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(2nd round of prioritization)

Prioritized substance group: Aprotic solvents

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1. Introduction

Human Biomonitoring for Europe (HBM4EU) has established a strategy for deriving prioritized substance groups that HBM4EU will work on in 2019 and 2020. This stepwise strategy included input from national and EU policy makers and from stakeholders. The substances were nominated and prioritised according to a transparent procedure that is described in Deliverable 4.3 on the Prioritisation strategy and criteria, produced by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES). The detailed description of how this prioritisation strategy was implemented in practice, the inputs received and the methodology applied for selecting substances to include in the second list of prioritised substances is the subject of the Deliverable D4.4 (lead European Environment Agency, EEA).

First, a survey was launched to understand the demands of the National Hubs, EU policy makers and members of the HBM4EU Stakeholder Forum. Subsequently an online survey requested the nomination of substances for research under HBM4EU. A long list of new nominated single substances and substance groups was produced. Substances on the long list were ranked according to the number of nominations received, enabling to reduce the list down to a short list of approximately 25 substances Background documents on the substances on the short list were produced. An expert group of HBM4EU scientists scored and ranked the substances according to their hazardous properties, exposure characteristics; and public concern. The ranked list was discussed at a joint meeting of the HBM4EU Management Board and the European Union (EU) Policy Board in March 2018, where agreement was reached on the draft 2nd list of HBM4EU priority substances. The Governing Board approved the final list. The Governing Board members were asked to identify a list of candidate institutions and experts for the positions of Chemical Substance Group Leaders for the new substances/groups of substances. The substance group leaders were approved and were asked to produce the scoping documents for the new list of prioritised substances. The process is documented in D4.5 Second list of HBM4EU priority substances and Chemical Substance Group Leaders for 2019-2021.

2. Background information

In chemistry, the solvents are qualitatively grouped into **non-polar, polar aprotic, and polar protic solvents**. A protic solvent is a solvent that has a hydrogen atom bound to oxygen (as in a hydroxyl group) or nitrogen (as in an amine group). In general terms, any solvent that contains a labile H+ is called a protic solvent. The molecules of such solvents readily donate protons (H+) to reagents. On the contrary, aprotic solvents cannot donate hydrogen as they do not have O-H or N-H bonds. The "a" means "without", and "protic" refers to protons or hydrogen atoms.

Examples of non-polar solvents are benzene, toluene, chloroform, dichloromethane, etc. Examples of polar protic solvents are water, most alcohols, formic acid, ammonia, etc, In their turn, some common polar aprotic solvents are acetone, acetonitrile, dimethylformamide, dimethylsulfoxide, etc.

There are numerous aprotic solvents, and they are widely used in different applications - as pH regulators and in water treatment products, anti-freeze products, coating products, lubricants and greases, adhesives and sealants, air care products (scented candles, air freshening sprays, electric and non-electric fragrance diffusers), non-metal-surface treatment products, inks and toners, leather treatment products, polishes and waxes, washing and cleaning products. They are also used as laboratory chemicals in scientific research, in agriculture, forestry and fishing as well as in the formulation of mixtures and/or re-packaging.

During the second prioritization process within HBM4EU the ECHA and Germany proposed to include in the second list of priority substances four aprotic solvents that have a similar toxicological profile and a harmonised classification for reproductive toxicity:

- 1-methyl-2-pyrrolidone (NMP),
- 1-ethylpyrrolidin-2-one (NEP),
- N,N-dimethylacetamide (DMAC),
- N,N-dimethylformamide (DMF).

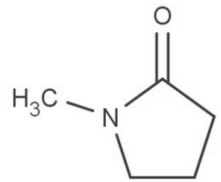
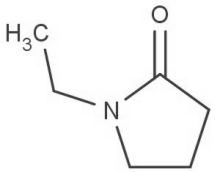
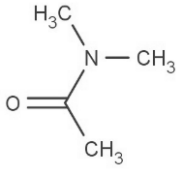
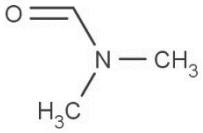
So, according to this proposal, the priority group of substances can be rephrased as “reprotoxic aprotic solvents”.

2.1 Hazardous properties

2.1.1 General characterisation

The toxicological profile of reprotoxic aprotic solvents is outlined in the Table 1.

Table 2.1: Identification of HBM4EU prioritised reprotoxic aprotic solvents

Name	Abbre-viation	CAS number	EC number	Formula	Structural formula	Harmonised classification acc. to CLP
1-methyl-2-pyrrolidone	NMP	872-50-4	212-828-1	C_5H_9NO		Repr. 1B, H360D STOT SE 3, H335 (C ≥ 10 %) Eye Irrit. 2, H319 Skin Irrit. 2, H315
1-ethylpyrrolidin-2-one	NEP	2687-91-4	220-250-6	$C_6H_{11}NO$		Repr. 1B, H360D
N,N-dimethylacetamide	DMAC	127-19-5	204-826-4	C_4H_9NO		Repr. 1B, H360D Acute Tox. 4, H332 Acute Tox. 4, H312
N,N-dimethylformamide	DMF	68-12-2	200-679-5	$HCON(CH3)_2$		Repr. 1B, H360D Acute Tox. 4, H332 Acute Tox. 4, H312 Eye Irrit. 2, H319

All substances in question have the harmonised classification according to PLC Regulation Repr. 1B, H360D (may damage the unborn child).

2.1.2 Human toxicological information

A number of key animal studies providing information on reproductive toxicity of selected aprotic solvents are summarized below.

NMP

Teratogenicity studies were performed by Becci et al. (1982) in rats given N-methylpyrrolidone, Dosages of 75,237 and 750 mg of N-methylpyrrolidone/kg body weight/day were administered dermally to groups of 25 pregnant Sprague-Dawley rats on days 6 through 15 of gestation. Additionally, the study used a positive dermal control. Hexafluoroacetone, was chosen based on its dermal teratogenic activity. An oral positive control, aspirin, was included in order to add significance to the data generated in the experimental positive dermal control group. All animals were killed and subjected to uterine examination on day 20 of gestation. Maternal toxicity was indicated at 750 mg of N-methylpyrrolidone/kg by reduced body weight gain during gestation. Treatment with N-methylpyrrolidone resulted in dose-dependent brightly colored yellow urine and dry skin. Treatment at the high dosage level resulted in fewer live fetuses per dam, an increase in the percentage of resorption sites and skeletal abnormalities. These effects could be the result of maternal toxicity. There was no evidence of teratogenic effects nor effects on the dams at 75 and 237 mg/kg of body weight.

The developmental toxicity of N-methyl-2-pyrrolidone (NMP) was studied in Sprague–Dawley rats after oral administration (Saillenfait et al., 2002). Pregnant rats were given NMP at doses of 0, 125, 250, 500, and 750 mg/kg/day, by gavage, on gestational days (GD) 6 through 20. Significant decreases in maternal body weight gain and food consumption during treatment, and a reduction in absolute weight gain were observed at 500 and 750 mg/kg. The incidence of resorptions per litter was significantly higher than control at 500 mg/kg, and rose to 91% at 750 mg/kg. Examination of the foetuses revealed treatment-related malformations, including imperforate anus and absence of tail, anasarca, and malformations of the great vessels and of the cervical arches. The incidence of malformed foetuses per litter, and of litters with malformed foetuses was significantly increased at 500 and 750 mg/kg. There was a dose-related decrease in foetal body weights (male, female, and total) that reached statistical significance at 250 mg/kg. A significant increase in incomplete ossification of skull bones and of sternebrae was also present at 500 and 750 mg/kg. In summary, the no-observed-adverse-effect level (NOAEL) for maternal and developmental toxicity was 250 and 125 mg/kg/day, respectively. Thus, oral administration of NMP produced developmental toxicity below maternally toxic levels.

In addition, Saillenfait with coauthors studied the developmental toxicity of inhaled N-methyl-2-pyrrolidone (NMP) in Sprague–Dawley rats (Saillenfait et al., 2003). Pregnant rats were exposed whole body to NMP vapours at concentrations of 0, 30, 60 and 120 ppm, 6 h/day, on gestational days (GD) 6 through 20. Maternal body weight gain was significantly decreased at 60 and 120 ppm on GD 6–13 and maternal food consumption was reduced at 120 ppm on GD 13–21. No significant difference in the gestational weight change corrected for the weight of the gravid uterus was observed, whatever NMP concentration. There were no adverse effects on embryo/fetal viability or evidence of teratogenicity at any concentration tested. Fetal toxicity indicated by reduced fetal weight was observed at 120 ppm. Thus, the no-observed-adverse-effect level (NOAEL) for maternal and developmental toxicity was 30 and 60 ppm, respectively.

The relative embryotoxicity of the N-methyl-2-pyrrolidone (NMP) and its metabolites was evaluated using rat whole embryo culture (WEC) and the balb/c 3T3 cytotoxicity test (Flick et al., 2009). The resulting data were evaluated using two strategies; namely, one based on using all endpoints determined in the WEC and the other including endpoints from both the WEC and the cytotoxicity test. The substance with the highest embryotoxic potential is NMP, followed by 5-hydroxy-N-methyl-pyrrolidone (5-HNMP), 2-hydroxy-N-methylsuccinimide (2-HMSI) and N-methylsuccinimide (MSI). Specific dysmorphogeneses induced by NMP and 5-HNMP were aberrations in the head region of the embryos, abnormal development of the second visceral arches and open neural pores. Only NMP and 5-HNMP induced specific embryotoxic effects and were classified as weakly embryotoxic, whereas the other two metabolites, 2-HMSI and MSI, were determined to be non-embryotoxic.

NEP

The developmental toxicity of N-ethyl-2-pyrrolidone (NEP) was studied in Sprague-Dawley rats after oral administration (Saillenfait et al., 2007). Pregnant rats were given NEP at doses of 0, 50, 250, 500 and 750 mg/kg/day, by gavage (5 ml/kg), on gestational days (GD) 6–20. Maternal toxicity, as evidenced by reduction in body weight gain and food consumption, was observed in all NEP groups at the beginning of treatment (GD 6–9). The incidence of resorptions was significantly increased at 500 mg/kg/day, and reached 83% at 750 mg/kg/day. There was a dose-related decrease in fetal weight, which was significantly lower than control at 250 mg/kg/day and higher doses. The incidence of malformed fetuses per litter and the number of litters with malformed fetuses were significantly increased at 500 and 750 mg/kg/day. Malformations mainly consisted of edema, anal atresia with absent tail, cardiovascular defects and fused cervical arches. Ossification of skull bones and sternebrae was significantly reduced at 500 and 750 mg/kg/day. The incidence of supernumerary ribs was significantly elevated at 250 mg/kg/day and higher doses. The authors of the study made conclusion that NEP administered by gavage is embryotoxic and teratogenic at maternal toxic doses.

Additionally, NEP was evaluated in a 4-week repeated dose study in rats (Saillenfait et al., 2016). NEP diluted in distilled water was orally administered by gavage to male and female Sprague-Dawley rats at doses of 0, 5, 50, and 250 mg/kg/day for 28 consecutive days. Transient decreases in the body weight and in the body weight gain of the males was observed during the first days of treatment at the 50 and 250 mg/kg/day doses. There was a marked increase in urine volume at the beginning of treatment in males and female rats at doses of 50 and 250 mg/kg/day. No biologically significant diferences were observed in hematological and clinical chemistry values in males and females at necropsy. Histological examination revealed an increase in hyaline droplets in the renal tubules of the kidneys and hepatocellular centrilobular hypertrophy in the liver of males at 250 mg/kg/day. Cytochrome P450 concentration in liver microsomes was slightly increased at 250 mg/kg/day in males. The results of this study demonstrate that NEP has mild to no effects at doses up to 250 mg/kg/day when administered orally to rats for 28 days with males being more susceptible than females.

With regard to human data, a number of publications are available, as well.

NMP

A 23-year-old laboratory technician was occupationally exposed to NMP during her first 20 weeks of pregnancy. The uptake via the lungs was probably of minor importance, as the NMP was handled at room temperature. Hand rinsing of glassware with NMP and cleaning up of an NMP spill in week 16 of pregnancy may have brought about a much larger uptake through the skin. During the 4 days following the spill, malaise, headache, and nausea were experienced. Examination of the pregnancy at week 14 showed no signs of delayed development; however, at week 25, signs of delayed fetal development were observed, and at week 31, a stillborn fetus was

delivered. Stillbirth in this period of pregnancy is unusual. However, as the level of exposure is unknown, it is impossible to establish if exposure to NMP is the causative factor (Solomon et al., 1996; Bower, 1997).

A total of 15 24-h exposures in a repeated-insult patch test in human subjects (n = 50) caused minor to moderate transient irritations. No signs of contact sensitization were observed. Direct contact of skin with NMP caused redness, swelling, thickening, and painful vesicles when NMP was used as a cleaner (Leira et al., 1992) or as a paint stripper (Åkesson & Jönsson, 2000).

Workers exposed to NMP in working areas with air concentrations up to 280 mg/m3 reported severe eye irritation and headache. With the methods of assessing the exposure level (sampling on charcoal and tracer gas method) and the response (observation and informal interview), it is impossible to develop a concentration–response relationship (Beaulieu & Schmerber, 1991). Six volunteers exposed to 10, 25, or 50 mg/m3 during 8 h in a chamber study registered their symptoms, before the start of exposure and then every 2 h for 16 h, in a questionnaire on a scale from 0 to 10 (0 = no symptoms and 10 = not tolerated). The volunteers displayed none of the following symptoms: eye or respiratory tract irritation; hacking cough, nose secretion, or blockage, sneezing, itching, or dryness in the mouth and throat, or other symptoms in upper airways; itching, secretion, smarting pain, visual disturbances, or other symptoms such as headache, dizziness, and nausea; and other symptoms. Two volunteers reported detecting an odour at 50 mg/m3. There were no significant differences in the spirometric data displayed by the forced expiratory volume in 1 s, vital capacity, and the highest forced expiratory capacity measured before or after any level of exposure. There were no acute changes in the nasal cavity assessed by continuous acoustic rhinometry. Even though the effects observed in this study were not very pronounced, it is mentioned that the possibility of undetected effects still remains (the number of volunteers was only six) (Åkesson & Paulsson, 1997).

In a dermal application experiment, 12 volunteers were exposed to 300 mg NMP through a dermal patch (filter paper, diameter 5 cm, protected with aluminium foil and attached by Dermalock) applied on the anterior face of the left forearm for 6 h. Five urine fractions were collected during 48 h following the onset of application. The mean dermal absorption of NMP was 67.9% (60.8 – 77.4%) (Ligocka et al., 2003).

NEP

No toxicity data in humans are available concerning NEP, however, it can be indicated that the toxicological profile of this substance is similar to NMP because both substances are structurally similar.

DMAC

No human data are available in relation to DMAC reproductive toxicity.

Liver toxicity was assessed in workers exposed to DMAC in an acrylic fiber manufacturing facility. Measurements were made over a 1-year study period. Evidence of liver toxicity was assessed by serum clinical chemistry tests (serum levels of total bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, & gamma-glutamyl transpeptidase) at least once during the study period for all 127 male workers in the two study departments and for 217 males in plant controls with no previous or current exposure to DMAC. Mean DMAC in air levels for the exposure groups appeared to differ (geometric mean DMAC in air levels of 1.9 and 1.3 ppm 12 hr TWA, respectively). No significant DMAC exposure related trends in hepatic serum clinical chemistry results were detected (Spies et al., 1995).

Two cases of toxic hepatitis from DMAC occurred among 25 employees on a new acrylic-fiber production line at a western U.S. manufacturing plant, probably due to inadequate personal protective equipment (PPE) for dermal exposures, resulting in skin penetration during maintenance

and repair procedures. The authors concluded that hepatotoxicity due to dermal absorption of DMAC and other amide-type solvents deserves special consideration in industrial settings (Baum and Suruda, 1997).

Elastane fibre workers exposed to DMAC were monitored for hepatic injury. Four hundred and forty new workers employed from 1 January 2002 to 31 July 2004 were included as study subjects. DMAC exposure estimates were based on urinary N-methylacetamide concentrations. There were 28 cases of DMAC induced hepatic injury. The overall incidence of DMAC induced hepatic injury among new elastane fibre workers was 0.089/person-year. Incidence rates were 7–10 times higher in high exposure groups than in low exposure groups. Fewer DMAC induced hepatic injuries occurred among workers employed for a longer period. The inverse relation between the incidence of DMAC induced hepatic injury and duration of employment may reflect a type of healthy survivor effect or tolerance to DMAC induced hepatic injury (Lee et al., 2006).

DMF

Only one study is available on the reproductive effects of DMF in humans. This study reported an increased rate of spontaneous abortion among pregnant women occupationally exposed to DMF. However, these results cannot be attributed solely to DMF, as these women were exposed to a number of additional chemicals (U.S. EPA, 1986, 1999).

56 of 66 workers in a fabric coating factory participated in the study. All had standard liver function tests at least once. 46 workers completed a questionnaire; 27 had more extensive clinical evaluation for recognized liver abnormalities. An outbreak of toxic liver disease has been associated with exposure to DMF in the workplace. The diagnosis of toxic liver disease was established by the clinical histories, negative viral serologies, an enzyme pattern of alanine aminotransferase levels being greater than aspartate aminotransferase levels, epidemiologic data on coworkers, and liver biopsy specimens. The high prevalence of unsuspected liver enzyme abnormalities in these workers suggests that occupational liver disease may occur more frequently than is generally recognized (Redlich et al., 1988).

Chronic occupational exposure to DMF by inhalation has resulted in effects on the liver and digestive disturbances in workers. The Reference Concentration (RfC) for DMF was set 0.03 mg/m3 based on digestive disturbances and minimal hepatic changes suggestive of liver abnormalities in humans (U.S. EPA, 1999).

2.2 Exposure characteristics

2.2.1 Trends in production volume/environmental behaviour and concentrations

According to substance information given in the ECHA website, the tonnage bands of the 4 registered reprotoxic aprotic solvents in question are the following:

- NMP 10 000 – 100 000 t/year,
- NEP 1000 – 10 000 t/year,
- DMF 10 000 – 100 000 t/year,
- DMAC 10 000 – 100 000 t/year.

1 of 4 registrants of NEP under REACH indicated the substance as persistent, bio accumulative and toxic, not giving justification for it. In general, NMP, NEP, DMAC and DMF are considered to be readily biodegradable, non-persistent in the environment with low bioaccumulation potential (log Kow -0.11 for NMP and log Kow -0.11 for other three substances), according to US EPA (The EPI (Estimation Programs Interface) Suite™ KOWWIN™)[1].

Information on environmental concentrations is lacking.

2.2.2 Human related exposure sources and uses

Aprotic solvents including reprotoxic aprotic solvents in question are used as pH regulators and in water treatment products, anti-freeze products, coating products, lubricants and greases, adhesives and sealants, air care products (scented candles, air freshening sprays, electric and non-electric fragrance diffusers), non-metal-surface treatment products, inks and toners, leather treatment products, polishes and waxes, washing and cleaning products. They are also used as laboratory chemicals in scientific research, in agriculture, forestry and fishing as well as in the formulation of mixtures and/or re-packaging. Releases to the environment of the substances is likely to occur from indoor use (e.g. machine wash liquids/detergents, automotive care products, paints and coating or adhesives, fragrances and air fresheners), outdoor use, indoor use in close systems with minimal release (e.g. cooling liquids in refrigerators, oil-based electric heaters) and outdoor use in close systems with minimal release (e.g. hydraulic liquids in automotive suspension, lubricants in motor oil and break fluids). In addition, releases to the environment of those substances can occur from industrial use - manufacturing of the substance, formulation of mixtures, in processing aids at industrial sites and as an intermediate step in further manufacturing of another substance (use of intermediates).

Detailed information on possible uses and releases to environment can be found on ECHA web pages[2].

[1] The EPI (Estimation Programs Interface) Suite™ is a Windows®-based suite of physical/chemical property and environmental fate estimation programs developed by EPA's and Syracuse Research Corp. (SRC). EPI Suite™ uses a single input to run the following estimation programs: KOWWIN™, AOPWIN™, HENRYWIN™, etc. (https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface)
estimation programs: KOWWIN™, AOPWIN™, HENRYWIN™, etc. (https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface)

[2] For NMP - https://echa.europa.eu/brief-profile/-/briefprofile/100.011.662;
For NEP - https://echa.europa.eu/brief-profile/-/briefprofile/100.018.409
For DMAC - https://echa.europa.eu/brief-profile/-/briefprofile/100.004.389
For DMF - https://echa.europa.eu/brief-profile/-/briefprofile/100.000.617

2.2.3 Human exposure routes

Both occupational exposure and exposure to the general public is relevant for reprotoxic aprotic solvents. Prevalence of high exposure is expected due to wide use and high production volume of substances under consideration. Exposure sources are ingredients in paints, graffiti remover, cleaning formulations, children's toys, textiles, carpets, inks, toner, pH-regulators, floccants, precipitants, neutrallisation agents, laboratory chemicals, Especially regarding NMP and NEP, exposure of the general population since 1991 is confirmed (Ulrich et al., 2018).

Dermal exposure (possibly including cosmetic products containing aprotic solvents also) as well as inhalation exposure to indoor emissions from consumer products and articles mentioned is playing a role. It should be remarked that regarding the general public, reproductive toxicants category 1B shall not be placed on the market as substances, constituents of other substances or components of a mixture above 0.3 %.

Dermal exposure is considered to be especially significant. According to the *Opinion* of the Scientific Committee on Consumer Safety (*SCCS*) of EC on NMP adopted in 2011, NMP is readily absorbed by all routes of exposure, but, due to its low vapour pressure, absorption through the skin represents the most likely and potentially the most important route of exposure to NMP under most known consumer use conditions. At the workplace, however, inhalation and dermal uptake can be assumed to be the important routes of exposure (*Scientific Committee on Occupational Exposure Limits* (SCOEL), SCOEL/REC/119, N-Methyl-2-Pyrrolidone, 2015).

For NEP, the German HBM Commission states that due to its use as substitute for NMP similar exposure routes can be assumed to be relevant for the human exposure, i.e. the inhalative and dermal exposure route (HBM Commission, 2015b).

SCCS in its conclusion in 2011 on NMP claims that based on a worst case assessment with a maximum use concentration of 5 % NMP in cosmetic products and a dermal absorption of 100 %, the Margin of Safety is considered to be too low. There is an absence of specific information on the actual possible maximum concentrations of NMP present in cosmetic products and specific measurement of dermal absorption of it through skin at relevant concentrations. With the information available at the time of assessment, the SCCS was of the opinion that the presence of NMP with a maximum use concentration of 5 % in cosmetic products is not safe for the consumer. A re-evaluation may be possible should relevant data that addresses the above be provided.

However, it is indicated that oral exposure through mists that deposit in the upper respiratory tract and are swallowed should be considered as well (US EPA, 2017). In addition, transplacental exposure shall be taken into account.

Professional and industrial workers, pregnant women and young children shall be considered as vulnerable sub-groups of population.

According to US EPA, for NMP adverse reproductive and other systemic effects could be a concern at higher exposures levels, but exposures that are protective of pregnant women and women who may become pregnant are expected to be also protective of other life stages and subpopulations (US EPA 2015).

2.2.4 Human biomonitoring (HBM) data availability

The scientific publications concerning biomonitoring of reprotoxic aprotic solvents in question are listed in the References. As reprotoxic aprotic solvents are widely used as industrial chemicals, almost all available studies are performed in relation to occupational environment and/or very often

in experimental settings with volunteers. Many of them were aimed at finding the appropriate exposure biomarkers and settings for biological monitoring in the occupational environment.

Only one study in relation to the general population and its sub-groups has been identified. **NMP** and **NEP** metabolite concentrations were determined in 540 24-h urine samples of the German Environmental Specimen Bank collected from 1991 to 2014. NMP metabolites 5-hydroxy-*N*-methyl-2-pyrrolidone (5-HNMP) and 2-hydroxy-*N*-methylsuccinimide (2-HMSI) as well as NEP metabolites 5-hydroxy-*N*-ethyl-2-pyrrolidone (5-HNEP) and 2-hydroxy-*N*-ethylsuccinimide (2-HESI) were determined by stable isotope dilution analysis using solid phase extraction followed by derivatization (silylation) and GC–EI–MS/MS. The respective metabolites were identified: 5-HNMP in 98.0 % and 2-HMSI in 99.6% of the samples; 5-HNEP in 34.8 % and 2-HESI in 75.7% of the samples. Metabolite concentrations were rather steady over the timeframe investigated, even for NEP which has been introduced as an NMP substitute only in the last decade. Calculated median daily intakes in 2014 were 2.7 µg/kg bw/day for NMP and 1.1 µg/kg bw/day for NEP. For the combined risk assessment of NMP and NEP exposure, the hazard index based on the human biomonitoring assessment I values (HBM I values) was less than 0.1. Therefore, the individual and combined NMP and NEP exposures in Germany were within acceptable ranges in the investigated timeframe (Ulrich et al., 2018).

NMP

Six female and six male volunteers (groups 1 and 2) were topically exposed for 6 hours to 300 mg of NMP. An additional group of six male volunteers (group 3) was exposed to 300 mg of NMP in a 50% water solution. Blood and urine were sampled before, during, and up to 9 days after the exposure. Plasma and urine were analyzed using mass spectrometry. For groups 1 and 2, 16% and 18% of the applied dose were recovered in the urine as the sum of NMP and its metabolites. For group 3, 4% was recovered. The maximal concentration of 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP) was 10, 8.1, and 2.1 µmol/l for groups 1, 2 and 3, respectively, in plasma and 420, 360 and 62 µmol/l in urine adjusted for density.

For 2-hydroxy-N-methylsuccinimide (2-HMSI), the maximal concentration was 5.4, 4.5, and 1.3 µmol/l for groups 1, 2 and 3, in plasma, respectively, and 110, 82 and 19 µmol/l in urine adjusted for density. For 5-HNMP there was a difference in time to reach the maximal concentration depending on whether pure NMP or 50% NMP in water was used. No such difference was seen for 2-HMSI. The differences in kinetics between male and female volunteers were small. The authors concluded that preferably 2-HMSI should be used as the biomarker of exposure to NMP (Akesson et al., 2004).

Six male volunteers were exposed for 8 hours to NMP concentrations of 0, 10, 25, and 50 mg/m^3. Blood and urine were sampled before, during, and up to 40 hours after exposure. Aliquots of urine and plasma were purified, derivatized, and analyzed for 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP) on a gas chromatograph/mass spectrometer in the electron impact mode. The mean plasma concentration [P-(5-HNMP)] after 8-hour NMP exposure to 10, 25, and 50 mg/m^3 was 8.0, 19.6, and 44.4 µmol/l, respectively. The mean urinary concentration [U-(5-HNMP)] for the 2 last hours of exposure was 17.7, 57.3, and 117.3 mmol/mol creatinine, respectively. The maximal P-(5-HNMP)and U-(5-HNMP) concentrations occurred 1 hour and 0-2 hours, respectively, after the exposure. The half-lives of P-(5-HNMP) and U-(5-HNMP) were 6.3 and 7.3 hours, respectively. The 5-HNMP urinary concentrations were 58% of the calculated retained dose. There was a close correlation (r) between P-(5-HNMP) (r=0.98) and U-(5-HNMP) (r=0.97) with NMP exposure. The authors concluded that 5-HNMP as biomarker in plasma is recommended (Akesson et al., 2000).

An experimental study with 16 volunteers exposed to 80 mg/m^3 NMP for 8 h under either whole-body, i.e. inhalational plus dermal, or dermal-only conditions was carried out. Additionally, the influence of moderate physical workload on the uptake of NMP was studied. The urinary

concentrations of NMP and its metabolites 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP) and 2-hydroxy-N-methylsuccinimide (2-HMSI) were followed for 48 h and analysed by gas chromatography-mass spectrometry (GC-MS). Percutaneous uptake delayed the elimination peak times and the apparent biological half-lives of NMP and 5-HNMP. Under resting conditions, dermal-only exposure resulted in the elimination of 71 +/- 8 mg NMP equivalents as compared to 169 +/- 15 mg for whole-body exposure. Moderate workload yielded 79 +/- 8 mg NMP (dermal-only) and 238 +/- 18 mg (whole-body). Thus, dermal absorption from the vapour phase may contribute significantly to the total uptake of NMP, e.g. from workplace atmospheres (Bader et al., 2008).

The study by Haufroid et al. (2014) was performed in order to examine the value of urinary 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP) and 2-hydroxy-N-methylsuccinimide (2-HMSI) in a population of workers exposed to N-methyl-2-pyrrolidone (NMP) and to look for health effects of exposure to this organic solvent. Airborne NMP was determined according to the NIOSH method. Urinary 5-HNMP and 2-HMSI (after and before next shift) were determined by liquid chromatography with tandem mass spectrometry. Outcomes were effects on lung, kidney, skin and mucous membranes, nervous system, haematopoiesis and liver determined by clinical examination and laboratory measurements. Univariate statistical methods and multiple regressions were used to analyse results. Skin resorption, smoking and other potential confounders were taken into account. 327 workers were eligible out of which 207 workers (63%) participated. 91 of these worked with NMP. Occupational exposure to NMP did often not occur daily and ranged from non-detectable to 25.8 mg/m^3 (median = 0.18). Urinary 2-HMSI (mg/l; before next shift) was the best biomarker of exposure to NMP, explaining about 70% of the variance, but most likelihood ratios did not allow for ruling exposure in or out, at these low levels of exposure. Creatinine adjustment did not improve the results clearly. No clear and consistent health effects could be associated with NMP exposure. No indication for a bias due to non-participation was found. The authors stated that biological monitoring, primarily urinary 2-HMSI (mg/l; before next shift), is of value to estimate exposure to NMP even when exposure is irregular and low. Likelihood ratios of urinary 5-HMNP or 2-HMSI are, however, not quite satisfactory at these low levels. No irritant or other health effects were found.

Meier et al (2013) reported on a study investigating current exposures to NMP in the spraying department of an automobile plant using biological monitoring. 5-HNMP and 2-HMSI were analysed in 69 urine samples of 14 workers exposed to NMP and of 9 non-exposed controls. Measurements of airborne exposure levels were not included. Three different working tasks ('loading' and 'cleaning' of the sprayer system and 'wiping/packing' of the sprayed materials) and three sampling times (pre-shift, post-shift, and pre-shift of the following day) were studied in exposed workers. Median levels of 5-HNMP and 2-HMSI in post-shift urine of exposed workers were 0.91 and 0.52 mg/g creatinine, respectively, whereas median levels in controls were below the limit of detection. Decreased levels of 5-HNMP were observed in pre-shift urine samples on the following day (0.39 mg/g creatinine) in exposed workers, while the concentration of 2-HMSI did not change (0.49 mg/g creatinine). Highest exposures occurred during sprayer cleaning with a maximum level of 8.31 mg/g creatinine of 5-HNMP in post-shift urine. In contrast to 'wipers/packers', no decrease in 5-HNMP could be observed in pre-shift urine samples on day 2 of the 'loaders' and 'cleaners'. Overall, exposure in terms of 5-HNMP post-shift and 2-HMSI pre-shift of the following day were well below existing biological limit values of the European Union (70 and 20 mg/g creatinine, respectively). The authors suggested that the analysis of 5-HNMP in pre-shift samples also provided essential information, particularly in situations involving direct handling of liquid NMP-containing formulations.

NEP

Koch et al. (2014) orally dosed 20.9 mg NEP to three male volunteers. These volunteers collected all their urine samples over a period of 4 days post dose. In these samples NEP metabolites 5-hydroxy-N-ethyl-2-pyrrolidone (5-HNEP) and 2-hydroxy-N-ethylsuccinimide (2-HESI) were identified and quantified, and their urinary elimination kinetics and their metabolic conversion factors were determined. After 4 days the researchers recovered 50.7 % of the dose of these two metabolites in urine, 29.1 % of 5-HNEP and 21.6 % of 2-HESI. The largest share of 5-HNEP was excreted within 24 h post dose, while the major share of 2-HESI was excreted on day 2 post dose. An elimination half-time for 5-HNEP of approx. 7 h and for 2-HESI of approx. 22-27 h was estimated. While the elimination of 5-HNEP was basically finished 72 h post dose, significant amounts of 2-HESI were still eliminated after 96 h. Both biomarkers can be used in human biomonitoring studies to extrapolate from urinary measurements to the NEP dose taken up and thus to evaluate the risk caused by exposure to this chemical.

DMAC

Worker exposure to DMAC in an acrylic fiber manufacturing facility was measured, over a 1-year study period, by full-shift (12 hours) personal air monitoring for DMAC and by biological monitoring for levels of DMAC, N-methylacetamide (MMAC), and acetamide in spot urine samples. Ninety-three of 127 male workers in seven job classifications in the solution preparation and spinning departments of the plant were monitored on the second consecutive workday after at least 3 days off for the first 10 months of the study and on both the first and second days during the study's final 2 months. Postshift urinary MMAC levels were significantly correlated ($P < .0001$, $r2 = .54$) with DMAC in air levels. An air level of 6.7 ppm 12-hour time-weighted average (TWA) corresponded to a urine MMAC level of 62 mg/g creatinine in a postshift spot urine sample obtained after the second consecutive workday. To minimize exposure misclassification due to variability in the regression relationship, a level of 35 mg MMAC/g creatinine in a postshift spot urine sample was recommended as a biomonitoring index. Postshift urine MMAC levels did not appear to plateau at higher air levels, nor did it appear that the DMAC demethylation metabolic mechanisms became saturated at threshold limit value (TLV)-level air-exposure levels. Urine MMAC levels in postshift samples obtained the second workday appeared to be greater than levels in postshift first-day samples, but the number of days until this postshift level would plateau could not be determined from this study (Spies et al., 1995).

Perbellini et al (2003) studied the concentration of N,N-dimethylacetamide (DMAC) and its metabolite, N-methylacetamide (NMA), in urine of workers occupationally exposed to DMAC in a factory producing synthetic acrylic fibres. During the first phase, 223 workers exposed to low environmental concentrations of DMAC provided urine samples at the end of a work shift. High concentrations of the unmodified solvent and its metabolite were found in a group of workers whose job was to start up machinery. The second and third phases focused on conditions favouring high uptake of DMAC. The highest concentrations of unmodified solvent and NMA were found in the urine of workers recently engaged in starting up machinery. NMA in urine was 1.5-173.6 mg/g creatinine (median 20.5). In spite of the low environmental concentration, about 20% of the urine concentration of NMA was higher than 30 mg/g creatinine. Dermal absorption of DMAC was high. A shower and a change of clothing at the end of the work shift, and washing away any solvent left on the skin, ensured that dermal absorption of DMAC did not continue. This significantly reduced the NMA urinary concentration at values lower than 30 mg/g creatinine. In some urine samples, S-acetamidomethyl-mercapturic acid (AMMA) was identified by NMR analysis; this is probably a metabolite of N,N-dimethylacetamide--it has never before been identified in humans or animals. The authors remarked that even at low environmental concentrations of DMAC, dermal absorption can be considerable. Unmodified DMAC and NMA concentrations in urine are good biomarkers for monitoring occupational exposure to the solvent.

Princivalle et al (2010) studied toxicokinetics of two major urinary metabolites of DMAC namely, S-(acetamidomethyl)mercapturic acid (AMMA) and N-methylacetamide (NMA). Urine samples were collected from workers exposed to DMAC in a factory manufacturing acrylic fibers. AMMA and NMA were determined by HPLC/MS and GC/MS, respectively. The working scheme in the factory consisted of periods of three consecutive working shifts alternated regularly with two days off work. In the first stage of the study, NMA and AMMA were determined in urine samples collected before, in the middle, and at the end of one working shift. In the second stage, urine was collected five times during three consecutive days after a two-day rest: before and at the end of the first and second working shifts and before the third shift. It was found that the end-of-shift NMA levels were several folds higher than the pre-shift levels of the same day and dropped significantly until the next shift. On the other hand, there were no significant differences in AMMA levels before and at the end of the same shift but a continuous rise during the three-day working period was observed. Median values of NMA concentrations at the end of working shifts were between 10.1 and 17.3 mg/g creatinine, median AMMA concentrations in the second or third day of the working period varied between 12.4 and 38.1 mg/g creatinine. The approximate half-lives of NMA and AMMA (means) in the exposed workers were about 9 and 29 h, respectively. Thus, while NMA in the end-of-shift urine samples remains a preferential biomarker of DMAC exposure during that shift, AMMA determined at the end of a work-week reflects cumulative exposure over the last few days. The authors made conclusion that further studies are needed to determine AMMA concentrations corresponding to the threshold limit value of DMAC.

DMF

DMF exposure was monitored in a synthetic leather factory; at the same time, urinary DMF and its metabolites were measured in urine samples collected before and at the end of workshifts. The study was run during two different periods. During the first phase ten workers were observed for 3 days (Monday, Tuesday and Wednesday) in the same week. In the second phase 16 workers were involved in the study on a Friday and on the following Monday. Urinary DMF, as well as hydroxymethyl-N-methylformamide and hydroxymethylformamide [measured as N-methylformamide (NMF) and formamide, respectively], were measured as a "physiological" product in subjects not exposed to dimethylformamide. Environmental exposure to DMF ranged between 10 and 25 mg/m^3.

The unmodified solvent found in urine collected at the end of the exposure was significantly related to the environmental concentrations of DMF; its urinary concentrations were found to range between 0.1 and 1 mg/l. Higher concentrations of NMF (mean 23.3 mg/l) and formamide (24.7 mg/l) were measured in urine samples collected at the end of workshifts. The same concentrations were related to individual exposures to DMF. N-Acetyl-S-(N-methylcarbamoyl)cysteine (AMCC) in the urine of workers exposed to DMF showed a mean concentration of 40.4 mg/l on Friday (before and after the workshift) and a mean concentration of 10.3 mg/l on Monday. Its slow kinetic profile favours its body accumulation during the working week (Lareo and Perbellini, 1995).

To estimate the contribution of skin absorption to total body burden of DMF across a working week in two groups with similar levels of respiratory exposure but dissimilar skin contact 25 workers in a synthetic leather (SL) factory, 20 in a copper laminate circuit board (CLCB) factory, and 20 age and sex matched non-DMF exposed subjects, were recruited. Environmental monitoring of DMF exposure via respiratory and dermal routes, as well as biological monitoring of pre-shift urinary N-methylformamide (U-NMF), were performed for five consecutive working days. Environmental and biological monitoring showed no detectable exposure in controls. The average airborne DMF concentration (geometric mean (GM) 3.98 ppm, geometric standard deviation (GSD) 1.91 ppm), was insignificantly lower for SL workers than for CLCB workers (GM 4.49, GSD 1.84 ppm). Dermal

DMF exposure and U-NMF values, however, were significantly higher for SL workers. A significant pattern of linear accumulation was found across a five day work cycle for SL workers but not for CLCB workers. Dermal exposure to DMF over five consecutive days of occupational exposure can result in the accumulation of a significant DMF body burden (Chang et al., 2005).

In order to measure exposure to DMF in occupational settings in 35 healthy workers employed in the polyacrylic fibre industry, N-methylformamide (NMF) and N-acetyl-S-(N-methylcarbamoyl)cysteine (AMCC) in urine, and N-methylcarbamoylated haemoglobin (NMHb) in blood were measured. Workplace documentation and questionnaire information were used to categorise workers in groups exposed to low, medium, and high concentrations of DMF. All three biomarkers can be used to identify occupational exposure to DMF. However, only the analysis of NMHb could accurately distinguish between workers exposed to different concentrations of DMF. The median concentrations were determined to be 55.1, 122.8, and 152.6 nmol/g globin in workers exposed to low, medium, and high concentrations of DMF, respectively. It was possible by the use of NMHb to identify all working tasks with increased exposure to DMF. While fibre crimpers were found to be least exposed to DMF, persons washing, dyeing, or towing the fibres were found to be highly exposed to DMF. In addition, NMHb measurements were capable of uncovering working tasks, which previously were not associated with increased exposure to DMF; for example, the person preparing the fibre forming solution. Measurement of NMHb in blood is recommended rather than measurement of NMF and AMCC in urine to accurately assess exposure to DMF in health risk assessment. However, NMF and AMCC are useful biomarkers for occupational hygiene intervention (Kafferlein et al., 2005).

Seitz et al. (2018) assessed the relation between occupational exposure to DMF after an 8 h work shift in the acrylic fibre industry and its three biological markers N-methylformamide (NMF_{total}), N-acetyl-S-(N-methylcarbamoyl)cysteine (AMCC), and N-methylcarbamoyl adduct at haemoglobin (MCVal). External DMF exposure of 220 workers was determined during the whole shift. A standardised questionnaire was used to obtain information about the worker's general health status, medical treatment, smoking habits, protective measures, and possible symptoms caused by DMF exposure. NMF and AMCC were analysed in post-shift urine samples and MCVal in blood. For longitudinal assessment the average AMCC concentration was determined over a period of 4 weeks (weekly sampling) in a sub-collective of 89 workers. The median of DMF concentration in air was 3.19 mg/m^3 (range < 0.15-46.9 mg/m^3).

The biological markers showed a median of 4.80 mg/L (range 0.20-50.6 mg/L) for NMF_{total}, 4.75 mg/g creatinine (range 0.06-49.6 mg/g creatinine) for AMCC, and 57.5 nmol/g globin (range 0.5-414 nmol/g) for MCVal. A significant linear relationship was observed between DMF in air and NMF as well as between DMF in air and AMCC in post-shift urine samples. The mean AMCC values measured weekly over a period of 4 weeks correlated significantly with MCVal adducts too. Excluding workers who had been using breathing masks on the day of the study led to even tighter correlations. The results of the present study demonstrate the applicability of the DMF biomonitoring parameters NMF_{total} in post-shift urine for the present-day exposure assessment, AMCC in the post-shift urine after several shifts for assessment of the cumulative exposure of the previous working days, and MCVal for assessment of long-term exposure during previous weeks and months.

2.2.5 Health based guidance values available for HBM data

For the protection of the general population, the German Environment Agency (Umweltbundesamt; UBA) recommends two types of health-based guidance values for **NMP** expressed as the sum of the concentration of two main metabolites of NMP in urine - 5-hydroxy-NMP and 2-hydroxy-N-methylsuccinimide (2-HMSI) and for NEP expressed as the sum of the concentration of two main metabolites of NEP in urine – 5-hydroxy-NEP (5-HNEP) and 2-hydroxy-n-ethylsuccinimide (2-HESI):

- **NMP: control values (HBM-I) of 10 mg/L for children and 15 mg/L for adults**
- **NMP: action values (HBM-II) of 30 mg/L for children and 50 mg/L for adults**

Exposure to NEP can be quantified by the determination of the excretion of its urinary metabolites 5-Hydroxy-N-ethyl-2-pyrrolidone (5-HNEP) and 2-Hydroxy-N-ethylsuccinimide (2-HESI). The resulting HBM-I and HBM-II values for the sum of the metabolites 5-HNEP and 2-HESI in the urine are the following:

- **NEP: control values (HBM-I) of 10 mg/L for children and 15 mg/L for adults**
- **NEP: action values (HBM-II) of 25 mg/L for children and 40 mg/L for adults**

For workers exposure to **NMP** a BLV of **20 mg/g creatinine** expressed as urine concentration 18 hours after the end of the previous exposure (pre-shift sample) for the 2-HMSI is established.

As NMP and NEP act very similar, UBA suggests using a mixture approach considering the sum of the 4 metabolites when a combined exposure to both compounds is expected. (Kommission Human Biomonitoring, 2015a)

As regards **DMAC**, BLV for workers of **20 mg/g creatinine** expressed as N-methylacetamide (NMAC) concentration in urine at the end of shift at the end of workweek is proposed (Qian YL et al., 2012)[3].

In their turn, The American Conference of Governmental Industrial Hygienists (ACGIH®)[4] recommends a Biological exposure index (BEI) of **30 mg/g creatinine** for NMAC concentration in urine at the end of shift at the end of the work week samples as reference value for workers, and the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) recommends the same limit value of **30 mg/g creatinine** in relation to NMAC urine concentration at the end of shift at the end of the work week (Perbellini L et al., 2003)[5].

According to the SCOEL, for occupational exposure to **DMF** the BLV expressed as N-methylformamide (NMF) concentration in urine **15 mg/L** at the end of shift at the end of work week is established. In its turn, the ACGIH's Biological Exposure Index for the same metabolite is **30 mg/L** (end of shift, end of the work week).

2.3 Policy relevance

2.3.1 Existing regulation (sectoral and inter-sectoral policies)

All 4 aprotic solvents in question are classified as Repr.1B, H360D (May damage the unborn child) according to CLP Regulation. Regarding general public and according to the entry 30 of Annex XVII of REACH, reproductive toxicants category 1B shall not be placed on the market as substances, constituents of other substances or components of a mixture above 0.3 %.

[3] https://www.ncbi.nlm.nih.gov/pubmed/23257108
[4] https://www.acgih.org/
[5] https://www.researchgate.net/publication/9085557_Biological_monitoring_of_occupational_exposure_to_NN-dimethylacetamide_with_identification_of_a_new_metabolite

In addition, with respect to consumer protection DMAC and DMF are listed in Annex II (list of substances prohibited in cosmetic products – entries 747 and 355, respectively) of the Cosmetic Products Regulation No1223/2009.

NMP, DMAC and DMF are used in the production of medicinal products and are therefore subject to the provisions of directives 2001/83/EC on medicinal products for human use and 2001/82/EC on veterinary medicinal products, as well as those of Commission Delegated Regulation (EU) No 1252/2014 on principles and guidelines of good manufacturing practice for active substances for medicinal products for human use.

NMP, DMAC and DMF are listed on the Candidate List under REACH as SVHC and included in the candidate list for authorisation.

Some uses of the NMP are restricted under Annex XVII of REACH, namely, the following conditions for restriction are set:

- Shall not be placed on the market as a substance on its own or in mixtures in a concentration equal to or greater than 0,3 % after 9 May 2020 unless manufacturers, importers and downstream users have included in the relevant chemical safety reports and safety data sheets, Derived No-Effect Levels (DNELs) relating to exposure of workers of 14,4 mg/m3 for exposure by inhalation and 4,8 mg/kg/day for dermal exposure (paragraph 1).
- Shall not be manufactured, or used, as a substance on its own or in mixtures in a concentration equal to or greater than 0,3 % after 9 May 2020 unless manufacturers and downstream users take the appropriate risk management measures and provide the appropriate operational conditions to ensure that exposure of workers is below the DNELs specified in paragraph 1 (paragraph 2).
- By way of derogation from paragraphs 1 and 2, the obligations laid down therein shall apply from 9 May 2024 in relation to placing on the market for use, or use, as a solvent or reactant in the process of coating wires (paragraph 3).

Within assessment of restriction proposal for NMP ECHA Risk Assessment Committee (RAC) has set a DNEL value for NMP **10 mg/m^3** (chronic inhalation exposure for workers covering pregnant women)[6]. A dermal DNEL of **4.8 mg/kg/day** is also proposed by RAC for the workers.

The European Commission adopted the restriction for NMP on 18 April 2018[7].

In addition, DMF is considered as Category 2A substance with respect to carcinogenicity according to IARC[8] and many organic solvents are mentioned as neurotoxicants (Grandjean, 2006; US EPA 2015).

Furthermore, DMF is included in the priority list of chemicals developed within the EU-Strategy for Endocrine Disruptors and placed in category 3 - no evidence of endocrine disrupting activity or no data available and listed in Annex 13 (List of 146 substances with endocrine disruption categorizations prepared in the Expert meeting)[9].

According to Directive 2010/75/EU of 24 November 2010 on industrial emissions (integrated pollution prevention and control), substances with CMR (carcinogenic, mutagenic, or toxic for reproduction) properties shall be replaced as far as possible by less harmful substances or mixtures within the shortest possible time.

[6] https://echa.europa.eu/documents/10162/aa77c7c4-4026-4ab1-b032-8a73b61ca8bd
[7] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018R0588&from=EN
[8] Volume 47, 71, 115 (In prep.)
[9] http://ec.europa.eu/environment/chemicals/endocrine/strategy/substances_en.htm

According to Directive 98/24/EC of 7 April 1998 on the protection of the health and safety of workers from the risks related to chemical agents at work, employers are required to eliminate risks or reduce them to a minimum, with a preference for substitution.

For the workers the Occupational exposure limits (OELs) are set for the following reprotoxic aprotic solvents in question:

- NMP: **40 mg/m³** or **10 ppm** (8-hours TWA) and **80 mg/m³** or **20 ppm** (short term);
- DMAC: **36 mg/m³** or **10 ppm** (8-hours TWA) and **72 mg/m³** or **20 ppm** (short term);
- DMF: **15 mg/m³** or **5 ppm** (8-hours TWA) and **30 mg/m³** or **10 ppm** (short term).

It should be remarked that different national limits may be lower.

In addition, on 23 September 2015, SCOEL confirmed their recommendation of 2007 for an OEL time-weighted average (TLV-TWA[10]) of **10 ppm** (**40 mg/m³**), a short-term exposure limit (TLV-STEL[11]) of **20 ppm** (**80 mg/m³**) and a BLV of **70 mg/g creatinine** in urine for 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP), monitored 2-4 h after exposure/shift with a supplemented 'skin' notation and adopted the revised Recommendation SCOEL/REC/119[12].

In addition with respect to the general population, the US EPA has proposed a Reference concentration (RfC)[13] for DMF **30 µg/m³** (last revised 10/01/1990[14]).

2.3.2 Upcoming regulation

NEP, DMF and DMAC are likely to be considered for restriction in the future. ECHA RAC started to evaluate the DMF restriction proposal in November 2018.

2.4 Technical aspects

2.4.1 Biomarkers available for parent compounds or metabolites in human matrices

A metabolic pathway suggested for humans is the following: **NMP** is first hydroxylated to 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP), and then oxidised to N-methylsuccinimide (MSI), which in turn is hydroxylated to 2-hydroxy-N-methylsuccinimide (2-HMSI) (Fig. 1).

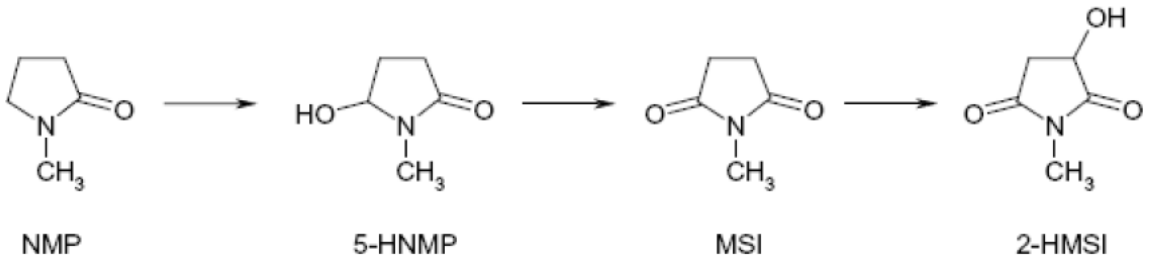


Figure 2.1: Proposed metabolism of NMP (Akesson et al., 1997; Carnerup et al., 2005).

[10] Threshold limit value - Time-weighted average
[11] Threshold limit value - Short term exposure limit
[12] http://files.chemicalwatch.com/2016-03-30_SCOEL-OPIN-2016-119.pdf
[13] The RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily inhalation exposure of the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (US-EPA)
[14] https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0511_summary.pdf

It is stressed that main metabolites of **NMP** are 5-hydroxy-NMP and 2-hydroxy-N-methylsuccinimide (2-HMSI) in urine (Apel et al., 2017). 2-HMSI is suggested as a biomarker of exposure to NMP, and the levels in plasma and urine may be used to indicate an exposure over three days as the half-life of 2-HMSI is longer than for the other metabolites (Jönsson et al., 2003).

As regards **NEP**, similarly to NMP, the main metabolites are 5-hydroxy-NEP and 2-hydroxy-N-ethylsuccinimide (Koch et al., 2014).

For the **DMAC** the main metabolites are N-methylacetamide (NMAC), N-hydroxymethylacetamide, acetamide (DMAC-OH) and N-acetyl-S-(acetamidomethyl)-L-cysteine (AMMA). According to French National institute of research and security (INRS) and its Biotox database[15], urine acetamide as a marker of DMAC exposure has been proposed but it is less well correlated with atmospheric DMAC levels in occupational environment than urinary NMAC. The determination of AMMA in urine at the end of the work week is considered to be interesting for biological monitoring of occupational exposure. Concentrations of the order of 10 to 40 mg/g creatinine are found in employees whereas NMAC levels are around 10 to 17 mg/g creatinine. BLV for workers is set for NMAC (see information provided above).

DMF main metabolites are N-methylformamide (NMF), N-hydroxymethylformamide (HMMF), acetamide and N-acetyl-S-(acetamidomethyl)-L-cysteine (AMCC).

2.4.2 Main characteristics of analytical methods

Gas chromatography-mass spectrometry was used for quantitative analysis of urine samples in relation to NMP (Bader M. et al., 2006) and cooled-injection gas chromatography and isotope dilution mass spectrometry is used to quantify all four metabolites of NMP and NEP (Schindler et al., 2012).

It can be assumed that gas chromatography-mass spectrometry is applicable for determination of other metabolites associated to DMAC and DMF as well. However, it is indicated that the precision of traditional gas chromatography is low due to the thermal decomposition of metabolites in the high-temperature gas chromatography injection port. To overcome this problem, a new method for the simultaneous separation and quantification of urinary DMAC metabolites using liquid chromatography-tandem mass spectrometry is developed (Yamamoto S. et al., 2018)[16].

As the biomarker of DMAC - DMAC-OH is decomposed during gas chromatography analysis, the total concentration of NMAC is the sum of DMAC-OH and NMAC. The same consideration is relevant to the biomarker of DMF – HMMF which will be decomposed during gas chromatography procedure as well. Therefore, the total concentration of NMF is the sum of HMMF and NMF.

2.5 Societal concern

NMP, NEP, DMAC and DMF are listed in the SIN List. The SIN (Substitute It Now!) List is a comprehensive database of chemicals likely to be restricted or banned in the EU. It is publicly available, regularly updated and provided completely free of charge by non-profit organisation ChemSec (https://chemsec.org).

NMP, DMAC and DMF are included in the Trade Union Priority List for REACH authorization.

[15] *Biotox* database is a biological monitoring guide for occupational physicians that is used in the health surveillance of exposed workers. It covers over 100 chemical substances and specifies the marker, the metabolic background with the influencing factors, and the biological medium to be sampled (http://www.inrs.fr/publications/bdd/biotox.html).

[16] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5886881/

3. Categorization of Substances

Table 3.1: Substances included in the substance group, listed according to availablitity of toxicology and human biomarker data, in category A, B, C, D, E substances (see general introduction)

Category	Abbreviation/ Acronym	Systematic name	CAS No.	Regulation
B	NMP	1-methyl-2-pyrrolidone	872-50-4	**REACH**: SVHC, included in the candidate list for authorization, restricted under Annex XVII **CLP**: harmonized classification Repr. 1B, H360D
	DMF	N,N-dimethylformamide	68-12-2	**REACH**: SVHC, included in the candidate list for authorization **CLP**: harmonized classification Repr. 1B, H360D **Cosmetic Products Regulation**: listed in Annex II - substances prohibited in cosmetic products
C	DMAC	N,N-dimethylacetamide	127-19-5	**REACH**: SVHC, included in the candidate list for authorization **CLP**: harmonized classification Repr. 1B, H360D **Cosmetic Products Regulation**: listed in Annex II - substances prohibited in cosmetic products
D	NEP	1-ethylpyrrolidin-2-one	2687-91-4	**CLP**: harmonized classification Repr. 1B, H360D

4. Policy-related questions

1. What is the current external exposure of the workers in EU to reprotoxic aprotic solvents and do they exceed Guidance values (reference values), where they are available? What data gaps exist?
2. What is the current internal exposure of the workers in EU to reprotoxic aprotic solvents, especially with respect to female workers at reproductive age, and do they exceed Guidance values (reference and HBM values), where they are available? What data gaps exist?
3. Are there geographical differences and differences caused by industrial sector in the exposure of workers in EU to reprotoxic aprotic solvents?
4. What is the current exposure of the general EU population to reprotoxic aprotic solvents, especially with respect to females at reproductive age as well as mothers and their young children, and do they exceed Guidance values (reference and HBM values), where they are available? What data gaps exist?
5. What are the environmental concentrations of reprotoxic aprotic solvents in different environmental media and what is their geographical distribution and time trend in EU, and can they contribute to the overall exposure of the general population? What data gaps exist?
6. What are the indoor air and dust concentrations of reprotoxic aprotic solvents?
7. What is the content of reprotoxic aprotic solvents in widely used commodities (cosmetics, washing & cleaning products, paints, textiles, leather, etc.)?
8. How the exposure of general population to reprotoxic aprotic solvents is correlated with lifestyle and consumption patterns, what is the main exposure route?
9. Are there differences in exposure of the general EU population to regulated and non-regulated reprotoxic aprotic solvents (banned use in cosmetics)?
10. Are there differences in exposure of the workers in EU in relation to regulated and non-regulated reprotoxic aprotic solvents after the restriction for NMP will enter into force after 9 May 2020?
11. What are differences in profiles of reprotoxic aprotic solvents observed in exposure assessment regarding occupational environment and in relation to general public taking into account spatial and temporal distribution?
12. What are the mixture effects of aprotic solvents as a whole in relation to human exposure and how it can be estimated?
13. What are the best indicator`s substances (markers) to identify hazardous exposures to aprotic solvents as a whole?
14. What are the analytical options available with respect to aprotic solvents (gas chromatography-mass spectrometry versus liquid chromatography-tandem mass spectrometry for biological matrices, other methods in addition, methods for environmental media)?
15. What are the levels of reprotoxic aprotic solvents and associated health effects in vulnerable population groups, namely, mothers and their young children?
16. Are there other potentially hazardous aprotic solvents apart from the four reprotoxic aprotic solvents in question?
17. What is the state - of - the – art regarding chemical safety`s legislation on reprotoxic aprotic solvents in question and other potentially hazardous aprotic solvents identified?
18. Can reference values be established for any reprotoxic aprotic solvent in the case they are missing?
19. Can biomarkers of health effects be developed?

5. Research Activities to be undertaken

Table 5.1: Listing of research activities to be carried out to answer the policy questions summed up in 4

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
1, 2, 3, 4, 5, 6, 7, 8, 10	NMP	Toxicological information. Established biomarkers of exposure and HBM values. Analytical methods in place. Notion on the most significant exposure route. Some information on external and internal exposure in the occupational environment.	Very general knowledge about releases to environment – the related information should be gathered. No information on contamination of different environmental media – published information must be searched and environmental monitoring should be arranged in different geographical locations within EU. No information on content in widely used consumers` products – investigations should be arranged. Information on indoor pollution is lacking – special investigations should be arranged. Lacking information on exposure in the general population - published information must be searched and biomonitoring shall be arranged, especially in relation to vulnerable population groups, namely, females at reproductive age, mothers and their young children. Spatial (geographical) and temporal distribution shall be followed-up. No systematic investigations on exposure levels caused by different industrial sectors and geographical locations within EU – such information should be gathered by additional literature search. Information on REACH restriction success is lacking – such investigations shall be done after the transitional period. Association between exposure of general population and lifestyle and consumption patterns is unclear – special investigations shall be arranged.
1, 2, 3, 4, 5, 6, 7, 8, 9	DMF	Toxicological information. Established biomarkers of exposure and HBM values. Analytical methods in place. Notion on the most significant exposure route. Some information on external and internal exposure in the occupational environment.	Very general knowledge about releases to environment – the related information should be gathered. No information on contamination of different environmental media – published information must be searched and environmental monitoring should be arranged in different geographical locations within EU. No information on content in widely used consumers` products – investigations should be arranged. Information on indoor pollution is lacking – special investigations should be arranged. Lacking information on exposure in the general population - published information must be searched and biomonitoring shall be arranged, especially in relation to vulnerable population groups, namely, females at reproductive age, mothers and their young children. Spatial (geographical) and temporal distribution shall be followed-up. No systematic investigations on exposure levels caused by different industrial sectors and geographical locations within EU – such information should be gathered by additional literature search. Information on success in relation to prohibition in cosmetic products is unclear - such investigations shall be done. Association between exposure of general population and lifestyle and consumption patterns is unclear – special investigations shall be arranged.

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
1, 2, 3, 4, 5, 6, 7, 8, 9	DMAC	Toxicological information. Established biomarkers of exposure and HBM values. Analytical methods in place. Notion on the most significant exposure route. Limited information on external and internal exposure in the occupational environment.	Very general knowledge about releases to environment – the related information should be gathered. No information on contamination of different environmental media – published information must be searched and environmental monitoring should be arranged in different geographical locations within EU. No information on content in widely used consumers` products – investigations should be arranged. Information on indoor pollution is lacking – special investigations should be arranged. Lacking information on exposure in the general population - published information must be searched and biomonitoring shall be arranged, especially in relation to vulnerable population groups, namely, females at reproductive age, mothers and their young children. Spatial (geographical) and temporal distribution shall be followed-up. No systematic investigations on exposure levels caused by different industrial sectors and geographical locations within EU – such information should be gathered by additional literature search. Information on success in relation to prohibition in cosmetic products is unclear - such investigations shall be done. Association between exposure of general population and lifestyle and consumption patterns is unclear – special investigations shall be arranged.
1, 2, 3, 4, 5, 6, 7, 8	NEP	Toxicological information. Established biomarkers of exposure and HBM values. Analytical methods in place. Notion on the most significant exposure route.	Very general knowledge about releases to environment – the related information should be gathered. No information on contamination of different environmental media – published information must be searched and environmental monitoring should be arranged in different geographical locations within EU. No information on content in widely used consumers` products – investigations should be arranged. Information on indoor pollution is lacking – special investigations should be arranged. Lacking information on exposure in the general population and in the occupational environment - published information must be searched and biomonitoring shall be arranged, especially in relation to vulnerable population groups, namely, females at reproductive age, mothers and their young children. Spatial (geographical) and temporal distribution shall be followed-up. No systematic investigations on exposure levels caused by different industrial sectors and geographical locations within EU – such information should be gathered by additional literature search. Association between exposure of general population and lifestyle and consumption patterns is unclear – special investigations shall be arranged.

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
11, 12, 13, 14, 15, 17, 18, 19	NMP, DMF, DMAC, NEP	Toxicological information. Established biomarkers of exposure and some HBM values. Analytical methods in place. Restricted external and internal exposure information in the occupational environment is in place.	Differences in profiles of reprotoxic aprotic solvents in relation to exposure and mixture effect is unclear – special investigations shall be done, possibilities to come to one common indicator substance (biomarker) should be assessed. No knowledge on biomarkers of health effects – special investigations shall be arranged. Contradictory information on applicability of different analytical methods – available methods shall be assessed, possibility an necessity to develop new methods should be assessed, interlaboratory validation exercises shall be arranged. Association between exposure of vulnerable population groups and related health effects is unclear – special investigations shall be arranged. No reference values including HBM values for all reprotoxic aprotic solvents – the missing reference values shall be developed.
16, 17	Other aprotic solvents	-	Lacking knowledge on possible other hazardous aprotic solvents– additional screening should be done and potential other priority aprotic solvents should be identified, their legal status should be investigated.

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