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SCOEL Contact Points

Subject: **Activities of the Scientific Committee on Occupational Exposure Limits (SCOEL)**

Dear Sir or Madam,

In the context of cooperation and transparency concerning the Commission's activities in the establishment of OELs, the recommendation on MOCA CAS: 101-14-4 (SCOEL/SUM/174) was sent to you on 10.09.2010.

After consideration of the comments received and following SCOEL procedures, I send you for further consultation:

Annex to SCOEL/SUM/174: **MOCA** **CAS: 101-14-4**

This document is an annex to SCOEL/SUM/174 on MOCA (adopted by SCOEL in 2010) and it gives recommendation on the biological guidance value for MOCA.

The purpose of sending this document is to allow interested parties to submit additional information, if necessary or to contribute to scientific discussion. There are four main areas where SCOEL welcomes scientific comments, namely:

- Are there any important or critical published papers that have not been taken into consideration;
- Has any of the scientific data been misinterpreted;
- Is the approach taken consistent with SCOELs methodology;
- Are you aware of any other relevant information.

I would therefore be grateful to receive any scientific comments or data that you may have on this substance on 3rd September 2012 at the latest to:

Employment, Social Affairs and Inclusion DG
Health, Safety and Hygiene at Work
European Commission
Ms Jill Järnberg – SCOEL Scientific Secretary
Euroforum Building - Room EUFO 2191A
L-2920 LUXEMBOURG

Comments should be addressed directly to: Ms. Jill Järnberg (e-mail: jill.jarnberg@ec.europa.eu) with copy to Ms. Zofia Podolan (e-mail: zofia.podolan@ec.europa.eu).

Yours faithfully,

Costas CONSTANTINOU
Head of Unit

c.c.: SCOEL Members
Members of ACSH Working Party on Chemicals

Annex to Recommendation from the Scientific Committee on Occupational Exposure Limits for the Biological Guidance Value for 4,4'-Methylene-bis-(2-chloroaniline) [MOCA]

Annex to SCOEL/SUM/174
June 2012

Employment,
Social Affairs
and Inclusion

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Annex to SCOEL/SUM/174: Recommendation from the Scientific Committee on Occupational Exposure Limits for the Biological Guidance Value for 4,4'-Methylene-bis-(2-chloroaniline) [MOCA]

BGV:	detection limit of the method [LOD]
Carcinogenic risk assessment:	see Recommendation section

The present annex to the Recommendation from SCOEL on methylene-bis-(2-chloroaniline) [MOCA] presents further details on the possibilities of quantitation of exposure by biological monitoring and on associated cancer risk assessment. A Biological Guidance Value (BGV) is recommended.

1. Toxicokinetics

The toxicokinetics of MOCA has been described in SCOEL/SUM/174 (Section 2.1). Because of its low vapour pressure and ability to pass through the skin, skin contact to MOCA is often the most significant route of exposure. Therefore, biological monitoring plays an important role in the assessment of exposure to MOCA in occupational settings.

MOCA is activated by the cytochrome P450 system to reactive intermediates including *N*-hydroxy-MOCA, which is the main toxic DNA- and protein-reactive intermediate in MOCA metabolism (Cocker *et al* 1985). *N*-Hydroxy-MOCA has been shown to form adducts in human urothelial cells (Kaderlik *et al* 1993). After an acute high level exposure, DNA adduct levels were increased 4–98 hours after the exposure, the levels being highest at 4 hours (Kaderlik *et al* 1993).

Inactivation of MOCA occurs mainly through glucuronide- and acetyl-conjugation. MOCA is excreted in the urine as a free compound and as glucuronide or acetyl derivatives, the main metabolite in the urine being the *N*-glucuronide of MOCA (Cocker *et al* 1990). In the urine of exposed workers, MOCA-*N*-glucuronide levels 2–3 times higher than those of free MOCA have been found (Cocker *et al* 1990, Robert *et al* 1999a,b). The level of *N*-acetyl MOCA in urine is generally less than 10 % of the level of MOCA recovered in urine of exposed workers (Ducos *et al* 1985). After an acute high-level exposure the excretion of MOCA in the urine was highest 4 hours after the exposure; 23 hours after the exposure 50 % of the dose was excreted (Osorio *et al* 1990). This suggests a rapid excretion of MOCA after an acute (dermal and/or inhalation) exposure.

2. Biological monitoring of MOCA

For biological monitoring of MOCA exposure, total MOCA (free and conjugated MOCA) can be determined in the urine. Earlier, only the analysis of so-called free MOCA (i.e. MOCA detected without hydrolysis) was used. Later, it was found that MOCA is mostly excreted as labile glucuronide and acetyl conjugates, which can break down forming free MOCA during sample storage, thus affecting the final levels of free MOCA in the sample. Therefore, it was recommended to pre-treat samples to take into account these labile conjugates (Cocker *et al* 1988, Cocker *et al* 1990). There are different methods, which have been used for this purpose. The method described by Cocker *et*

al (1990) involves heat hydrolysis of labile conjugates followed by solid-phase extraction into 90 % acetonitrile, with separation of MOCA by reverse-phase HPLC and electrochemical detection. The detection limit of this method was reported as 10 nmol/l (≈ 3 µg/l) (Cocker *et al* 2009). Also alkaline hydrolysis has been used for the measurement of total MOCA in urine samples. Robert *et al* used a method involving stabilisation of MOCA by sulphamic acid followed by alkaline hydrolysis at 80 °C, a single isooctane extraction and HPLC analysis, either with UV or electrochemical detection. The detection limit of this method was 1 µg/l (UV detection) and 0.1 µg/l (electrochemical detection) (3.745 nmol/l and 0.37 nmol/l, respectively) (Robert *et al* 1999a). Robert *et al* (Robert *et al* 1999b) compared the different methods to measure MOCA in urine. The methods tested involved the measurement of MOCA from non acid-stabilised or acid stabilised (sulphamic acid or citric acid) samples before or after alkaline hydrolysis or using heat hydrolysis. In this way, free MOCA (no acid-stabilisation, no hydrolysis), acid-labile MOCA (acid stabilisation, no hydrolysis), heat-labile MOCA (heat hydrolysis (Cocker *et al* 1990)) and total MOCA (acid stabilisation and alkaline hydrolysis) were determined from each urine sample. The comparative results showed that the mean sulphamic acid-labile MOCA concentrations were close to the total and heat-labile MOCA concentrations. MOCA measured in sulphamic acid-protected urine samples without hydrolysis could, therefore, be used as a reliable biological marker of exposure to MOCA. According to the correlations observed in this study, values of 100 and 45 µg/l of "free"' MOCA correspond to 130 and 60 µg/l sulphamic acid-labile MOCA, respectively, and values of 90 and 45 µg/l as heat-labile MOCA are equivalent to 60 and 30 µg/l of sulphamic acid-labile MOCA.

Because of the rapid elimination of MOCA, urine samples must be collected immediately at the end of the work-shift.

Since MOCA has been shown to bind to haemoglobin, haemoglobin adduct analysis has also been suggested for the biological monitoring of MOCA (Bailey *et al* 1993, Vaughan and Kenyon 1996). The advantage of this method is that it reflects the levels of biologically active MOCA and integrates exposure over a period of several weeks. However, it is currently not in routine use for the biomonitoring of MOCA in Europe. Also, a method to detect DNA adducts by ^{32}P-post labelling analysis in exfoliated urothelial cells has been described (Kaderlik *et al* 1993).

3. Exposure to MOCA

Usually, MOCA cannot be detected in the urine of occupationally non-exposed people (levels below current detection limits).

In 1996, Cocker *et al* (Cocker *et al* 1996) published results on the biological monitoring of MOCA (free and heat-labile MOCA) in UK industry during 1977–1994. These results showed a steady decline in urinary MOCA levels during this period. The 90th percentiles declined from 180 µmol/mol creatinine at 1977 to 15 µmol/mol creatinine at 1993–1994. Based on these results the UK HSE proposed a biological guidance value of 15 µmol/mol creatinine for MOCA.

Robert *et al* (Robert *et al* 1999b) published results on the biological monitoring programme of 40 workers in three polyurethane factories in France with potential exposure to MOCA. The results (measurements using sulphamic-acid pre-treatment without alkaline hydrolysis, see above) showed levels varying with job categories, with highest levels in mixers and maintenance workers. Also a variation between the factories was seen. Combined results showed a geometric mean of 12.8 µg/l, with a range of 0.5–570 µg/l. There were, however, significant differences between factories with factory B showing the lowest exposure levels (geometric mean 2.9, range 0.5–47

µg/l). Differences were explained by differences in exposure conditions, including use of enclosed MOCA handling systems with hoods, glove boxes and local exhaust ventilation during the loading of MOCA vessels. It was concluded that at the present time (1999), it was possible in France to reach urinary MOCA levels of around 20 µg/l, expressed as sulphamic acid-labile MOCA. Therefore, a guidance value (based on current feasibility) of 20 µg/l was proposed (~30 µg/l of total MOCA, corresponding to 112 nmol/l).

Recently, Cocker *et al* (2009) published results from an occupational survey of 2 suppliers and 20 workplaces using MOCA in the UK. The survey included an assessment of types of exposure controls and nature of work activities. Gathering samples were from workplace air (personal and static), glove samples, surface wipes, and urine samples. Urine samples were from workers involved in the weighing, melting and pouring of MOCA and from some indirectly exposed workers. Of 80 personal assessed exposures to MOCA by inhalation, only 16 % were above the detection limit for MOCA and only two exceeded the UK workplace exposure limit of 5 µg/m^3. Mean exposure was 2.4 µg/m^3. About 60 % of surface samples had detectable MOCA contamination, ranging from 0.019 to 400 µg/cm^2. Contaminations of both inner and outer gloves were also detected (48 % and 90 % had detectable levels, respectively). Urine samples were obtained from 78 workers in 18 companies using MOCA, and from one supplier. Urinary analyses were performed according to the method of Cocker *et al* (Cocker *et al* 1988, Cocker *et al* 1990). MOCA was detected in 51 % of the samples, but only 3 samples exceeded the proposed guidance value of 15 µmol/mol creatinine. The maximum urinary concentration of MOCA was 25 µmol/mol creatinine from a moulder. The 90th percentile of all the urine results was 8.6 µmol/mol creatinine. Among workers directly exposed to MOCA (n = 59) the 90th percentile was 11.7 µmol/mol creatinine and among those (n = 19) who were not directly exposed but who might have been exposed if best practice was not followed the 90th percentile was 2.9 µmol/mol creatinine. Since there was a clear need for improvements in occupational hygiene at these workplaces, it was concluded that a guidance value based on the 90th percentile of data from workplaces with good control should be less than the 90 % value of 8.6 µmol/mol creatinine found in this study. It was also noted that the current UK guidance value of 15 µmol/mol creatinine would no longer be a stimulus to further reduce exposure (Cocker *et al* 2009).

The Finnish Institute of Occupational Health (FIOH) publishes yearly results from monitorings of the Finnish industry. The total number of MOCA measurements during the years 2000–2008 was 49 (FIOH 2000-2008). Most of the samples were derived from workers involved in the manufacturing of polyurethane coatings. MOCA was measured as total MOCA using alkaline hydrolysis. Most of the values were < 5 µmol/mol creatinine, the range being between below the limit of detection (1 µmol/mol creatinine) and 10 µmol/mol creatinine (FIOH 2000-2008). The 95th percentile of these measurements (n = 49) was 3.4 µmol/mol creatinine (FIOH, unpublished data). Based on these data, FIOH proposed in 2008 a "biological action limit" value of 5 µmol/mol creatinine or total MOCA (FIOH 2008). The cancer risk for this exposure level was assessed on the basis of the available information. DECOS has estimated using linear extrapolations from animal testing that the cancer risk of MOCA at a daily dose level of 1 mg/kg is 3.7×10^{-2} (DECOS 2000). This corresponds to a total risk of 1.9×10^{-4} for a worker weighing 70 kg, with 40 years working life, working 48 weeks/year, 5 days/week and 8 hours/day. Assuming that the half-time is 23 hours (open one-compartment model; steady state after one week exposure), the average urinary concentration of MOCA at steady state is 2.6 µmol/mol creatinine, when the concentration in the Friday afternoon sample is 5 µmol/mol creatinine. Noting that the average daily excretion of creatinine for a 50-year old man of 70 kg is 12 mmol (Moriyama *et al* 1988, Wang *et al* 1996, Welle *et al* 1996, Remer *et al* 2002)

and assuming that 50 % of the MOCA absorbed in the body is measured in the urine analysis (hydrolysis of the acetyl, glucuronide and sulphate conjugates), this corresponds to a daily dose of 17 µg (2.6 µmol/mol creatinine × 0.012 mol × 267.17 g/mol × 2). Thus, the proposed BGV of 5 µmol/mol creatinine corresponds approximately to a cumulative life-time cancer risk of 3×10^{-6} (Friday specimen; for a Tuesday specimen with 5 µmol MOCA/mol creatinine, the risk estimate is 4×10^{-6}.

4. Recommendation

Since MOCA is a genotoxic carcinogen, no health based biological limit value can be set (SCOEL carcinogen group A). Since the general population is not exposed to MOCA, MOCA is not detected in the urine of occupationally non-exposed people. This means that urinary levels of occupationally non-exposed stay below the detection limit of the method, which typically lay around 5–10 nmol/l (≈0.5–1 µmol/mol creatinine) with modern analytical methods. Thus, the Biological Guidance Value (BGV) for MOCA corresponds to the detection limit of the biomonitoring method.

In occupationally exposed populations, urinary MOCA levels (total MOCA in the urine) below 5 µmol/mol creatinine can be reached using good working practises at the workplace. According to the risk assessment presented above, this corresponds to a cancer risk of $3–4 \times 10^{-6}$. Urinary samples should be collected at the end of the work-shift.

5. References

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