ΚΥΠΡΙΑΚΟ
ΕΜΠΟΡΙΚΟ ΚΑΙ
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ΕΠΙΜΕΛΗΤΗΡΙΟ

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European Commission

Λευκωσία, 11 Νοεμβρίου 2014

ΠΡΟΣ: **Όλους τους ενδιαφερόμενους**

ΘΕΜΑ: **Σύσταση Επιστημονικής Επιτροπής για τα Όρια Επαγγελματικής Έκθεσης (SCOEL) για την ανιλίνη.**

Κυρίες / Κύριοι,

Επισυνάπτεται η Σύσταση της Επιστημονικής Επιτροπής για τα Όρια Επαγγελματικής Έκθεσης που αφορά την ανιλίνη.

Μας έχει ζητηθεί όπως έχουμε τα σχόλια σας για τη Σύσταση και ιδιαίτερα για τα πιο κάτω σημεία:

- Υπάρχουν σημαντικά ή κρίσιμα δημοσιευμένα έγγραφα τα οποία δεν έχουν ληφθεί υπόψη;
- Έχει οποιοδήποτε από τα επιστημονικά δεδομένα παρερμηνευθεί;
- Έχετε υπόψη σας οποιαδήποτε άλλη σχετική πληροφορία;

Παρακαλούμε όπως αποστείλετε τα σχόλια σας στο email: freedom@ccci.org.cy το αργότερο μέχρι την Παρασκευή 21 Νοεμβρίου 2014.

Με εκτίμηση,

Παναγιώτης Παναγής,
Λειτουργός Τμήματος Εργασιακών Σχέσεων.

/ΕΞ

Recommendation from the Scientific Committee on Occupational Exposure Limits for Aniline (Addendum 2014)

SCOEL/SUM/153
September 2014

Draft for 4-week consultation
Deadline 25 November 2014

Employment, Social Affairs and Inclusion

Table of contents

Recommendation from the Scientific Committee on Occupational Exposure Limits for Aniline (Addendum 2014)

8-hour TWA:	2 ppm (7.74 mg/m^3)
STEL:	5 ppm (19.35 mg/m^3)
BLV:	0.2 mg aniline/l urine (sampling: end of shift)
Additional categorisation:	SCOEL carcinogen group C (carcinogen with a practical threshold)
Notation:	"Skin"

The SCOEL recommendation of an OEL for aniline (SCOEL/SUM/153), dated August 2010, was based on older human volunteer data by Dutkiewicz and Piotrowski (1961). This study provided the only data of this kind in 2009/2010 (when discussed by SCOEL). The study was not performed according to modern standards. In the meantime, new data have become available that allow a re-evaluation. The present addendum is therefore focussed on recent publications from 2009 on. These publications shed new light on the mechanisms of experimental spleen toxicity and carcinogenicity, and on quantitative aspects of methaemoglobin induction by aniline in humans, which are connected to OEL derivation. For other aspects, the reader is referred to the SCOEL Recommendation dated 2010.

1. Substance identification, physico-chemical properties

Name:	Aniline
Synonyms:	Aminobenzene, phenylamine
Molecular formula:	$C_6H_5NH_2$
EC No.:	200-539-3
CAS No.:	62-53-3
Molecular weight:	93.127 g/mol
Conversion factors:	1 ppm = 3.87 mg/m^3
(20 °C, 101.3kPa)	1 mg/m^3 = 0.258 ppm

EU harmonised classification:

Acute Tox. 3	H301	Toxic if swallowed
Acute Tox. 3	H311	Toxic in contact with skin
Skin Sens. 1	H317	May cause an allergic skin reaction
Eye Dam. 1	H318	Causes serious eye damage
Acute Tox. 3	H331	Toxic if inhaled
Muta. 2	H341	Suspected of causing genetic defects
Carc. 2	H351	Suspected of causing cancer
STOT RE 1	H372	Causes damage to organs through prolonged or repeated exposure
Aquatic Acute 1	H400	Very toxic to aquatic life

2. Occurrence

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

3. Health significance

3.1. Mode of action of spleen toxicity and carcinogenicity

As discussed in SCOEL/SUM/153, aniline has experimentally induced spleen tumours in rats. Aniline was categorised by SCOEL as a Group C carcinogen with a practical threshold, because carcinogenic doses caused early effects on haematological parameters, inflammatory reactions in the spleen and perturbations of iron metabolism as a result of haemolytic anaemia. In the meantime, this has been further substantiated.

Ma *et al* (2008) exposed male Sprague-Dawley rats subchronically to aniline (0.5 mmol/kg/day *via* drinking water for 30 days). This aniline treatment led to a significant increase in splenic oxidative DNA damage, measured as 8-hydroxy-deoxyguanosine and to up-regulation of OGG and NEIL1/2 DNA glycosylases in spleen.

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

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

A fourth study (Ma *et al* 2013) examined whether NTH1 and APE1 contribute to the repair of oxidative DNA lesions in the spleen following aniline treatment. This was identical to that in the preceding studies. The treatment led to significant increase in

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

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

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

3.2. Toxicokinetics

The toxicokinetics of aniline have been previously described in detail (SCOEL/SUM/153). In the meantime, a comparative study on percutaneous penetration of a number of chemicals, including aniline, provided additional support for the assignment of a "skin" notation (Korinth *et al* 2012). The flux estimation for aniline was 752.2 ± 213.5 µg/cm/h (mean ± SEM).

3.2.1. Biological monitoring

New data on biological monitoring became available with the human experimental exposure study described in Section 3.3.

3.3. Human experimental exposure

Käfferlein *et al* (2014) conducted a controlled human exposure study in volunteers. In a first pilot section of this study, 2 males and 2 females were exposed for 8 hours to 2 ppm airborne aniline. In this pilot experiment, exposure to aniline was carried out at 4 x 2-hour intervals, with 15 min breaks in between to facilitate blood sampling. The persons exercised for 4 x 20 min on a cycle ergometer to a previously determined aerobic/anaerobic threshold while being simultaneously exposed. This resulted in an increase of methaemoglobin levels up to 1.6 %, with a plateau after 6 hours. The maximal excretion of aniline in urine after exposure was 306 µg/l. The following main study included 19 non-smoking persons (10 males, 9 females) who were exposed to 2 ppm aniline for 6 hours. This period was broken into 3 x 2-hour intervals, with 15 min breaks for blood sampling. The persons exercised for 3 x 20 min on a cycle ergometer during exposure in their previously determined individual work load (leading to a mean ventilation rate of approximately 30 l/min during exercise). The basal methaemoglobin level prior to exposure was 0.72 ± 0.19 %. Following exposure, the maximum methaemoglobin level in blood was 1.21 ± 0.29 % (range: 0.8–2.07 %), and aniline excretion in the urine was 168.0 ± 51.8 µg/l (range: 79.5 ± 418.3 µg/l). After 24 hours, the mean level of methaemoglobin returned to the basal level (0.65 ± 0.18 %). No significant differences between males and females were noted. Details of the time course of methaemoglobin in blood during and after exposure are shown in Figure 1; the time course of aniline excretion in urine is shown in Figure 2. There was an elevation of the level of aniline-haemoglobin adducts, which depended on the acetylator status (NAT2; slow or fast acetylators), as shown in Figure 3. By contrast, the methaemoglobin levels and aniline excretion showed no significant effect of the acetylator status.

In essence, the new study of Käfferlein *et al* (2014) shows that a single exposure to aniline for 6–8 hours leads to methaemoglobin levels, which are far below the level of 4–5 %, which is regarded as being critical (SCOEL/SUM/153). The data also confirm that there will be no carry-over of elevated methaemoglobin to the next shift upon daily repetitive exposure. As the individual maximal methaemoglobin level under the

given experimental conditions was 2.21 %, even a moderate physical activity will not lead to exceeding the critical level.

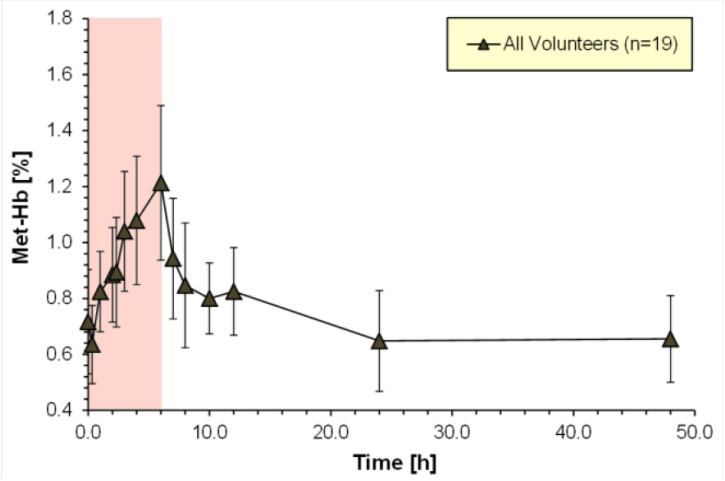

Figure 1. Time course of methaemoglobin (Met-Hb) levels in blood of 19 volunteers during and after experimental exposure to 2 ppm airborne aniline (Käfferlein *et al* 2014).

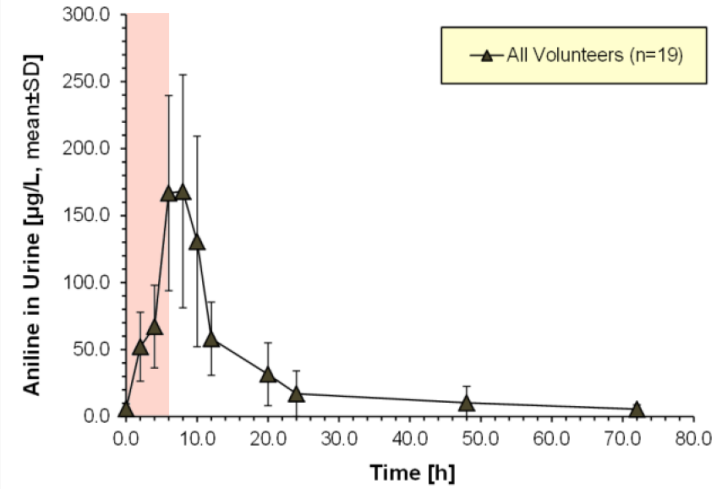

Figure 2. Time course of aniline excretion in the urine of 19 volunteers during and after experimental exposure to 2 ppm airborne aniline (Käfferlein *et al* 2014).

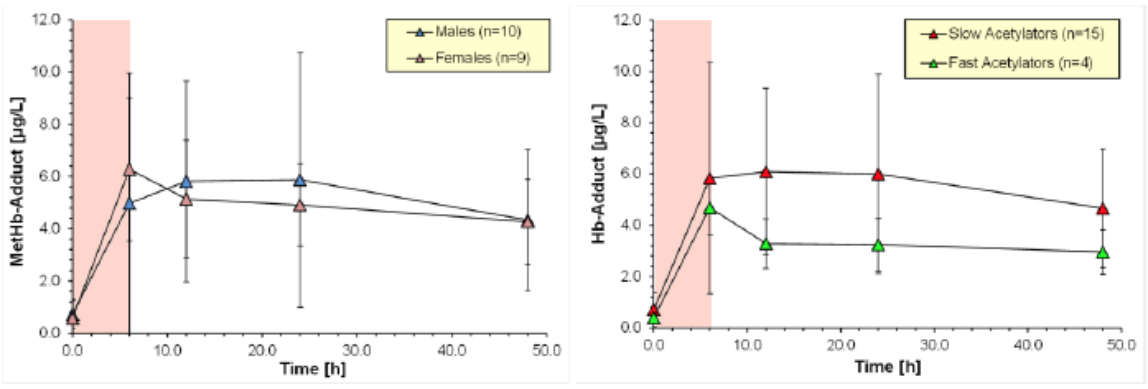

Figure 3. Time course of the aniline-haemoglobin adduct in the blood of 19 volunteers during and after experimental exposure to 2 ppm airborne aniline (Käfferlein *et al* 2014).

4. Recommendation

Since the SCOEL Recommendation SCOEL/SUM/153 new data have become available, which give rise to a re-assessment of the former Recommendation.

Studies into the mechanism of the experimental splenotoxicity and carcinogenicity in rats (Section 3.1) have provided further confirmation for a catagorisation into SCOEL group C of carcinogens (with a practical threshold). Therefore, it is now well established that chronic splenotoxicity and subsequent carcinogenicity is a secondary process following an increased breakdown of erythrocytes because of aniline-induced methaemoglobenaemia. As outlined in SCOEL/SUM/153, it follows that avoidance of excessive methaemoglobinaemia will protect against carcinogenesis in the spleen.

The new experimental human exposure study by Käfferlein *et al* (2014) supersedes the data of Dutkiewicz and Pietrowski (1961), which was the basis of the former recommendation of SCOEL. According to the new study, an exposure to 2 ppm aniline for 8 hours will not lead to elevation of methaemoglobin beyond critical levels, even if a moderate workload is considered (Section 3.3). *Therefore, an airborne level of 2 ppm aniline is recommended as OEL (8-hour TWA)*.

A comparison of the methaemoglobin levels reached under the conditions of the study of Käfferlein *et al* (see Figure 1) and the critical methaemoglobin levels of 4–5 % (SCOEL/SUM/153) shows that the recommended OEL of 2 ppm aniline implies an inbuilt safety factor of about 2. Therefore, no additional uncertainty factor is needed to compensate for possible additional human inter-individual variation.

As outlined by SCOEL (SCOEL/SUM/153), a STEL is preferred to limit short-term exposures with possible methaemoglobin formation. In view of the short half-life of aniline and the rapid decrease of methaemoglobin, an excursion factor of 2 will provide adequate protection (SCOEL/SUM/153). Applying the preferred value approach of SCOEL, a *STEL of 5 ppm is therefore recommended*.

In line with the previous argumentation (SCOEL/SUM/153), which is supported by additional data (Section 3.2), a *"skin" notation* is warranted.

Also regarding biological monitoring, the new study study of Käfferlein *et al* (2014) supersedes the study of Dutkiewicz and Piotrowski (1961). The time course of aniline in urine during and after the 6-hour exposure to 2 ppm aniline (Section 3.3, Figure 2) showed a mean concentration of aniline in urine of 170 µg/l. Considering an 8-hour workshift, *a new BLV of 0.2 mg aniline/l urine is therefore recommended.*

The present Addendum was adopted by SCOEL on Date Month Year.

5. References

Dutkiewicz T, Piotrowski J (1961). Experimental investigations on the quantitative estimation of aniline absorption in man. Pure Appl Chem 3:319-323.

Käfferlein HU, Broding HC, Bünger J, Jettkant B, Koslitz S, Lehnert M, Marek EM, Blaszkewicz M, Monsé C, Weiß T, Brüning T (2014). Human exposure to airborne aniline and formation of methemoglobin: a contribution to occupational exposure limits. Arch Toxicol 88:1419-1426.

Korinth G, Schaller KH, Bader M, Bartsch R, Göen T, Rossbach B, Drexler H (2012). Comparison of experimentally determined and mathematically predicted percutaneous penetration rates of chemicals. Arch Toxicol 86(3):423-430.

Kütting B, Göen T, Schwegler U, Fromme H, Uter W, Angerer J, Drexler H (2009). Monoarylamines in the general population – a cross-sectional population-based study including 1004 Bavarian subjects. Int J Hyg Environ Health 212(3):298-309.

Ma H, Wang J, Abdel-Rahman SZ, Boor PJ, Khan MF (2008). Oxidative DNA damage and its repair in rat spleen following subchronic exposure to aniline. Toxicol Appl Pharmacol 233(2):247-253.

Ma H, Wang J, Abdel-Rahman SZ, Hazra TK, Boor PJ, Khan MF (2011). Induction of NEIL1 and NEIL2 DNA glycosylases in aniline-induced splenic toxicity. Toxicol Appl Pharmacol 251(1):1-7.

Ma H, Wang J, Abdel-Rahman SZ, Boor PJ, Khan ME (2013). Induction of base excision repair enzymes NTH1 and APE1 in rats spleen following aniline exposure. Toxicol Appl Pharmacol 267(3):276-283.

Wang J, Ma H, Boor PJ, Ramanujam VM, Ansari GA, Khan MF (2010). Up-regulation of heme oxygenase-1 in rat spleen after aniline exposure. Free Radic Biol Med 48(4):513-518.

Weiss T, Angerer J (2002). Simultaneous determination of various aromatic amines and metabolites of aromatic nitro compounds in urine for low level exposure using gas chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 778(1-2):179-192.