as absolutely possible), 2.07% was measured as highest individual Met-Hb value. This means that, when the MAK value is observed, this is clearly below the Met-Hb value of 5% considered as adverse.

A special feature of deriving a BAT value on the basis of a thus attained Met-Hb value is, that, in analogy to the derivation of the BAT value for carbon monoxide, this BAT value is to be derived as ceiling value on account of acute toxic effects: It must be ensured that, also in individual cases, on compliance with the BAT value, the Met-Hb level remains below 5%. The highest individual value found by Käfferlein et al. (2014 a) was 2.07% Met-Hb. Taking the average baseline value of 0.72% Met-Hb into account, the highest value of the Met-Hb increment induced by aniline was 1.35%. Contrary to the pilot study by Käfferlein et al. (2014 a), the Met-Hb level attained no plateau after exposure in the main study (see Figure 1), so that a linear extrapolation of the experimental 6-hour exposure to an 8-hour working day exposure becomes necessary. The Met-Hb increment to be expected after 8 hours is therefore 1.80%. The 8-hour increment found by Käfferlein et al. (2014 a) under the experimental conditions of exposure to aniline at the level of the MAK value, is

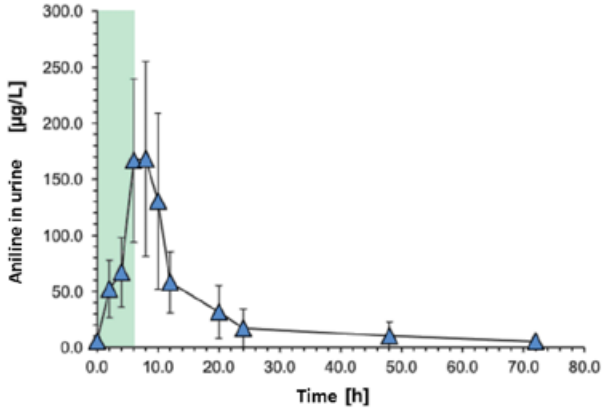

Figure 2 Kinetics of the urinary excretion of total aniline in 19 persons before, during and after a chamber exposure to 2 ml aniline/m³ for 6 hours (Käfferlein et al. 2014 b)

lower than the maximum permissible Met-Hb increment of 4% by a factor of 2.22 when taking the general baseline value into account.

In the study by Käfferlein et al. (2014 a), the mean value of aniline excretion was 168 µg/l urine after 6-hour exposure; therefore, an excretion of 224 µg/l would be expected at the end of an 8-hour exposure. When taking the factor of 2.22 stated above as basis, an excretion of 497.3 (rounded off to 500) µg/l would be expected under conditions in which the critical Met-Hb increment is not yet attained. The thus determined value for an aniline excretion of 500 µg/l at the end of shift can therefore be derived as BAT value. The BAT value relates to a Met-Hb level of 5% and is to be seen as ceiling value, i.e. it must be ensured that this value is not exceeded.

A **BAT value** of

500 µg aniline (after hydrolysis)/l urine[3)]

is therefore established.

Sampling should be at the end of exposure or end of shift.

The adduct increment of about 5 µg/l blood (see Figure 3) found in the study with volunteers after a single 6-hour exposure is not in obvious contradiction to the previous BAT value, as the adduct accumulates to a great extent after repeated exposure to aniline. As Käfferlein et al. (2014 a) did not monitor the adduct levels in the volunteers any longer than 48 hours, no adduct half-life or adduct decay rate can be calculated from this study.

A quantitative consideration with regard to the existing BAT adduct value is therefore not possible from the study by Käfferlein et al. (2014 a). The previous BAT

3) derived as ceiling value because of acute toxicity

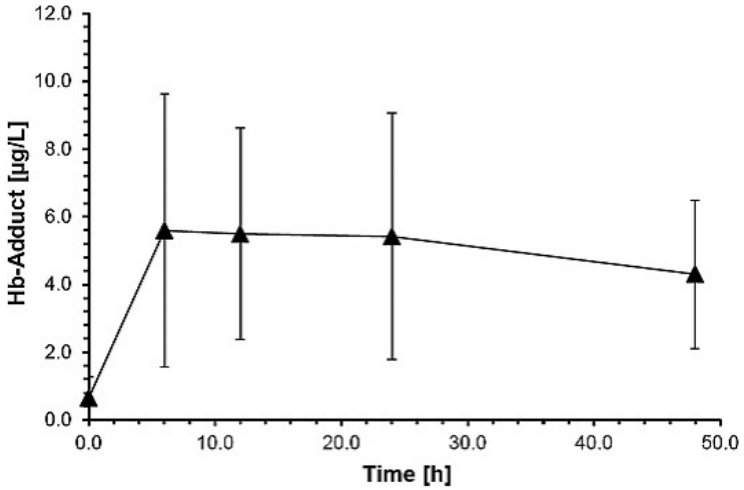

Figure 3 Kinetics of the aniline-haemoglobin adduct levels in 19 persons before and after a chamber exposure to 2 ml aniline/m3 for 6 hours (Käfferlein et al. 2014 c)

value of 100 µg aniline adduct/l blood is based on occupational-medical field results and is therefore to be considered as an occupational health-based BLW.

For this reason a **BLW** of

100 µg aniline (released from haemoglobin conjugate)/l blood

is established for aniline.

Sampling should be carried out after exposure for at least 3 months (see BAT Documentation 1986, translated).

At the workplace, attention must be paid to the fact that aniline penetrates the skin very readily (Korinth et al. 2012). This explains with great probability the demonstrable discrepancy in Met-Hb formation between laboratory and field conditions. Therefore, biomonitoring is of special importance for this substance.

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