Collective expert appraisal: summary of discussion with conclusions

Regarding the "expert appraisal on recommending occupational exposure limits for chemical agents"

Evaluation of biomarkers and recommendation of biological reference values for acrylamide

[CAS no.: 79-06-1]

This document summarises the work of the Expert Committee on expert appraisal for recommending occupational exposure limits for chemical agents (OEL Committee) and the Working Group on biomarkers (biomarkers WG).

Presentation of the issue

On 12 June 2007, AFSSET received a solicited request from the French Directorate General for Labour to conduct the scientific expert appraisal work required for setting occupational exposure limit values (OELVs) for acrylamide. Set by a 1995 Circular[1], France has an indicative 8h-OELV of 0.3 $mg.m^{-3}$ (0.1 ppm) for acrylamide.

The Directorate General for Labour asked AFSSET to reassess this value and, if necessary, to propose new occupational exposure limit values based on health considerations.

This request was entrusted to AFSSET's OEL Committee which, in June 2011, issued a report in which the additional lifetime risk of cancer, under conditions of occupational exposure to acrylamide, was estimated to be:

- 10^{-4} individual excess risk for 40 years of exposure to 4 $\mu g.m^{-3}$
- 10^{-5} individual excess risk for 40 years of exposure to 0.4 $\mu g.m^{-3}$
- 10^{-6} individual excess risk for 40 years of exposure to 0.04 $\mu g.m^{-3}$

The report also recommended:

- assigning the "skin notation".

ANSES decided to supplement its expert appraisal with an assessment of the biological monitoring data on acrylamide in occupational environment, in order to establish the relevance of recommending monitoring of one or more biomarkers in addition to the OEL and the establishment of biological limit values for the selected biomarker(s).

[1] DRT Circular no. 95-4 of 12 January 1995 amending and supplementing the Circular of 19 July 1982 as amended, on the acceptable values for concentrations of certain hazardous substances in workplace atmospheres

Scientific background

Biological monitoring of exposure in workplaces has emerged as a complementary method to atmospheric metrology for assessing exposure to chemical agents. Biological monitoring assesses a worker's exposure by including all the routes by which a chemical penetrates the body (lung, skin, digestive tract). It is particularly worthwhile when a substance has a systemic effect, and:

- when routes other than inhalation contribute significantly to absorption,
- and/or when the pollutant has a cumulative effect,
- and/or when the working conditions (wearing of respiratory protection, inter-individual differences in respiratory ventilation, etc.) determine large differences in internal dose between individuals that are not taken into account by atmospheric metrology.

With regard to prevention of chemical risk in the workplace, the French Labour Code provides for the use of biological monitoring of exposure and biological limit values.

OEL Committee definitions

Biological limit value (BLV): This is the limit value for the relevant biomarkers. As for the 8h-OEL, it aims to protect workers exposed to the chemical agent in question regularly and over the course of a working life from the adverse effects associated with medium- and long-term exposure. Two types of biological limit values can be recommended depending on the available data:

- BLV based on a health effect: the level of a biomarker for which the scientific data do not report any health effects;
- BLV based on exposure to the 8h-OEL: average level of a biomarker corresponding, according to the scientific data, to exposure to the 8h-OEL.

Biological reference values from:

- the general population: the closest value to the 95th percentile of the distribution of biomarkers concentrations found in a general adult population whose characteristics are similar to those of the French population;
- otherwise a control population not occupationally exposed to the substance under study: the closest value to the 95th percentile of the distribution of biomarkers concentrations found in a control population not occupationally exposed to the substance under study.

These values cannot be considered to offer protection from the onset of health effects, but do allow a comparison with the concentrations of biomarkers assayed in exposed workers. These values are of particular interest in cases where it is not possible to establish a BLV.

Organisation of the expert appraisal

ANSES entrusted examination of this request to the Expert Committee on expert appraisal for recommending occupational exposure limits for chemical agents (OEL Committee). The Agency also mandated the Working Group on biomarkers for this expert appraisal.

The methodological and scientific aspects of this group's work were regularly submitted to the OEL Committee. The report produced by the working group takes account of the observations and additional information provided by the Committee members.

This expert appraisal was therefore conducted by a group of experts with complementary skills. It was carried out in accordance with the French Standard NF X 50-110 “Quality in Expertise Activities”.

Description of the method

A rapporteur in the biomarkers WG was mandated by the Agency to produce a summary report on biomarkers of exposure and the recommendation of biological limit values (BLVs) and biological reference values for the biomarker(s) considered as relevant. An ANSES officer also contributed to this report.

The summary report on the biomarkers for acrylamide results from bibliographical information taking into account the scientific literature published on this substance until 2011. The bibliographical research was conducted in the following databases: Medline, Toxline, HSDB, ToxNet (CCRIS, GENE-TOX, IRIS), ScienceDirect. The rapporteur reassessed the original articles or reports cited as references whenever he considered it necessary, or whenever the Committee requested it.

The Expert Committee on expert appraisal for recommending occupational exposure limits for chemical agents adopted the summary report on the biomarkers at its meeting on 12 January 2012.

The summary and conclusions of the collective expert appraisal were adopted by the Committee on expert appraisal for recommending occupational exposure limits for chemical agents on 12 January 2012.

The collective expert appraisal work and the summary report were submitted to public consultation from 18 October 2012 to 20 December 2012. No comment was received. The OEL Committee adopted this version on 4 April 2013.

Result of the collective expert appraisal

Introduction

For the assessment of the biological monitoring data on acrylamide, 41 scientific papers were selected from the *Medline* database using the following keywords:

- acrylamide and biomarker
- acrylamide and biological monitoring
- acrylamide and exposure (worker, general population)
- acrylamide and metabolism
- acrylamide and haemoglobin adducts
- acrylamide concentration and blood, urine
- acrylamide modelling
- acrylamide and health effects (tumor, carcinogenicity)

Toxicokinetics data

Two studies report quantitative information on the dermal absorption of acrylamide (AA) in humans. The studies by Fennell *et al.* (2005 and 2006) report that 30 to 35% of the dermally administered dose of acrylamide may be absorbed by this route (solution diluted to 50%).

Pulmonary absorption data in humans are only qualitative.

Gastrointestinal absorption of acrylamide may account for at least 40% of the dose ingested in food (Fennell *et al.*, 2006). Absorption increases with the dilution of acrylamide in an aqueous solution.

The main acrylamide degradation pathway includes conjugation with glutathione, with the formation of cysteine-S-propionamide, and its elimination as mercapturic acid (AAMA) or its sulfoxide. The other transformation pathway involves oxidation, mainly via CYP2E1 to form glycidamide (GA) (a highly reactive epoxide). This can be reduced to glyceramide or conjugated with glutathione (detoxification pathway) yielding two isomers (GAMA and iso-GAMA), which are excreted in the urine. Acrylamide and glycidamide are able to react with the terminal valine of haemoglobin and thus form the haemoglobin adducts, AAVal and GAVal. Glycidamide can also react by nucleophilic substitution with certain nucleophilic atoms of the DNA bases, e.g. the N7 of adenine or the N3 of adenine, to form N7-(2-carbamoyl-2-hydroxyethyl)guanine (N7-GA-Gua) and N3-(2-carbamoyl-2-hydroxyethyl)adenine (N3-GA-Ade). These adducts have only been described in rats.

Acrylamide is rapidly excreted or metabolised. Its elimination constant in the blood is equal to 0.15 h^{-1} and its half-life in blood is approximately 4.5 hours (Fennell *et al.*, 2005). Given glycidamide's high reactivity with glutathione or proteins, it has a limited life span in blood (free state). Few studies have measured it in biological fluids.

In humans, after exposure to acrylamide, adduct levels increase steadily until they reach a plateau at the fourth month of exposure. Upon cessation of exposure, adduct concentrations decrease regularly for four months, which is the average life span of red blood cells (approximately 120 days) and means that the adducts formed are stable over time (Hagmar *et al.*, 2001; Kjuus *et al.*, 2004). The kinetics of formation of acrylamide and glycidamide haemoglobin adducts (terminal valine) may follow a second-order constant. Fennell *et al.* (2005) determined the formation constants *in vitro* of acrylamide adducts (4.3×10^{-6} $l.g^{-1}$ $globin.h^{-1}$) and glycidamide adducts (6.7×10^{-6} $l.g^{-1}$ $gb.h^{-1}$). Fennell *et al.* (2005) also determined that the daily increments of acrylamide and glycidamide haemoglobin adducts were equal to 74.7 nmol of $AAVal.g^{-1}$ $gb.(mmol\ of\ AA.kg^{-1})^{-1}$ and 28.9 nmol of $GAVal.g^{-1}$ $gb.(mmol\ of\ AA.kg^{-1})^{-1}$.

Two studies on volunteers report different elimination kinetics for acrylamide following oral exposure, with maximum concentration reached 3 hours after the onset of exposure in one case, and between 8 and 16 hours in the other case (Fuhr *et al.*, 2006; Fennell *et al.*, 2006). Both studies report a half-life of around 2 to 3 hours and that 3 to 5% of the ingested acrylamide dose is recovered unchanged in urine after 24 hours (Fuhr *et al.*, 2006; Fennell *et al.*, 2006).

According to Fuhr *et al.* (2006), the maximum concentration of AAMA in urine may be reached seven hours after the onset of exposure and its half-life may be approximately 17 hours. During dermal or oral exposure, AAMA seems to be the major urinary metabolite, accounting for 68 to 69% of all metabolites detected in the urine (AA, GA, cysteine-S-propionamide, AAMA and its sulfoxide, GAMA and iso-GAMA) (Fennell *et al.*, 2006). In 24 hours, between 30 and 45% of the ingested acrylamide dose is excreted as AAMA in the urine and this percentage reaches 50% after 72 hours (Boettcher *et al.*, 2006; Fennell *et al.*, 2006; Fuhr *et al.*, 2006).

For the same exposure, urinary AAMA-sulfoxide may account for 17.5 to 18.5% of all metabolites measured in urine (AA, cysteine-S-propionamide, AAMA and its sulfoxide, GA, GAMA and iso-GAMA), while 7 to 9% of the ingested dose is found as sulfoxide after 24 hours (Fennell *et al.*, 2006).

In contrast, 0.1% of the dermally administered acrylamide dose is found as AAMA-sulfoxide after 24 hours and this percentage reaches 1% after 4 days (Fennell *et al.*, 2006). The metabolic fractions found by dermal exposure are therefore well below those corresponding to the oral route.

Maximum urinary excretion of glycidamide in humans is reached 4 to 16 hours after the onset of oral exposure (Fennell *et al.*, 2006).

According to Fuhr *et al.* (2006), the maximum concentration of GAMA in urine is reached 14 hours after the start of exposure, with a half-life of approximately 22 hours.

The relative percentages of the urinary metabolites measured (AA, GA, cysteine-S-propionamide, AAMA and its sulfoxide, GAMA and iso-GAMA) were calculated during oral exposure and are respectively 1.5% and 0.35% for GAMA and iso-GAMA (Fennell *et al.*, 2006).

Table 1: Summary of the molar excretion fractions of each metabolite calculated for oral (24 hours and 72 hours) or subcutaneous (96 hours) exposure according to Boettcher *et al.*, 2006; Fennell *et al.*, 2006 and Fuhr *et al.*, 2006.

Urinary metabolite	Molar excretion fraction % Oral route (24 hours)	Molar excretion fraction % Oral route (72 hours)
AA	3 to 5	4.5
AAMA	30 to 45	50
AAMA-sulfoxide	7 to 9	NR
GA	0.45 to 0.65	NR
GAMA	0.6 to 2.7	4.8
Iso-GAMA	0.15 to 0.17	NR

Choice of biomarkers

The acrylamide biomarkers identified in the scientific literature are the following (abbreviations given in brackets):

- Acrylamide — Urine — (AAu)
- N-acetyl-S-(2-carbamoylethyl)cysteine — Urine — (AAMAu)
- N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)cysteine — Urine — (GAMAu)
- N-acetyl-S-(1-carbamoyl-2-hydroxyethyl)cysteine — Urine — (iso-GAMAu)
- Acrylamide — Blood — (AAb)
- Glycidamide — Blood — (GAb)
- N-(2-carbamoylethyl)valine — Blood — (AAVal)
- N-(2-carbamoyl-2-hydroxyethyl)valine — Blood — (GAVal)

Glycidamide is a molecule that reacts with DNA. It may be important to measure this biomarker in the blood since glycidamide-induced DNA lesions play a part in the onset of acrylamide's mutagenic and other genotoxic effects. The measurement of acrylamide in the blood and urine is specific to acrylamide exposure. Monitoring these three biomarkers (AAb, GAb, AAu) would therefore be interesting in the workplace (specificity and relationship with the effect). However, due to their reactivity with glutathione or proteins, acrylamide and glycidamide have a limited lifespan in the blood (free state). It therefore seems difficult to use these biomarkers to assess occupational exposure. .

Studies in the workplace primarily report measurements of urinary metabolites, such as mercapturic acids, and measurements of haemoglobin adducts, which may be relevant for biological monitoring of occupational exposure.

Mercapturic acids have half-lives of between 10 and 20 hours and their measurement at the end of the work shift reflects daily exposure. Regarding mercapturic acids from glycidamide, GAMA and iso-GAMA, few data are available to support a recommendation for the monitoring of these biomarkers, whether for occupationally exposed workers or the general population. Studies in the general population show that GAMA concentrations are often below the limit of detection and are influenced by many individual factors (diet, smoking, etc.) and cannot therefore be used for comparison. Similarly, few studies report data on AAMA, meaning recommendations cannot be made with certainty for the monitoring of these biomarkers of exposure. Moreover, when AAMA is hydrolysed prior to analysis, the hydrolysis product is not specific to acrylamide exposure since it is also found in exposure to acrylonitrile. Although not chosen as relevant biological indicators of exposure, the information available on AAMA and GAMA is described in the annex of the expert report.

Acrylamide and glycidamide haemoglobin adducts reflect exposure from the previous three months. Only information on AAVal, which was considered sufficient, is described in the report. As the information on GAVal was only fragmentary, it is presented in the annex of the expert report.

In conclusion, only acrylamide haemoglobin adducts, measured in the blood, are the subject of recommendations in the expert report.

Information on biological indicators of exposure identified as relevant for the biological monitoring of exposed workers

Name	**N-(2-carbamoylethyl)valine (AAVal) (pmol.g^{-1} gb)**	
Other substances giving rise to this biomarker	N-methyloacrylamide	
Concentrations found in exposed workers or volunteers	• Field studies Calleman *et al.* (1994), Chinese workers exposed (n=51): Atmospheric concentrations: NR Arithmetic mean 9553, median 7100, from 300 to 34,000 Jones *et al.* (2006), English workers (n=60) Atmospheric concentrations: mean 30 µg.m^{-3} AAVal: 90th percentile 514 • Studies in volunteers Fennell *et al.* (2006): ingestion of 3 doses of acrylamide, measurement of mean concentrations of AAVal (n=3 groups of 6) 0.5 mg.kg^{-1}: 514; 1 mg.kg^{-1}: 914; 3 mg.kg^{-1}: 2479	
Conversion factor	1 µg.L^{-1} AAVal=36.90 pmol.g^{-1} gb	
Concentrations in the general population	Chevolleau *et al.* (2007), French general population (ENNS pilot study) (n=68) Non smokers (n=52): median 27 Smokers (n=16): median 53 Scherer *et al.* (2007), German general population Non smokers (n=100): mean 27.8; 90th percentile 37.2 Smokers (n=274): mean 84.1; 90th percentile 136.4 Urban *et al.* (2006): German general population Non smokers (n=60): median 26.8 Smokers (n=60): median 79.1 Schettgen *et al.* (2003), German general population Non smokers (n=25): median 21; 95th percentile 46 Smokers (n=47): median 85; 95th percentile 159 Hartmann *et al.* (2008), German general population Non smokers (n=91): median 30; 95th percentile 51 CDC (2009), USA-NHANES 20 to 59 years (n=2570): 95th percentile 223 Vesper *et al.* (2010), USA-NHANES Non smokers (n=5686): geometric mean 50.5 Smokers (n=1316): geometric mean 113 Vesper *et al.* (2008), European general population Non smokers (n=255): median 42.5; 95th percentile 88.3 Smokers (n=255): median 121; 95th percentile 285	
Recommended limit values for exposed workers	USA - ACGIH (BEI)	Germany BLW: 15 µg.L^{-1} or 550 pmol.g^{-1} gb (peripheral neuropathy) According to the study by Hagmar *et al.* (2001)
	Germany - DFG (BAT)	
	Quebec - IRSST (IBE)	
	Finland - FIOH (BAL)	
	Other value(s) (Swiss, etc.)	

Study of the relationship between AAVal concentrations and health effects

Carcinogenicity

There are no data in humans that can be used to make the link between haemoglobin adduct concentrations and acrylamide carcinogenicity data. It should be noted that the literature review by Pelucchi *et al.* (2011) presents the results of two epidemiological studies in the general population whose aim was to try to calculate the excess cancer risk related to AAVal concentrations. This review did not report any significant association between excess risk for the cancers studied (primarily breast and prostate cancer) and concentrations of acrylamide haemoglobin adducts. The same review was unable to show any excess cancer mortalities in two cohorts of workers for cancer of the lungs, kidneys and pancreas.

Neurotoxicity

The effects reported in the workplace usually concern neurotoxicity. Only two studies have linked neurotoxicity measurements (clinical examination, neurotoxicity index) with AAVal concentrations (Calleman *et al.*, 1994; Hagmar *et al.*, 2001).

Calleman *et al.*, (1994) found a good correlation (r=0.67) between AAVal concentrations and the neurotoxicity index in 41 workers (34 men and 7 women) exposed to acrylamide (duration of use greater than 6 months). In 1996, Calleman used the results of this study to present different doses (AAVal concentrations) for various neurotoxicity indicators: NOAEL, LOAEL and the concentration at which 50% of exposed people exhibit the considered effect (EL_{50}). Only the EL_{50} presented slight differences depending on the neurotoxicity indicator observed.

The second study focused on workers involved in the construction of a road tunnel in Sweden (Hagmar *et al.*, 2001). As several workers developed health problems (impairment of the peripheral nervous system), a survey was conducted to monitor these workers and perform a medical investigation. A total of 213 workers and 18 non-occupationally exposed and non-smoker controls were included in the survey. Exposure occurred for 2 months (August to September) in a situation of co-exposure to acrylamide, methyloacrylamide and formaldehyde. Adduct levels were not measured during the exposure period but only 1 to 2 weeks after cessation of exposure, and effects were studied only 2 to 4 weeks after cessation of exposure using a self-administered questionnaire and a clinical examination. The authors reported neurotoxicity symptoms (numbness and tingling of the limbs) from 510 $pmol.g^{-1}$ gb.

Both studies have certain limitations:

- In the study by Calleman *et al.* (1996), irrespective of the neuropathy symptoms studied, the NOAELs were equal to 2000 pmol of $AAVal.g^{-1}$ gb and the LOAELs equal to 6000 pmol of $AAVal.g^{-1}$ gb. As this dose-response relationship was the same in spite of the different indicators being indicators of more or less early effect, it is difficult to interpret these points of departure for establishing a dose-response relationship;
- In the study by Hagmar *et al.* (2001), the time interval between the end of exposure, the measurement of adduct levels and the study of the health effects makes interpretation and extrapolation difficult.

Study of the relationship between AAVal concentrations and exposure to acrylamide

Field studies

There are few studies in an occupational environment providing airborne concentrations of acrylamide. Studies of acrylamide exposure mainly concern the general population and enable the estimatation of the acrylamide doses ingested from the haemoglobin adduct levels (generally, acrylamide adducts) measured.

Jones *et al.* (2006) investigated the association between atmospheric concentrations of acrylamide and adduct levels in 60 workers in the United Kingdom. The atmospheric concentrations of acrylamide are not reported, but it is stated that the mean is about 30 µg.m^{-3}. The authors indicate that there is a strong correlation (r=0.61) between atmospheric concentrations of acrylamide and concentrations of AAVal.

In this study, the limit of detection (LOD) of acrylamide in air is 5 µg.m^{-3}, higher than the atmospheric concentration of acrylamide calculated by the OEL Committee, for a 10^{-4} individual excess risk for 40 years of exposure (4 µg.m^{-3}). Moreover, the reported correlation was calculated for exposure levels ranging from 5 to 200 µg.m^{-3} (determined graphically), which are greater concentrations than the atmospheric concentrations of acrylamide calculated for the individual excess risks (IERs) of 10^{-5} (0.4 µg.m^{-3}) and 10^{-6} (0.04 µg.m^{-3}). It is therefore not possible to establish the validity of the reported correlation for exposure to atmospheric concentrations below this study's LOD (1/10th to 1/100th of the LOD).

Experimental data

Establishment of the 8h-OELV is based on the choice of the critical effect of testicular mesothelioma presented by the study by Friedman *et al.* (1995). Acrylamide was administered to a group of male rats (Fisher 344) in drinking water at doses of 0 (204 animals), 0.1 (204 animals), 0.5 (102 animals) and 2 (75 animals) mg.kg^{-1}.d^{-1} for two years. Female rats received doses of 0 (100 animals), 1 (100 animals) and 3 (100 animals) mg.kg^{-1}.d^{-1}.

An increase in testicular mesothelioma is considered as significant at the dose of 2 mg.kg^{-1}.d^{-1}. A benchmark dose for a 10% prevalence of testicular mesothelioma (BMD_{10}) in rats and its lower limit at 10%, BMDL (0.628 mg.kg^{-1}.d^{-1}), was calculated from the results of this study.

To extrapolate this BMDL to humans taking into account the same route of exposure (oral), it was decided to apply an allometric adjustment factor, thereby determining a human equivalent dose from the dose retained in rats.

As the rats were exposed throughout their lifetime (24 hours/day, 7 days/week for 104 weeks, the average lifespan of a rat), the human equivalent dose should be adjusted for an exposure scenario classically taken into account in workers (lifetime of 75 years, with exposure for 8 hours/day, 5 days/week, 48 weeks/year for 40 years).

From these two adjustments, the daily dose in humans, adjusted to an occupational exposure scenario, is 1.7 mg.kg^{-1}.d^{-1}. The study by Fennell *et al.* (2005) reported kinetic parameters calculated in humans for the formation of acrylamide haemoglobin adducts. Thus AAVal concentrations can be calculated for three doses of acrylamide corresponding to the IERs 10^{-4}, 10^{-5} and 10^{-6} calculated to establish the OEL. Under the assumption that pulmonary absorption is equivalent to oral absorption, for chronic exposure, AAVal concentrations are calculated (Fennell *et al.* 2005) such that:

Ingested dose (mg.kg^{-1}.d^{-1})= [AAVal] x M(AA)/[(T_{eryth}/2) x Fr_{AAVal}]

- [AAVal]: concentration of AAVal adducts (mmol.g^{-1} gb)
- M(AA): molecular weight of acrylamide 71
- T_{eryth}: average lifespan of erythrocytes (d) 120
- Fr_{AAVal}: daily adduct increment of AAVal (mmol.g^{-1} gb.(mmol(AA).kg^{-1})$^{-1}$) 74.7 x 10^{-6}

Calculating these concentrations presents many uncertainties. The kinetic parameters of acrylamide and of the adducts were measured for oral absorption of a single dose of acrylamide. The constant of adduct formation is not determined:

- for continuous exposure, with the adducts concentration at equilibrium;
- for exposure by inhalation.

Establishment of BLVs and choice of biological reference values

The calculations of AAVal concentrations based on either the study in rats chosen for establishing the OEL, or the atmospheric concentrations of acrylamide and the kinetic data obtained in humans, present many uncertainties. These two methods are not robust enough to accurately predict concentrations of acrylamide haemoglobin adducts associated with three IERs (10^{-4}, 10^{-5} and 10^{-6}).

The studies reporting index of neurotoxic effects of acrylamide were also excluded since they presented some limitations. Accordingly, it was not considered relevant to recommend a biological limit value on the basis of an effect other than cancer.

Moreover, the Committee wishes to reiterate that the ALARA[2] principle should be applied in the presence of a non-threshold carcinogen. Thus, when it is not possible to calculate biomarker concentrations on the basis of a quantitative risk assessment or to recommend a pragmatic biological limit value, biological reference values may be proposed.

[2] As Low As Reasonably Achievable

Proposed biological reference values

These values are not intended to protect from health effects but can be used to assess exposure levels in workers.

Thus the concentrations found in the European population in the study by Vesper *et al.* (2008) enable the following biological reference values to be proposed for AAVal [95th percentile in the general population (40 to 60 years)]:

- Non smokers: 88.3 pmol.g^{-1} gb
- Smokers: 285 pmol.g^{-1} gb
- Without distinction as to smoking status: 244 pmol.g^{-1} gb

Separate values for smokers and non-smokers can be rounded to respectively 285 and 85 pmol.g^{-1}gb.

Conclusions of the collective expert appraisal

Biological indicator of exposure: AAVal, haemoglobin adduct (blood)

BLV based on a health effect: None

BLV based on exposure to the 8h-OEL: None

Biological reference values:

- Non-smokers: 85 pmol.g^{-1} gb
- Smokers: 285 pmol.g^{-1} gb

Sampling method and factors that may affect the interpretation of AAVal assays

The sampling times are not critical because acrylamide haemoglobin adducts are stable and last the entire lifetime of the erythrocytes (approximately 120 days).

Some studies recommend the use of heparin glass tubes and a sample of at least 10 mL.

The tubes are kept at 5°C until centrifugation. If there is a lack of information regarding the stability of samples, it is recommended to perform centrifugation as soon as possible. The erythrocyte fraction, separated by centrifugation, can be stored at -70°C in plastic tubes until analysis.

A diet rich in potato crisps, French fries, cereals and coffee can cause large differences in AAVal concentrations within the general population.

Tobacco consumption may increase levels of acrylamide haemoglobin adducts by a factor of 3 to 4.

Drugs that induce CYP2E1 may influence the formation of GA and thus reduce the formation of AAVal adducts.

Biometrology

Blood AAVal		
Analytical methods		
	Method 1	**Method 2**
Analytical technique Bibliographic references	Gas chromatography – mass spectrometry detection (GC – MS)	Gas chromatography – tandem mass spectrometry detection (GC – MS/MS)
Limit of detection	3.5 pmol/g globin	0.2 pmol/g globin
Limit of quantification	11.7 pmol/g globin	0.7 pmol/g globin
Fidelity	NR	NR
Precision	NR	NR
Reference standard	Availability of a commercial reference standard *N*-2-Carbamoylethylvaline-leucide-anilide	
Existence of an inter-laboratory quality control programme	NR	NR
References	Bergmark *et al.*, 1993 Schettgen *et al.*, 2002 Schettgen *et al.*, 2003 Paulsson 2003 Schettgen *et al.*, 2004 Boettcher and Angerer, 2005 Jones *et al.*, 2006 Urban *et al.*, 2006	Bergmark 1997 Perez *et al.*, 1999 Paulsson *et al.*, 2003 Hagmar *et al.*, 2005 Bjellaas *et al.*, 2007a Chevolleau *et al.*, 2007 Hartmann *et al.*, 2008

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References

Bergmark E. Hemoglobin adducts of acrylamide and acrylonitrile in laboratory workers, smokers and nonsmokers. Chem Res Toxicol. 1997;10(1):78-84.

Bergmark E, Calleman CJ, He F, Costa LG. Determination of hemoglobin adducts in humans occupationally exposed to acrylamide. Toxicol Appl Pharmacol. 1993;120(1):45-54.

Bjellaas T, Olesen PT, Frandsen H, Haugen M, Stølen LH, Paulsen JE, Alexander J, Lundanes E, Becher G. Comparison of estimated dietary intake of acrylamide with hemoglobin adducts of acrylamide and glycidamide. Toxicol Sci. 2007a;98(1):110-7.

Boettcher MI, Angerer J. Determination of the major mercapturic acids of acrylamide and glycidamide in human urine by LC-ESI-MS/MS. J Chromatogr B Analyt Technol Biomed Life Sci. 2005;824(1-2):283-94.

Boettcher MI, Bolt HM, Drexler H, Angerer J. Excretion of mercapturic acids of acrylamide and glycidamide in human urine after single oral administration of deuterium-labelled acrylamide. Arch Toxicol. 2006;80(2):55-61.

Calleman CJ. The metabolism and pharmacokinetics of acrylamide: implications for mechanisms of toxicity and human risk estimation. Drug Metab Rev. 1996;28(4):527-90.

Calleman CJ, Wu Y, He F, Tian G, Bergmark E, Zhang S, Deng H, Wang Y, Crofton KM, Fennell T, *et al.* Relationships between biomarkers of exposure and neurological effects in a group of workers exposed to acrylamide. Toxicol Appl Pharmacol. 1994;126(2):361-71.

Chevolleau S, Jacques C, Canlet C, Tulliez J, Debrauwer L. Analysis of hemoglobin adducts of acrylamide and glycidamide by liquid chromatography-electrospray ionization tandem mass spectrometry, as exposure biomarkers in French population. J Chromatogr A. 2007;1167(2):125-34.

Fennell TR, Sumner SC, Snyder RW, Burgess J, Friedman MA. Kinetics of elimination of urinary metabolites of acrylamide in humans. Toxicol Sciences. 2006;93(2):256-67.

Fennell TR, Sumner SC, Snyder RW, Burgess J, Spicer R, Bridson WE, Friedman MA. Metabolism and hemoglobin adduct formation of acrylamide in humans. Toxicol Sci. 2005;85(1):447-59.

Fuhr U, Boettcher MI, Kinzig-Schippers M, Weyer A, Jetter A, Lazar A, Taubert D, Tomalik-Scharte D, Pournara P, Jakob V, Harlfinger S, Klaassen T, Berkessel A, Angerer J, Sörgel F, Schömig E. Toxicokinetics of acrylamide in humans after ingestion of a defined dose in a test meal to improve risk assessment for acrylamide carcinogenicity. Cancer Epidemiol Biomarkers Prev. 2006;15(2):266-71.

Hagmar L, Törnqvist M, Nordander C, Rosén I, Bruze M, Kautiainen A, Magnusson AL, Malmberg B, Aprea P, Granath F, Axmon A. Health effects of occupational exposure to acrylamide using hemoglobin adducts as biomarkers of internal dose. Scand J Work Environ Health. 2001;27(4):219-26.

Hagmar L, Wirfält E, Paulsson B, Törnqvist M. Differences in haemoglobin adduct levels of acrylamide in the general population with respect to dietary intake, smoking habits and gender. Mutat Res. 2005;580(1-2):157-65.

Hartmann EC, Boettcher MI, Schettgen T, Fromme H, Drexler H, Angerer J. Hemoglobin adducts and mercapturic acid excretion of acrylamide and glycidamide in one study population. J Agric Food Chem. 2008;56(15):6061-8.

Jones K, Garfitt S, Emms V, Warren N, Cocker J, Farmer P. Correlation of haemoglobin-acrylamide adducts with airborne exposure: an occupational survey. Toxicol Lett. 2006;162(2-3):174-80.

Kjuus H, Goffeng LO, Heier MS, Sjöholm H, Ovrebø S, Skaug V, Paulsson B, Törnqvist M, Brudal S. Effects on the peripheral nervous system of tunnel workers exposed to acrylamide and N-methylolacrylamide. Scand J Work Environ Health. 2004;30(1):21-9.

Paulsson B, Athanassiadis I, Rydberg P, Törnqvist M. Hemoglobin adducts from glycidamide: acetonization of hydrophilic groups for reproducible gas chromatography/tandem mass spectrometric analysis. Rapid Commun Mass Spectrom. 2003; 17(16):1859-65.

Pelucchi C, La Vecchia C, Bosetti C, Boyle P, Boffetta P. Exposure to acrylamide and human cancer—a review and meta-analysis of epidemiologic studies. Ann Oncol. 2011;22(7):1487-99.

Pérez HL, Cheong HK, Yang JS, Osterman-Golkar S. Simultaneous analysis of hemoglobin adducts of acrylamide and glycidamide by gas chromatography-mass spectrometry. Anal Biochem. 1999;274(1):59-68.

Schettgen T, Broding HC, Angerer J, Drexler H. Hemoglobin adducts of ethylene oxide, propylene oxide, acrylonitrile and acrylamide-biomarkers in occupational and environmental medicine. Toxicol Lett. 2002;134(1-3):65-70.

Schettgen T, Rossbach B, Kütting B, Letzel S, Drexler H, Angerer J. Determination of haemoglobin adducts of acrylamide and glycidamide in smoking and non-smoking persons of the general population. Int J Hyg Environ Health. 2004;207(6):531-9.

Schettgen T, Weiss T, Drexler H, Angerer J. A first approach to estimate the internal exposure to acrylamide in smoking and non-smoking adults from Germany. Int J Hyg Environ Health. 2003;206(1):9-14.

Urban M, Kavvadias D, Riedel K, Scherer G, Tricker AR. Urinary mercapturic acids and a hemoglobin adduct for the dosimetry of acrylamide exposure in smokers and nonsmokers. Inhal Toxicol. 2006;18(10):831-9.

Vesper HW, Slimani N, Hallmans G, Tjonneland A, Agudo A, Benetou V, *et al.* Cross-sectional study on acrylamide hemoglobin adducts in subpopulations from the European Prospective Investigation into Cancer and Nutrition (EPIC) Study. J Agric Food Chem. 2008;56(15):6046-53.

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On behalf of the Committee experts

François Paquet,

Committee Chairman