anses

Valeurs limites d'exposition
en milieu professionnel

Acétaldéhyde

Évaluation des effets sur la santé et des méthodes de mesure

Avis de l'Anses
Rapport d'expertise collective

Août 2025

Connaître, évaluer, protéger

Avis de l'Anses
Saisine n°2022-MPEX-0178

Le directeur général

Maisons-Alfort, le 14 août 2025

AVIS
de l'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail

relatif à l'expertise en vue de la fixation de valeurs limites d'exposition à des agents chimiques en milieu professionnel

Évaluation des effets sur la santé et des méthodes de mesure des niveaux d'exposition sur le lieu de travail pour l'acétaldéhyde

(CAS n° 75-07-0)

L'Anses met en œuvre une expertise scientifique indépendante et pluraliste.
L'Anses contribue principalement à assurer la sécurité sanitaire dans les domaines de l'environnement, du travail et de l'alimentation et à évaluer les risques sanitaires qu'ils peuvent comporter.
Elle contribue également à assurer d'une part la protection de la santé et du bien-être des animaux et de la santé des végétaux et d'autre part à l'évaluation des propriétés nutritionnelles des aliments.
Elle fournit aux autorités compétentes toutes les informations sur ces risques ainsi que l'expertise et l'appui scientifique technique nécessaires à l'élaboration des dispositions législatives et réglementaires et à la mise en œuvre des mesures de gestion du risque (article L.1313-1 du code de la santé publique).
Ses avis sont publiés sur son site internet.

L'Anses a été saisie en juin 2022 par la Direction générale du travail (DGT) pour la réalisation de l'expertise suivante : évaluation des effets sur la santé et des méthodes de mesure des niveaux d'exposition sur le lieu de travail pour l'acétaldéhyde en vue de la fixation de valeurs limites d'exposition à des agents chimiques en milieu professionnel.

1. CONTEXTE ET OBJET DE LA SAISINE

Dans le cadre du protocole d'accord entre l'Anses et le ministère du travail pour la mise en œuvre du programme de travail d'expertise scientifique en matière de valeurs limites atmosphériques et biologiques pour les expositions professionnelles, renouvelé en 2023, la DGT a saisi l'Anses, afin de mener les travaux d'expertise nécessaires à la fixation de valeurs limites d'exposition professionnelle (VLEP) fondées sur des considérations sanitaires pour l'acétaldéhyde.

Actuellement, la France dispose, pour l'acétaldéhyde, d'un repère en santé-travail sous la forme d'une valeur moyenne d'exposition sur 8 heures de 180 $mg.m^{-3}$ (100 ppm), valeur indicative fixée par la circulaire du 13 mai 1987[1].

2. ORGANISATION DE L'EXPERTISE

L'expertise a été réalisée dans le respect de la norme NF X 50-110 « Qualité en expertise – Prescriptions générales de compétence pour une expertise (Mai 2003) ».

L'expertise relève du domaine de compétences du comité d'experts spécialisé (CES) « Valeurs sanitaires de référence » (CES VSR). L'évaluation des effets sanitaires de l'acétaldéhyde a été réalisée par le CES VSR avec l'appui de deux rapporteurs.

Pour l'évaluation des méthodes de mesure dans l'air des lieux de travail, l'Anses a confié l'expertise au groupe de travail « Métrologie ».

Les travaux d'expertise ont été soumis régulièrement au CES tant sur les aspects méthodologiques que scientifiques.

Le présent avis se fonde pour les aspects scientifiques sur le rapport intitulé « Expert appraisal on recommending occupational exposure limits for chemical agents - Assessment of health effects and methods for the measurement of exposure levels in workplace atmospheres for acetaldehyde (CAS N° 75-07-0) » (Anses 2024).

Le rapport en anglais ainsi que la partie « Analyse et conclusions du CES » du présent avis en français ont été adoptés par le CES VSR le 12 décembre 2024. Un expert s'est abstenu[2].

L'Anses analyse les liens d'intérêts déclarés par les experts avant leur nomination et tout au long des travaux, afin d'éviter les risques de conflits d'intérêts au regard des points traités dans le cadre de l'expertise.

Les déclarations d'intérêts des experts sont publiées sur le site internet : https://dpi.sante.gouv.fr/.

Description de la méthode scientifique

Les VLEP proposées par le CES VSR sont des niveaux de concentration de l'agent chimique dans l'atmosphère des lieux de travail à ne pas dépasser sur une période de référence déterminée et en deçà desquels le risque d'altération de la santé est considéré comme négligeable, à partir des connaissances scientifiques les plus récentes. Même si des modifications physiologiques réversibles sont parfois tolérées, aucun atteinte organique ou fonctionnelle de caractère irréversible ou prolongée n'est admise à ce niveau d'exposition pour la grande majorité des travailleurs. Ces niveaux de concentration sont déterminés en considérant que la population exposée (les travailleurs) est une population qui ne comprend ni enfants, ni personnes âgées. Ces valeurs s'appliquent à l'ensemble de la population des travailleurs, y compris les populations sensibles.

[1] Circulaire du 13 mai 1987 complétant l'annexe de la circulaire du 19 juillet 1982 relative aux valeurs admises pour les concentrations de certaines substances dangereuses dans l'atmosphère des lieux de travail.
[2] Motivation de l'abstention par l'expert : « N'étant pas membre de la précédente mandature du CES VSR ayant validé la partie A "effets sanitaires" du rapport d'expertise collective, je ne souhaite pas me prononcer ».

Trois types de valeurs atmosphériques, sont recommandés par le CES : les valeurs limites d'exposition sur 8 heures (VLEP-8h)[3], les valeurs limites court terme sur 15 minutes (VLCT-15min)[4] et les valeurs plafond[5].

Les VLEP sont, idéalement, élaborées à partir de données permettant de caractériser la relation entre les variations de concentrations atmosphériques de l'agent chimique et les effets sanitaires. La construction des VLEP diffèrent en fonction des connaissances ou des hypothèses formulées sur les mécanismes d'action des substances. Actuellement, l'hypothèse par défaut est de considérer une relation monotone entre la dose d'exposition, et l'effet observé. En l'état actuel des connaissances et par défaut, on considère généralement que, pour les effets non cancérogènes, la toxicité ne s'exprime qu'au-delà d'un certain seuil de dose (Anses, à paraître). Pour les effets cancérogènes, il est possible d'établir des VLEP-8h à seuil (correspondant au seuil en dessous duquel il n'est pas attendu la survenue d'effets) ou sans seuil (correspondant à la probabilité de la survenue des effets) selon le mode d'action de l'agent chimique étudié.

En pratique, la proposition de VLEP comprend différentes étapes indiquées en Figure 1.

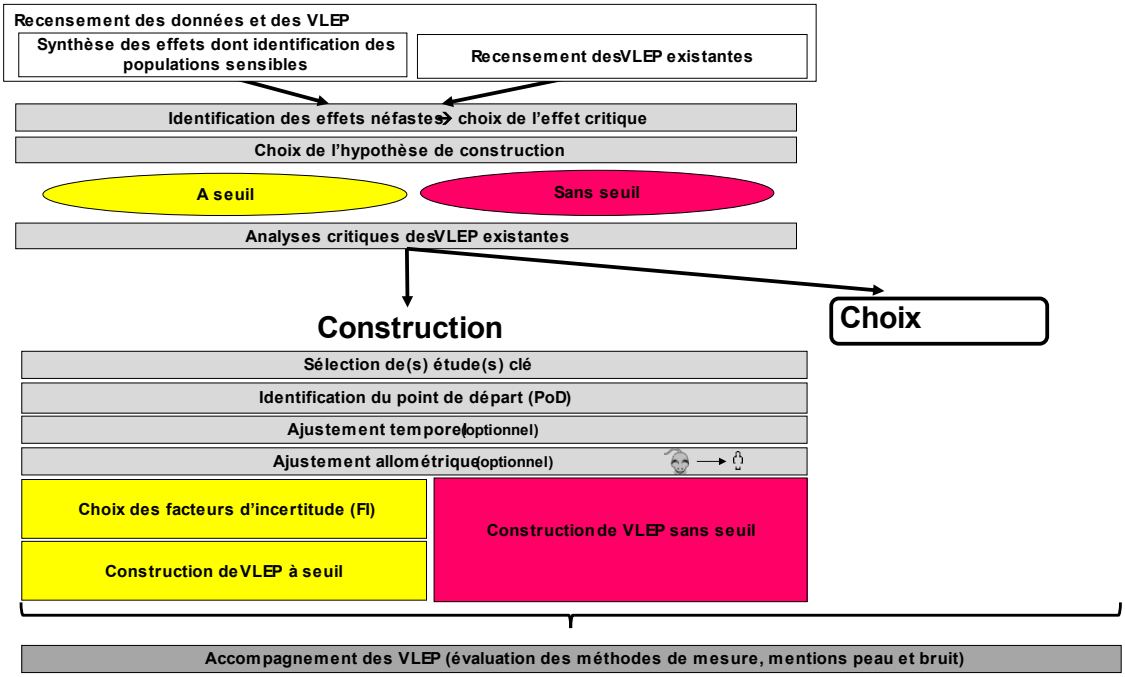


Figure 1 : Schéma des différentes étapes de construction d'une VLEP

Dans le cadre de l'élaboration de VLEP, une valeur pragmatique peut être proposée en l'absence de donnée pour calculer une VLEP-8h à partir de la VLCT-15 min et inversement.

[3] Valeur limite de la moyenne de la concentration atmosphérique d'un agent chimique prélevé dans la zone de respiration d'un travailleur pondérée par la durée d'un poste de travail c'est-à-dire 8 heures. Dans l'état actuel des connaissances scientifiques, la VLEP-8h est censée protéger d'effets sur la santé à moyen et long termes les travailleurs exposés régulièrement et pendant la durée d'une vie de travail à l'agent chimique considéré.

[4] Valeur limite de la moyenne de la concentration atmosphérique d'un agent chimique prélevé dans la zone de respiration d'un travailleur pondérée sur une période de référence de 15 minutes. Cette concentration est mesurée pendant le pic d'exposition et ce, quelle que soit sa durée. Elle vise à protéger la santé des travailleurs des effets toxiques aigus en limitant l'intensité des pics d'exposition ou certains effets à long terme dus à la répétition d'expositions de courtes durées.

[5] Valeur limite de la concentration atmosphérique d'un agent chimique dans la zone de respiration d'un travailleur, qui ne doit être dépassée à aucun moment de la période de travail.
La valeur plafond s'applique aux agents chimiques pour lesquels le profil toxicologique montre qu'une exposition peut entraîner, de façon instantanée, un effet grave et potentiellement irréversible et qui ne peut pas être contrôlé par l'application d'une VLEP-8h ou d'une VLCT-15min.

Une valeur plafond pragmatique peut également être déterminée à partir d'une VLCT-15min. La valeur pragmatique est proposée dans un objectif de prévention et n'est pas fondée sur une étude chez l'Homme ou l'animal (Anses, à paraître).

Le CES VSR évalue également la nécessité d'attribuer une mention « peau », lorsqu'une pénétration cutanée significative a été identifiée. Cette mention indique la nécessité de prendre en compte la voie cutanée lors de l'évaluation de l'exposition professionnelle, notamment au travers de la mise en œuvre d'une surveillance biologique des expositions et l'évaluation de la contamination surfacique au poste de travail. Son attribution rappelle également la nécessité de mettre en œuvre des mesures de prévention comme, par exemple, le port de gants de protection appropriés et la possibilité de vérifier la non contamination des milieux (prélèvements surfaciques) (Anses, à paraître).

Le CES VSR évalue également la nécessité ou non d'une mention « bruit » signalant un risque d'atteinte auditive en cas de co-exposition au bruit et à l'agent chimique en dessous des limites d'exposition recommandées, afin que les préventeurs mettent en place des mesures appropriées (collectives, individuelles et médicales) (Anses, à paraître).

Le CES VSR évalue les méthodes de référence applicables pour la mesure des niveaux d'exposition sur le lieu de travail. La qualité de ces méthodes et leur applicabilité à la mesure des expositions aux fins de comparaison à une VLEP sont évaluées et classées au regard des exigences de performances indiquées notamment dans la norme NF EN 482 (2021) : «Atmosphère des lieux de travail – Exigences générales concernant les performances des modes opératoires de mesurage des agents chimiques» et des critères de décision détaillés dans le rapport méthodologique (Anses, 2020).

Le classement de ces méthodes est réalisé de la manière suivante (cf. Figure 2) :

- catégorie 1A : méthodes validées (l'ensemble des critères de performance sont satisfaits) ;
- catégorie 1B : méthodes partiellement validées (les critères essentiels de performance sont satisfaits) ;
- catégorie 2 : méthodes indicatives (des critères essentiels de validation ne sont pas suffisamment explicités, ou bien la méthode nécessite des ajustements devant faire l'objet d'une validation) ;
- catégorie 3 : méthodes non recommandées car inadaptées (des critères essentiels de validation ne sont pas remplis)
- catégorie 3* : méthodes non validées car non évaluables (des critères essentiels de validation ne sont pas documentés).

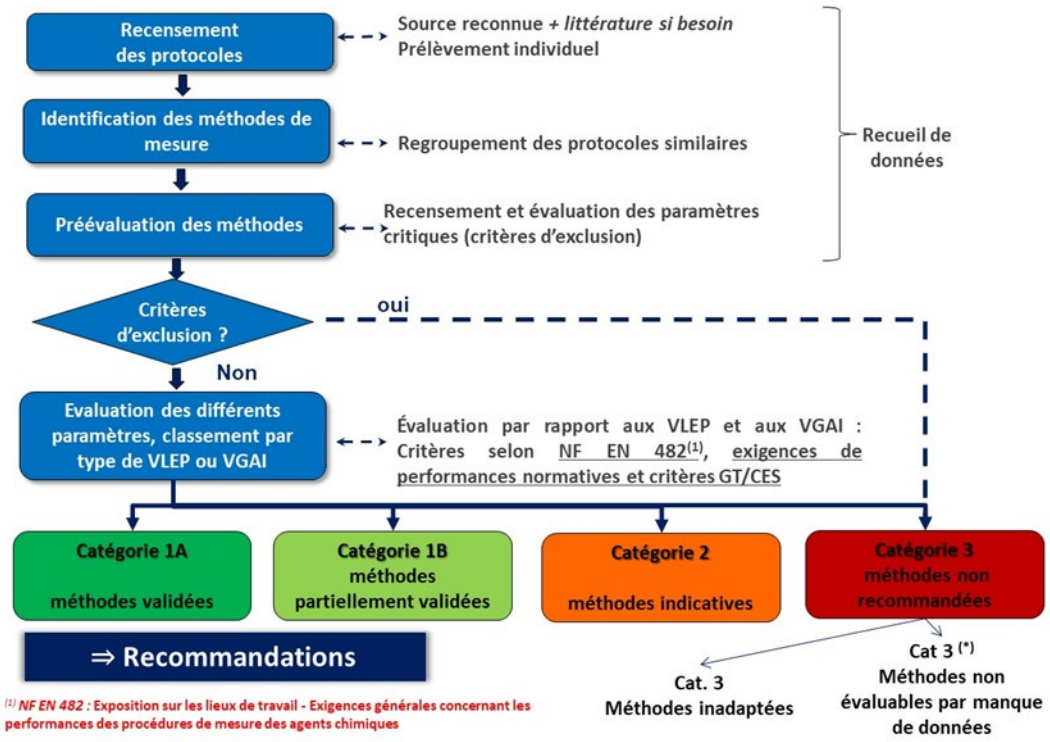


Figure 2 : Principe général de l'évaluation des méthodes de mesure dans l'air

Une étude comparative et détaillée des méthodes classées en catégorie 1A, 1B et 2 est réalisée au regard des différentes données de validation et de la faisabilité technique, de manière à recommander la ou les méthodes les plus appropriées pour la mesure des concentrations aux fins de comparaison aux VLEP.

3. ANALYSE ET CONCLUSIONS DU CES

3.1. Informations générales

L'acétaldéhyde est un aldéhyde aliphatique saturé qui existe à température ambiante sous forme de gaz incolore à l'odeur fruitée à faibles concentrations et piquante à fortes concentrations (Ruth et al. 1986 ; NTP 2011; CSSC 2012; WHO 1995; INRS 2022). Il est miscible à l'eau et à la plupart des solvants. Il est très inflammable et instable dans l'air (INRS 2022).

L'acétaldéhyde est produit à échelle industrielle à de nombreuses fins et utilisations (INRS 2022). Par exemple, il est utilisé comme intermédiaire dans la production d'acide acétique, d'acétate de cellulose, de dérivés de pyridine, de parfums, de peintures (colorants à l'aniline), de plastiques et de caoutchoucs synthétiques; pour le tannage du cuir et l'étamage des miroirs; comme dénaturant de l'alcool; comme identifiant dans les mélanges de carburants; comme durcisseur pour les fibres de gélatine; dans les produits à base de colle et de caséine; comme conservateur pour le poisson et les fruits; dans l'industrie papetière et en tant qu'agent aromatisant (ECHA 2016).

L'acétaldéhyde est un aldéhyde largement présent dans la nature. Il est formé lors de la combustion des matières organiques : il est ainsi présent dans la fumée de tabac (Health Council of the Netherlands 2014), dans les fumées de cuisson… Il se forme également de manière endogène chez l'Homme en petites quantités, par exemple, lors de la décomposition

de l'éthanol dans le corps. Il est, en outre, présent dans les matériaux de construction, de décoration et d'ameublement et dans des produits de consommation courante (nettoyants pour sols, parquets, stratifiés, colles, lasures, décapants, carreaux et flocages, etc.).

3.2. Revue des recommandations scientifiques existantes en matière de valeurs limites d'exposition professionnelle

Le comité scientifique européen sur les valeurs limites d'exposition professionnelle (*Scientific Committee on Occupational Exposure Limits* ou SCOEL) et le comité d'évaluation des risques (*Risk Assessment Committee* ou RAC) de l'Agence européenne des produits chimiques (*European Chemicals Agency* ou ECHA) n'ont à ce jour pas émis de recommandation de VLEP pour l'acétaldéhyde.

Plusieurs VLEP recommandées par différents organismes scientifiques ont été recensées.

Dans les suppléments (suppléments aux rapports de 2000 et 2008) publiés en 2013, la DFG (*Deutsche Forschungsgemeinschaft*) a confirmé la valeur MAK (*Maximale Arbeitsplatz-Konzentration* ou concentration maximale sur le lieu de travail) de 50 ppm (soit 91 $mg.m^{-3}$ à 25°C) recommandée en 1982 (DFG 2013a et b). Par analogie avec le formaldéhyde, la DFG considère que des lésions tissulaires locales induites dans l'arbre respiratoire par l'exposition chronique à l'acétaldéhyde sont également une condition préalable au développement de tumeurs dans la muqueuse nasale des rats pour l'acétaldéhyde. Selon la DFG, le respect de la valeur MAK de 50 ppm, valeur à partir de laquelle aucune irritation et aucune lésion tissulaire locale de la muqueuse nasale n'ont été observées dans les études animales, devrait donc également protéger contre la formation de tumeurs. Dans le rapport de 2000, la DFG recommandait une valeur momentanée de 100 ppm (une valeur momentanée est une valeur qui ne doit être dépassée à aucun moment), confirmée dans le supplément de 2008. L'acétaldéhyde est classé dans la catégorie I de limite des pics, avec un facteur dit d'excursion de 1, qui, multiplié par la valeur MAK, donne la concentration maximale moyennée admissible des pics sur une durée d'échantillonnage de 15 minutes.

Aux Etats-Unis, l'ACGIH (*American Conference of Governmental Industrial Hygienists*) recommande depuis 1993 (confirmation en 2014) une valeur plafond de 25 ppm (soit 45 $mg.m^{-3}$ à 25°C) afin de prévenir l'irritation des voies oculaires et des voies respiratoires supérieures qui se produit entre 25 et 50 ppm (ACGIH® 2018). L'ACGIH a estimé que la prévention de ces effets par la valeur plafond protégerait contre les effets cancérigènes. L'ACGIH n'a pas recommandé de mention spécifique.

La société japonaise pour la santé au travail (JSOH) a recommandé en 2021 (et confirmé en 2022) une valeur limite plafond (OEL-Ceiling) de 10 ppm (soit 18 $mg.m^{-3}$ à 25 °C) pour l'acétaldéhyde (JSOH 2021). Cette valeur est basée sur l'irritation oculaire observée chez l'Homme à des concentrations de 50 ppm pendant 15 minutes (Silverman *et al.* 1946) et 4 heures (Muttray *et al.* 2009) et la possibilité d'une légère altération du transport mucociliaire dans les voies respiratoires supérieures chez les rats exposés à 150 ppm pendant 6 heures par jour, 5 jours/semaine pendant 13 semaines dans l'étude de Dorman *et al.* de 2008 (Nomiyama 2021). Cette OEL-Ceiling devrait prévenir les effets observés sur les yeux et les voies respiratoires supérieures, compte tenu de la sensibilité plus élevée due au métabolisme plus faible de l'acétaldéhyde chez environ 40 % de la population japonaise, qui a le génotype *ALDH2*2.*

3.3. Synthèse des données toxicologiques

Le profil toxicologique de l'acétaldéhyde a été élaboré à partir du rapport de l'Anses publié en 2014 et des rapports produits par les institutions nationales et internationales (DFG 2013, ACGIH® 2014, INRS 2022). Une recherche bibliographique complémentaire a été effectuée dans les bases de données PubMed® et Scopus® afin de prendre en compte la littérature scientifique parue à partir de septembre 2022 jusqu'en octobre 2023. Les détails de la recherche bibliographique (requête, principaux mots-clés, critères d'inclusion et d'exclusion) sont décrits dans l'annexe 1 du rapport.

Les études décrites dans le présent avis sont principalement des études par voie respiratoire ou consécutives à une exposition cutanée à de l'acétaldéhyde. Quelques études par voies orale et intrapéritonéale sont également mentionnées.

Les conversions de ppm en $mg.m^{-3}$ des concentrations testées dans les études ont été effectuées à 25°C.

3.3.1. Toxicocinétique

Des études toxicocinétiques menées chez l'Homme et l'animal (rat) montrent que l'absorption de l'acétaldéhyde et son passage dans la circulation systémique sont limités (Anses 2014).

Les données disponibles montrent qu'une grande partie de l'acétaldéhyde inhalé est retenue au point de contact, se liant rapidement et irréversiblement aux protéines libres et aux molécules de thiol (ex. cystéine, glutathion) (Anses 2014 ; Serio and Gudas 2020).

Aucune donnée concernant la distribution de l'acétaldéhyde après une exposition chez l'Homme n'a été identifiée dans la littérature. Chez des rats exposés à de l'acétaldéhyde gazeux (débit de 1 $L.min^{-1}$ pendant 1 heure et passage d'air à travers 400 mL d'une solution aqueuse d'acétaldéhyde à 2,5 % dans une boîte hermétique), l'acétaldéhyde était détectable dans le sang, le foie, les reins, la rate, le cœur et les muscles squelettiques (seuls organes étudiés) (Hobara et al. 1985). L'acétaldéhyde semble traverser facilement la barrière hémato-encéphalique et peut être partiellement transféré du sang maternel au sang fœtal (CEPA 1993; Blakley and Scott 1984).

Les principales voies métaboliques sont la conjugaison à des groupes thiols au site de contact (cystéine, glutathion, etc.) et l'oxydation par l'aldéhyde déshydrogénase (ALDH) dépendante du NAD[6] qui métabolise rapidement l'acétaldéhyde en acide acétique, principalement dans le foie.

Il n'existe pas de donnée humaine concernant l'élimination de l'acétaldéhyde et les données animales sont très limitées. L'acétaldéhyde est éliminé principalement sous forme d'acétate dans l'urine (ECHA 2016).

Il existe plusieurs modèles PBPK (*physiologically based pharmacokinetic model*) chez l'Homme et/ou le rat pour l'acétaldéhyde. Cependant, ceux-ci apportent principalement des informations sur la distribution et le métabolisme de l'acétaldéhyde mais en tant que métabolite de l'éthanol.

[6] nicotinamide-adénine-dinucléotide

3.3.2. Toxicité aiguë

Les principaux effets observés chez l'Homme après une exposition à des vapeurs d'acétaldéhyde sont l'irritation oculaire (90 $mg.m^{-3}$), cutanée, et des voies respiratoires supérieures et inférieures (527 $mg.m^{-3}$) allant jusqu'à une bronchoconstriction chez les personnes asthmatiques.

- **Effets respiratoires et pulmonaires et irritation des yeux**

Plusieurs études sur des volontaires sains ou asthmatiques ont été menées sur des périodes d'exposition courtes.

Tableau 1 : Résumé des études exposant de manière aiguë des volontaires à l'acétaldéhyde

Nombre de volontaires	Concentration en acétaldéhyde de l'aérosol et durée d'exposition	Résultats observés	Référence
Volontaires sains (n = 12/sexe)	45, 90, 360 $mg.m^{-3}$ (25, 50, 200 ppm) 15 minutes	Irritation oculaire subjective à 90 $mg.m^{-3}$ (50 ppm) et chez plusieurs individus en dessous de 45 $mg.m^{-3}$ (25 ppm)	(Silverman, Schuttle and First 1946)
Volontaires sains (n = 14)	241 $mg.m^{-3}$ (134 ppm) 30 minutes	Légère irritation subjective dans les voies respiratoires supérieures	(Sim and Pattle 1957)
Volontaires sains (n = 20)	45, 90, 360 $mg.m^{-3}$ (25, 50, 200 ppm) 4 heures	Pas d'effet sur la muqueuse nasale à 50 ppm	(Muttray et al. 2009)
Volontaires asthmatiques japonais (n = 9 volontaires asthmatiques vs 9 témoins)	140, 280, 560, 1120 $mg.m^{-3}$ (77,8, 155,6, 311,2, 622,4 ppm) 2 minutes	PC20*: 565 $mg.m^{-3}$ (314 ppm)	(Myou et al. 1993)
Volontaires asthmatiques japonais (n = 9)	140, 280, 560, 1120 $mg.m^{-3}$ (77,8, 155,6, 311,2, 622,4 ppm) 2 minutes	PC20*: 651 $mg.m^{-3}$ (362 ppm)	(Myou et al. 1994)
Volontaires asthmatiques japonais sensibles à l'éthanol (n = 10)	1,12 ; 2,24 ; 4,48 ; 8,68 ; 17,64 ; 35 ; 70 ; 140 ; 280 ; 1120 ; 2240 $mg.m^{-3}$ (0,62 ; 1,24 ; 2,48 ; 4,82 ; 9,80 ; 19,44 ; 38,89 ; 77,78 ; 155,56 ; 622,22 ; 1244,44 ppm) 2 minutes	PC20*: 588 $mg.m^{-3}$ (327 ppm)	(Fujimura et al. 1999)
Volontaires asthmatiques japonais tolérants à l'éthanol (n = 16)		PC20*: 900 $mg.m^{-3}$ (500 ppm)	

Volontaires asthmatiques caucasiens (n = 61 vs 20 témoins)	140, 280, 560, 1120 $mg.m^{-3}$ (77,8, 155,6, 311,2, 622,4 ppm) 2 minutes	PC20*: 142 $mg.m^{-3}$ (79 ppm)	(Prieto et al. 2000)
Volontaires asthmatiques caucasiens (n = 16)	80 à 2560 $mg.m^{-3}$ (44 à 1422 ppm) 2 minutes	PC20*: 692 ppm (1246 $mg.m^{-3}$)	(Prieto et al. 2002a)
Volontaires asthmatiques caucasiens (n = 16), volontaires pour la rhinite allergique (n = 43), Volontaires sains (n = 19)	80 à 2560 $mg.m^{-3}$ (44 à 1422 ppm) 2 minutes	PC20*: 2166 $mg.m^{-3}$ (1203 ppm) chez volontaires avec rhinite 1136 $mg.m^{-3}$ (631 ppm) chez les volontaires asthmatiques 2560 $mg.m^{-3}$ (1422 ppm) chez les volontaires sains	(Prieto et al. 2002b)

* PC20 : concentration entrainant une diminution de 20% du volume expiratoire maximal par seconde (VEMS) ; Les valeurs indiquées correspondent aux moyennes géométriques exprimées en ppm ou en $mg.m^{-3}$ d'acétaldéhyde.

Chez l'Homme, l'exposition à des concentrations inférieures à 25 ppm (45 $mg.m^{-3}$) pendant 15 minutes a provoqué une irritation sensorielle oculaire chez des volontaires sains (Silverman, Schuttle and First 1946). L'exposition de 2 à 4 minutes à 12,5 ppm (22,4 $mg.m^{-3}$) d'acétaldéhyde sous forme d'aérosol a potentialisé l'hyperréactivité bronchique au test de provocation à la méthacholine chez des volontaires asthmatiques (Myou et al. 1994). L'inhalation d'un aérosol de solution aqueuse d'acétaldéhyde chez des adultes asthmatiques a provoqué une bronchoconstriction à partir de 79 ppm (142 $mg.m^{-3}$) (Prieto et al. 2000).

Chez l'animal, la toxicité aiguë de l'acétaldéhyde est faible, avec des valeurs de concentrations létales (CL50) comprises entre 24 et 31 $g.m^{-3}$ (Kruysse 1970 cité dans OEHHA 2008 ; Appelman, Woutersen and Feron 1982). Les principaux symptômes observés sont une baisse de la fréquence respiratoire, une augmentation du rythme cardiaque et de la pression artérielle, une protéinurie et un œdème pulmonaire et, aux doses les plus fortes, une dépression du système nerveux central. Des altérations histopathologiques sont également induites dans l'arbre respiratoire, principalement au niveau de la cavité nasale, chez le rat (Santé Canada 2000).

- **Irritation oculaire**

L'application de 0,04 mg d'acétaldéhyde sur les yeux de lapin a causé une irritation grave, mais aucun détail n'a été rapporté (CERI 2007).

- **Irritation cutanée**

Chez l'Homme, le contact direct de l'acétaldéhyde sous forme liquide provoque une irritation cutanée (Von Burg and Stout 1991). Des érythèmes ont été observés lors de la réalisation de patchs tests sur des volontaires sains exposés à différentes concentrations d'acétaldéhyde (Haddock and Wilkin 1982 ; Wilkin and Fortner 1985 cités dans DFG 2013b). Néanmoins, ces études comportent des limites (patchs tests non-occlusifs, véhicule non spécifié, type de patch test non précisé, *etc.*). Selon le comité scientifique pour la sécurité des consommateurs

(CSSC), des concentrations d'acétaldéhyde supérieures à 1 % sont susceptibles d'être irritantes pour la peau humaine (CSSC 2012). Le CSSC rapporte que, dans une étude menée sur des lapins selon la ligne directrice 404 de l'OCDE, l'acétaldéhyde ne s'est pas avéré être irritant pour la peau. Dans une autre étude menée non conformément à la ligne directrice 404 de l'OCDE, le CSSC rapporte que l'application de 500 mg d'acétaldéhyde a provoqué une légère irritation de la peau chez la même espèce sans fournir plus de détails (CSSC 2012).

- **Sensibilisation respiratoire et cutanée**

L'acétaldéhyde n'est pas classé comme sensibilisant chez l'Homme selon le règlement CLP.

Aucune étude sur le caractère sensibilisant de l'acétaldéhyde par inhalation n'a été identifiée dans la littérature. Seules des données sur la sensibilisation cutanée sont disponibles.

Sur la base de l'ensemble des données disponibles chez l'Homme et l'animal, le CSSC a conclu en 2012 que les preuves d'un potentiel de sensibilisation cutanée de l'acétaldéhyde, sont limitées (CSSC 2012).

3.3.3. Effets subchroniques et chroniques

Les effets associés à l'exposition humaine par inhalation à long terme à l'acétaldéhyde sont peu documentés. La seule étude épidémiologique identifiée dans la littérature, relative à la pollution de l'air intérieur, n'a pas permis d'établir de lien entre l'exposition à l'acétaldéhyde et la survenue d'effets respiratoires (Billionnet et al. 2011).

Les différentes études conduites chez l'animal indiquent que l'exposition par voie respiratoire à l'acétaldéhyde induit des altérations non-néoplasiques incluant des dégénérescences et des hyperplasies des épithéliums du tractus respiratoire chez le rat, la cavité nasale semblant être la cible principale après une exposition par inhalation à l'acétaldéhyde. A noter que la muqueuse nasale olfactive semble être plus sensible que la muqueuse nasale respiratoire aux effets de l'acétaldéhyde (Anses 2014).

Ainsi, les études animales disponibles ont permis d'identifier :

- une LOAEC comprise entre 150 et 750 ppm (270 et 1 350 $mg.m^{-3}$) et une NOAEC comprise entre 50 et 150 ppm (90 et 270 $mg.m^{-3}$) sur la base de la dégénérescence (et de l'hyperplasie) de l'épithélium olfactif chez le rat ;
- une LOAEC comprise entre 1 340 et 1 650 ppm (2 411 et 2 970 $mg.m^{-3}$) et une NOAEC de 390 ppm (702 $mg.m^{-3}$) sur la base de la dégénérescence de l'épithélium olfactif chez les hamsters.

3.3.4. Effets sur la reproduction et le développement

Chez l'Homme, aucune étude sur les effets sur la reproduction et le développement suite à une exposition par voie respiratoire n'a été identifiée.

Chez l'animal, les différentes études évaluant les effets de l'acétaldéhyde sur le développement visent principalement à renseigner le rôle de l'acétaldéhyde dans la tératogénicité induite par l'éthanol dont il est le principal métabolite (Anses 2014). Dans ces études, l'exposition à l'acétaldéhyde par voie parentérale ou amniotique chez les souris et les rates gestantes a induit des malformations et des résorptions fœtales. Aucune de ces études n'est conduite par voie respiratoire.

3.3.5. Effets génotoxiques

Chez l'Homme, les études de génotoxicité sont relativement limitées et visent à évaluer les effets de l'éthanol liés à la consommation de boissons alcoolisées. Ces études montrent que l'acétaldéhyde a un potentiel génotoxique.

In vitro, l'acétaldéhyde induit des mutations génétiques et est donc mutagène. De plus, il induit des cassures de brins d'ADN et a donc un mécanisme d'action clastogène. Il génère des adduits à l'ADN, des réticulations ADN-protéines, à la fois dans les cellules de rongeurs et les cellules humaines en l'absence d'activation métabolique. L'acétaldéhyde induit également des dommages à l'ADN, comme le montrent les tests des comètes, des micronoyaux, et les essais d'aberrations chromosomiques.

In vivo, il existe très peu d'études sur la génotoxicité de l'acétaldéhyde et encore moins par inhalation.

L'exposition intrapéritonéale à l'acétaldéhyde entraîne une augmentation des micronoyaux et de l'échange de chromatides sœurs à des doses très élevées. L'étude de Kunugita ***et al.*** montre que l'acétaldéhyde a un potentiel génotoxique par inhalation (augmentation des micronoyaux, augmentation des mutations) chez les souris Aldh2 Knock Out, mais pas chez les souris de type sauvage traitées à l'acétaldéhyde (500 ppm, 2 semaines par inhalation et 100 $mg.kg^{-1}$, une fois par jour pendant 2 semaines par voie orale) (Kunugita et al. 2008).

Comme pour le formaldéhyde (et d'autres aldéhydes), in vivo, les effets génotoxiques de l'exposition respiratoire à l'acétaldéhyde sont toujours associés à des lésions d'irritation des voies respiratoires, en particulier des fosses nasales : hyperplasie, métaplasie et dégénérescence de l'épithélium olfactif et à un moindre degré de l'épithélium respiratoire. La nécrose des cellules épithéliales induit une prolifération cellulaire régénérative.

Selon Budinsky et al., l'événement clé des effets génotoxiques et cytotoxiques de l'acétaldéhyde implique une base de Schiff intermédiaire dans la formation de pontages ADN-protéines (Budinsky et al. 2013). La réparation de l'ADN, l'apoptose et d'autres réponses adaptatives liées au stress, ainsi que le remplacement des protéines ou la redondance de la fonction protéique sont des mécanismes de protection contre ces effets génotoxiques.

3.3.6. Effets cancérogènes

Aucune étude épidémiologique sur la cancérogénicité de l'acétaldéhyde n'a été identifiée.

Les études de cancérogénicité chez les rongeurs montrent que les épithéliums olfactif et respiratoire sont les principales cibles de l'acétaldéhyde après une exposition respiratoire répétée. Les données disponibles sont relativement limitées et anciennes.

Il n'a pas été identifié de données récentes sur la cancérogénicité de l'acétaldéhyde.

Les classifications de l'US EPA (catégorie B2 : probablement cancérogène pour l'Homme en 1991) et du CIRC (groupe 2B : possiblement cancérogène pour l'Homme en 1999)) sont basées principalement sur 4 études : (Feron 1979; Feron, Kruysse, and Woutersen 1982; Woutersen et al. 1984,1986). L'exposition par inhalation à l'acétaldéhyde provoque des tumeurs nasales chez les rats et des tumeurs laryngées chez les hamsters.

Dans les études de Woutersen *et al.*, des rats Wistar mâles et femelles (n = 105/sexe/groupe) ont été exposés à 0, 735, 1 412 ou 3 000 ppm (0, 1 322, 2 541 ou 5 400 $mg.m^{-3}$) d'acétaldéhyde, 6 heures par jour et 5 jours par semaine, pendant 28 mois (Woutersen et al. 1984, 1986; Woutersen and Feron 1987). La concentration la plus élevée a été progressivement réduite à 977 ppm (1758 $mg.m^{-3}$), en raison d'un retard de croissance sévère, d'une perte de poids corporel et d'une mortalité précoce. Les rats survivants ont été euthanasiés et autopsiés à la fin de l'étude (semaines 120-122) ; les rats morts au cours de l'étude ont également été autopsiés. L'incidence de l'adénocarcinome de l'épithélium respiratoire nasal a augmenté de manière significative dans tous les groupes traités, quel que soit le sexe. L'incidence du carcinome épidermoïde de l'épithélium respiratoire nasal était significativement élevée chez les rats mâles aux deux plus fortes concentrations. Chez les femelles, une augmentation statistiquement significative de ces tumeurs n'a été observée qu'à la plus forte concentration. Aucune augmentation de l'incidence tumorale n'a été observée, ni dans le larynx, ni dans les poumons (Woutersen et al. 1984, 1986; Woutersen and Feron 1987). De nouveaux cas d'adénocarcinome nasal et de carcinome épidermoïde nasal ont été observés au cours d'une période de récupération de 26 à 52 semaines, après l'arrêt de l'exposition (Woutersen and Feron 1987). Cette étude présente des limites : taux de mortalité élevé, animaux des deux sexes morts avant la fin de l'exposition à la plus forte concentration, seuls quelques animaux survivants à la fin de l'étude à 1412 ppm, concentrations testées très élevées.

Deux études plus anciennes réalisées chez des hamsters ont montré une augmentation de l'incidence des tumeurs nasales et laryngées (Feron 1979; Feron, Kruysse and Woutersen 1982).

D'un point de vue mécanistique, les données disponibles indiquent que les effets cancérogènes de l'acétaldéhyde sur l'arbre respiratoire surviennent à des niveaux d'exposition supérieurs à ceux induisant des effets irritants et cytotoxiques, ce qui est en faveur d'un effet cancérogène à seuil, démontré pour d'autres aldéhydes, en particulier le formaldéhyde. L'induction de tumeurs résulte de la conjugaison des effets génotoxiques de l'aldéhyde et de la prolifération cellulaire secondaire à ses effets cytotoxiques.

3.3.7. **Populations sensibles**

Des études menées chez des volontaires ont montré que les personnes ayant déjà eu une hyperréactivité bronchique (personnes atteintes de rhinite ou d'asthme dans ces études) sont plus sensibles aux effets irritants respiratoires de l'acétaldéhyde inhalé que les sujets sains (Myou et al. 1993; Myou et al. 1994; Fujimura et al. 1999; Prieto et al. 2000; Prieto et al. 2002a; Prieto et al. 2002b).

L'ensemble des éléments suggère que les populations présentant des maladies respiratoires (asthme, rhinite allergique) ont une sensibilité exacerbée aux effets irritants respiratoires de l'acétaldéhyde.

3.4. Éléments de proposition pour fixer des VLEP

Conformément au document méthodologique de l'Anses (Anses, à paraître), le CES VSR estime qu'il est justifié de préconiser une VLEP-8h et une VLCT-15min pour l'acétaldéhyde. La VLEP-8h recommandée vise à protéger les travailleurs contre les effets à long terme et une VLCT-15min contre les effets à court terme.

3.4.1. Valeur limite d'exposition professionnelle sur 8 heures (VLEP-8h)

3.4.1.1. Choix de l'effet critique

Aucun effet systémique n'a été rapporté suite à une exposition par inhalation ou par voie cutanée à l'acétaldéhyde, chez l'Homme ou l'animal.

Les effets documentés après une exposition par inhalation à l'acétaldéhyde sont des effets irritants et cancérogènes pour l'arbre respiratoire (cf. §3.3).

Aucune étude épidémiologique récente sur la cancérogénicité n'a été identifiée dans la littérature.

Des tumeurs nasales chez le rat et des tumeurs du larynx chez les hamsters ont été rapportées à la suite d'une exposition à des vapeurs d'acétaldéhyde à des concentrations de 735 ppm (1322 $mg.m^{-3}$) et plus (Woutersen et al. 1986), et chez des hamsters exposés à 1 650 ppm (2970 $mg.m^{-3}$) (Feron, Kruysse and Woutersen 1982). Cependant, il convient de noter que la concentration la plus faible testée (750 ppm) est très élevée, et que la mortalité élevée observée dans les deux études rend difficile l'interprétation des données.

L'analyse du mécanisme d'action de la cancérogénicité indique une survenue des effets cancérogènes à des niveaux d'exposition supérieurs à ceux induisant une cytotoxicité et la nécessité pour leur production, d'une prolifération cellulaire régénérative, secondaire aux effets cytotoxiques, associée aux effets génotoxiques. Les effets cancérogènes de l'acétaldéhyde sont probablement à seuil de dose, comme cela est établi pour d'autres aldéhydes. La prévention des effets irritants, préalable nécessaire à la production des effets cancérogènes, préviendrait aussi ces derniers.

Le CES VSR retient l'existence d'un seuil pour la survenue de cancer des fosses nasales (cytotoxicité associée à la prolifération régénérative, génotoxicité aux doses cytotoxiques).

Sur la base de l'ensemble des données disponibles, l'altération de l'épithélium olfactif est retenue comme effet critique pour l'élaboration d'une VLEP-8h. Le CES VSR considère que protéger de la survenue d'une irritation prolongée et d'une dégénérescence de l'épithélium nasal protégera de la survenue du cancer des fosses nasales.

3.4.1.2. Choix de l'étude clé

Conformément à la méthodologie de l'Anses pour l'élaboration de VLEP (Anses, à paraître), à qualité égale, les données humaines sont considérées comme plus pertinentes que les données animales pour l'établissement d'une VLEP-8h. Cependant, aucune étude humaine chronique pertinente n'est disponible.

La plupart des études sur les animaux ont été réalisées sur des périodes d'exposition trop courtes pour être considérées comme pertinentes pour l'établissement d'une VLEP-8h. Parmi les études subchroniques disponibles (Kruysse, Feron and Til 1975; Feron 1979; Feron Kruysse, and Woutersen 1982; Woutersen et al. 1984; Dorman et al. 2008), l'étude de Dorman *et al*. est retenue comme l'étude la plus pertinente et la plus robuste (score de Klimisch 1) mettant en évidence l'effet critique retenu observé aux doses les plus faibles (Dorman et al. 2008).

Cette étude a investigué les effets de l'exposition à l'acétaldéhyde par inhalation sur les voies respiratoires supérieures (histopathologie, prolifération cellulaire) chez le rat. Des rats Fisher F344 (n = 60/concentration) ont été exposés à de l'acétaldéhyde à des concentrations de 0,

50, 150, 500 ou 1500 ppm (0, 90, 270, 900, 2700 mg.m^{-3}), 6 heures par jour, 5 jours par semaine pendant 13 semaines. À 1500 ppm, des effets ont été observés sur l'épithélium respiratoire (hyperplasie et métaplasie des narines jusqu'au tissu olfactif dorsal). À des concentrations égales et allant jusqu'à 500 ppm, des effets ont été observés sur les épithélia respiratoire et olfactif (métaplasie et hyperplasie minimes à modérées) et sur l'épithélium du larynx (métaplasie des cellules squameuses minimes à modérées). Les modifications histopathologiques les plus précoces étaient des pertes neuronales dans l'épithélium olfactif, qui ont été observées dès 150 ppm. La gravité et la distribution de ces lésions augmentaient avec la durée et la concentration de l'exposition.

Le CES VSR retient donc l'étude de 2008 de Dorman *et al.* comme étude clé pour l'élaboration de la VLEP-8h. Il s'agit en effet de l'étude la plus robuste disponible par inhalation, avec la durée d'exposition la plus longue (13 semaines).

3.4.1.3. Choix du point de départ (PoD)

Dans l'étude de Dorman *et al*., une dégénérescence de l'épithélium olfactif a été observée chez le rat à partir de 150 ppm. Cette concentration est donc considérée comme une LOAEC et la concentration de 50 ppm (90 mg.m^{-3}) comme une NOAEC (Dorman et al. 2008). En l'absence de résultat quantifié à une concentration de 50 ppm, il n'a pas été possible d'effectuer une modélisation de la dose repère (BMD).

Le CES VSR retient la NOAEC de 50 ppm (90 mg.m^{-3}) comme point de départ pour la construction de la VLEP-8h.

3.4.1.4. Ajustement temporel

Dans l'étude de Dorman *et al.,* des rats F344 ont été exposés pendant 13 semaines (6h/j, 5j/semaine) par inhalation. L'acétaldéhyde étant un irritant qui induit des lésions tissulaires de l'épithélium olfactif par expositions répétées, et afin de tenir compte de la différence de temps d'exposition entre les animaux (6h/jour) et les travailleurs (8h/jour), un ajustement temporel est effectué comme suit :

$$NOAEC_{ADJ} = NOAEC \times 6h/8h = (90 \times 6)/8 = 67{,}5 \text{ mg.m}^{-3} \text{ (soit } 37{,}5 \text{ ppm)}$$

3.4.1.5. Ajustement dosimétrique

Une NOAEC équivalente chez l'Homme ($NOAEC_{HEC}$) a été calculée sur la base de la $NOAEC_{ADJ}$ dérivée de l'étude clé pour tenir compte des différences dosimétriques entre l'animal (rat) et l'Homme.

L'acétaldéhyde étant considéré comme un gaz de catégorie 1 (US EPA 1994b; US EPA 2009), la formule suivante s'applique pour le calcul de la NOAEC équivalente humaine :

$$NOAEC_{ADJ\ HEC} = NOAEC_{ADJ} \times \text{Regional Gas Dose Ratio} = NOAEC \times (V_A/SA_A)/(V_H/SA_H)$$

Avec $NOAEC_{ADJ\ HEC}$ = NOAEC équivalent chez l'Homme

$NOAEC_{ADJ}$ = NOAEC ajusté chez l'animal

V_A = taux de ventilation chez le rat = 0,20 m^3/jour

SA_A = surface de la région extra thoracique chez le rat = 15 cm^2

V_H = taux de ventilation chez le travailleur = 10 m^3/jour

SA_H = surface de la région extra thoracique chez l'Homme= 200 cm^2

$$\mathbf{NOAEC_{ADJ\ HEC}} = 67,5 \times [(0,2/15) / (10/200)] = \mathbf{17,6\ mg.m^{-3}\ (10\ ppm)}$$

3.4.1.6. Choix des facteurs d'incertitude

Conformément au guide méthodologique (Anses, à paraître), les facteurs d'incertitude (FI) suivants sont retenus :

- variabilité inter-espèces : $\mathbf{FI_{A\text{-}TD} = 1}$

Pour tenir compte de la variabilité inter-espèce, un ajustement dosimétrique a été réalisé afin de pouvoir disposer d'une concentration équivalente humaine. Comme le rat et l'Homme respirent différemment (par le nez et la bouche pour les humains et le 'nez' uniquement pour les rats), une exposition plus élevée des muqueuses respiratoires et olfactives est attendue chez les rats. En conséquence et comme le prévoit le guide méthodologique, aucun facteur d'incertitude supplémentaire n'est appliqué.

- variabilité intra-individuelle : $\mathbf{FI_H = \sqrt{10}}$

Un FI_H de $\sqrt{10}$ est retenu car l'étude clé a été réalisée sur des rongeurs et que peu de paramètres ont pu être évalués au regard des effets qui pourraient être observés chez l'Homme (par exemple, induction d'une hyperréactivité bronchique, motilité ciliaire dans les voies respiratoires supérieures ou les bronches), la variabilité étant considérée comme moindre pour un effet irritant.

- utilisation d'une NOAEC : $\mathbf{FI_{L/B} = 1}$, le PoD étant une NOAEC

- transposition d'une exposition moyen terme à une exposition long terme : $\mathbf{FI_S = \sqrt{10}}$

En raison du manque de données sur les effets liés à une exposition chronique, une extrapolation à partir des effets subchroniques a été effectuée. La durée de l'étude clé retenue, considérée en toxicologie comme « subchronique » (les animaux ayant été exposés 5 jours par semaine pendant 13 semaines), correspond à environ 10% de la vie des animaux, ce qui, chez l'Homme, correspondrait à environ 7 ans d'exposition selon les conventions. Comme le montre l'étude clé de 2008 de Dorman *et al*., les effets irritants peuvent être cumulatifs. Au début de l'exposition, seuls des dommages minimes à l'épithélium olfactif sont observés, puis sur une période plus longue, on s'attend à observer des effets irritants plus sévères (tels que nécrose, métaplasie, hyperplasie et signes d'inflammation). De même, il n'existe pas suffisamment de données pour évaluer si des effets similaires pourraient se produire à la suite d'une exposition chronique à des concentrations inférieures à celles testées dans les études subchroniques. De plus, d'autres effets, non observés dans les études d'exposition subchronique, pourraient survenir à long terme à la suite d'expositions répétées (maladies respiratoires chroniques, mutations répétées de l'ADN). Par conséquent, un FI_S de $\sqrt{10}$ est appliqué.

- qualité de la base de données : $\mathbf{FI_D = 1}$, la base de données étant importante.

Le CES VSR retient l'application d'un facteur d'incertitude global de 10.

3.4.1.7. Proposition de VLEP-8h

La VLEP-8h est calculée en faisant le rapport entre le PoD ajusté et le FI global.

VLEP-8h = $NOAEC_{ADJ\ HEC}$ /FI = 17,6 $mg.m^{-3}$/10 = 1,76 $mg.m^{-3}$ arrondi à 1,8 $mg.m^{-3}$ (soit 1 ppm)

Par conséquent, le CES VSR recommande une VLEP-8h de 1,8 $mg.m^{-3}$ (1ppm). En prévenant de l'irritation respiratoire, cette VLEP-8h devrait protéger de la survenue de cancer des voies respiratoires.

3.4.2. Valeur limite court terme sur 15 minutes (VLCT-15min)

3.4.2.1. Choix de l'effet critique

L'acétaldéhyde est un irritant respiratoire et oculaire qui, en raison de sa forte réactivité, exerce principalement sa toxicité au point d'entrée dans l'organisme. Les symptômes observés à la suite d'une exposition par voie aérienne comprennent une irritation des voies respiratoires supérieures et inférieures. Bien que les muqueuses nasales et oculaires semblent être les cibles les plus sensibles, les études disponibles sur ces paramètres ont été jugées de qualité insuffisante. Les seules études jugées de bonne qualité portent sur la bronchoconstriction (secondaire à une inhalation par la bouche, nez bouché) chez des asthmatiques (Prieto et al. 2000; Myou et al. 1993; Myou et al. 1994). L'hyperréactivité bronchique, correspondant à un effet d'irritation sensorielle et apparaissant plus tôt que la lésion des voies respiratoires, est choisie comme effet critique.

Au regard des données disponibles, la bronchoconstriction induite chez les asthmatiques, effet le plus sensible chez l'Homme, est retenue comme effet critique pour l'établissement de la VLCT-15min.

3.4.2.2. Choix de l'étude clé

Conformément au document méthodologique de l'Anses (Anses, à paraître), à qualité égale, les données humaines sont considérées comme plus pertinentes que les données animales.

Trois études suivant le même protocole expérimental ont montré une bronchoconstriction chez les asthmatiques (Prieto et al. 2000; Myou et al. 1993; Myou et al. 1994). Des groupes de volontaires sains et asthmatiques ont été exposés par inhalation à un aérosol d'acétaldéhyde (140, 280, 560, 1120 $mg.m^{-3}$) pendant 2 minutes. Les résultats ont montré une diminution dose-dépendante significative du VEMS (volume expiratoire maximal en 1 seconde) chez les volontaires asthmatiques (par rapport aux témoins sains)(Myou et al. 1993). Dans une autre étude, des volontaires asthmatiques d'origine japonaise ont été exposés par nébulisation à une concentration ne provoquant pas de bronchoconstriction d'une solution saline de 0,8 mg/L d'acétaldéhyde. Le but de cette étude était de déterminer si l'exposition à l'acétaldéhyde diminuait la dose de méthacholine induisant une diminution de 20 % du VEMS (PD20), autrement dit, induisait une hyperréactivité bronchique (Myou et al. 1994). Les études de Myou *et al.* n'ont pas été retenues pour la construction d'une VLCT-15 min en raison du faible nombre de sujets (n = 9) et parce qu'elles ne visaient pas à étudier la relation dose-réponse pour la bronchoconstriction induite par l'acétaldéhyde.

L'étude de Prieto *et al*. a décrit l'exposition de volontaires asthmatiques à l'acétaldéhyde pendant quelques minutes. Soixante et un sujets adultes asthmatiques (et 20 sujets témoins

non asthmatiques) ont été exposés dans des conditions expérimentales identiques à celles de l'étude de 1994 de Myou *et al*. (Prieto et al. 2000) afin d'étudier l'effet de l'exposition à l'acétaldéhyde sur la bronchoconstriction en mesurant la diminution du VEMS. Les résultats présentés dans l'article de Prieto *et al*. indiquent que la concentration atmosphérique d'acétaldéhyde induisant une diminution de 20% (PC20) du VEMS était de 142 $mg.m^{-3}$ (79 ppm). Cette étude est jugée comme présentant une bonne sensibilité : en effet, une bronchoconstriction a été observée, chez presque tous les sujets asthmatiques, alors qu'aucun volontaire sain n'a présenté cet effet. Bien que présentant certaines limites (courte durée d'exposition : 2 minutes, absence de mesure d'exposition individuelle), l'étude est jugée suffisamment robuste pour être retenue comme étude clé.

Le CES VSR retient l'étude de 2000 de Prieto *et al.* comme étude clé pour l'élaboration de la VLCT-15min.

3.4.2.3. Choix du point de départ (PoD)

Dans l'étude de 2000 de Prieto *et al*., la LOAEC correspond à une concentration d'acétaldéhyde dans l'air de 142 $mg.m^{-3}$ (79 ppm) (Prieto et al. 2000).

Le CES VSR retient la LOAEC de 142 $mg.m^{-3}$ (79 ppm) comme PoD pour l'établissement de la VLCT-15min.

3.4.2.4. Ajustement temporel

La toxicité des irritants sensoriels tels que l'acétaldéhyde est plutôt dépendante de la concentration que de la durée d'exposition (Belkebir et al, 2011). En effet, le mode d'action responsable des effets d'irritation sensorielle est similaire pour les agents chimiques donnant lieu à des effets d'irritation. Il dérive principalement de l'activation du réflexe d'alerte et de protection du corps face aux produits chimiques qui entrent en contact avec les muqueuses des yeux et des voies respiratoires. Ce réflexe est médié par le nerf trijumeau. Les effets sensoriels apparaissent à des doses plus faibles que l'irritation « lésionnelle », cette dernière induisant des dommages irréversibles. Par conséquent, aucun ajustement temporel n'a été appliqué à la LOAEC.

3.4.2.5. Choix des facteurs d'incertitude (FI)

Les FI suivants sont retenus (Anses, à paraître) :

- variabilité inter-espèces : $\mathbf{FI_A = 1}$ dans la mesure où l'étude clé utilisée pour établir la VLCT-15min est basée sur une population humaine et sur un sous-groupe particulièrement sensible (asthmatiques) ;

- variabilité intra-individuelle **:** $\mathbf{FI_H = 1}$ L'étude clé a été réalisée sur des individus particulièrement sensibles aux irritants respiratoires (asthmatiques). La protection de cette population protège également l'ensemble de la population de travailleurs. Les personnes asthmatiques sont connues pour présenter une hyperréactivité bronchique. Quand on utilise le test de provocation à la méthacholine pour mesurer la réactivité bronchique, les PD20 et PC20 (dose et concentration de méthacholine induisant une diminution de 20 % du VEMS) des asthmatiques, sont plus faibles que celles des sujets non asthmatiques. Les asthmatiques constituent donc une population sensible.

- utilisation d'une LOAEC : $\mathbf{FI_{L/B} = \sqrt{10}}$ pour tenir compte de l'utilisation d'une LOAEC plutôt que d'une NOAEC. Une valeur de √10 est retenue en raison de la nature de l'effet critique observé et de son observation dans une population sensible (bronchoconstriction chez des asthmatiques).

- qualité de la base de données : $\mathbf{FI_D = \sqrt{10}}$ pour tenir compte d'une exposition aux aérosols *via* une solution saline dans l'étude clé et des modalités expérimentales d'exposition à l'acétaldéhyde à l'aide d'un nébuliseur (aérosol salin) qui reste une technique habituelle dans les tests de dépistage de l'asthme.

Le CES VSR retient donc un facteur d'incertitude global de 10.

3.4.2.6. Proposition de la VLCT-15min

La VLCT-15min est calculée en faisant le rapport entre le PoD ajusté et le FI global.

$$VLCT\text{-}15min = NOAEC_{ADJ\ HEC} / FI = 142/10 = 14{,}2\ mg.m^{-3}\ (soit\ 7{,}9\ ppm)$$

Par conséquent, le CES VSR recommande une VLCT-15min de 14,2 $mg.m^{-3}$ (soit 7,9 ppm).

3.4.3. Mention « peau »

En raison de sa grande réactivité au site de contact, l'acétaldéhyde devrait se lier aux composés de l'épiderme et ne pas atteindre le derme, entraînant a priori un faible passage dans la circulation systémique. Une seule étude fournit des données quantitatives sur l'absorption cutanée de l'acétaldéhyde (Thredgold et al. 2020). Cependant, cette étude présente certaines limites et ne permet pas de déterminer la contribution de la voie cutanée à l'exposition globale des travailleurs.

En l'absence de données suffisantes sur la pénétration percutanée, la mention « peau » n'est pas recommandée pour l'acétaldéhyde.

3.4.4. Mention « bruit »

En l'absence de donnée sur d'éventuelles interactions lors de co-expositions au bruit et à l'acétaldéhyde, la mention « bruit » n'est pas recommandée pour l'acétaldéhyde.

3.5. **Conclusion et recommandations**

Type de VLEP		**VLEP-8h**	**VLCT-15min**
Valeurs		1,8 mg.m^{-3} (1 ppm à 25°C ; 0,9 ppm à 20°C)*	14,2 mg.m^{-3} (7,9 ppm à 25°C ; 7,1 ppm à 20°C)*
Population cible		Travailleurs	
Effet critique		Altération de l'épithélium olfactif	Bronchoconstriction
Etude clé	**Référence**	Dorman *et al.* 2008	Prieto *et al*. 2000
	Population de l'étude	Rats F344 adultes	Adultes asthmatiques
	Exposition (temps, voie)	13 semaines (6h/j, 5j/semaine), inhalation	2 minutes (nébulisation)
Point de départ (POD)		NOAEC = 90 mg.m^{-3} (50 ppm)	LOAEC = 4,72 mg.mL^{-1} = 142,3 mg.m^{-3} ** (soit 79ppm)
Ajustement temporel		$NOAEC_{ADJ}$ = 90 x 6/8 = 67,5 mg.m^{-3} (37,5 ppm)	Non
Ajustement dosimétrique		$NOAEC_{ADJ\ HEC}$ = $NOAEC_{ADJ}$ x RGDR = 17,6 mg.m^{-3} (10 ppm)	Non
Facteurs d'incertitude (FI)		10 $FI_{A\text{-}TD}$:1; FI_H: √10 ; $FI_{L/B}$: 1; FI_S: √10 ; FI_D: 1	10 FI_A:1; FI_H : 1 ; $FI_{L/B}$: √10 ; FI_D: √10
Mention peau		Non	Non
Mention bruit		Non	Non

***** Les directives européennes fixant les VLEP prennent en compte un facteur de conversion à 20 °C, les valeurs recommandées sont également exprimées à l'aide de ce facteur de conversion. 1 ppm (m/m^3) équivalent à 2 mg/m^3 à 20 °C

** considérant un nébulisateur Hudson 1720 : flux air par minute = 6 L.min^{-1} ; temps de nébulisation = 2 à 4 minutes ; sortie de la solution d'acétaldéhyde = 0,18 mL.min^{-1}

RGDR : Regional Gas Dose ratio

3.6. Eléments de proposition pour fixer une méthode de mesure

Trois méthodes de mesure de l'acétaldéhyde dans l'air des lieux de travail ont été identifiées et évaluées au regard de la VLEP-8h et de la VLCT-15 min :

- méthode A : Prélèvement actif sur support (tube de gel de silice ou filtre) imprégné de 2,4-dinitrophényl hydrazine (DNPH), désorption à l'acétonitrile, analyse par chromatographie en phase liquide et détection par spectrophotométrie UV/visible.
- méthode B : Prélèvement actif sur résine polymérique XAD-2® imprégnée de 2-(hydroxyméthyl)pipéridine (HMP), désorption au toluène, analyse par chromatographie en phase gazeuse et détection par détection spécifique de l'azote (NPD) ou ionisation de flamme (FID).
- méthode C : Prélèvement passif sur support imprégné de DNPH, désorption à l'acétonitrile, analyse par chromatographie en phase liquide et détection par spectrométrie UV.

Le tableau en Annexe 1 précise les protocoles de mesure recensés et présente l'évaluation de ces méthodes de mesure.

Parmi ces trois méthodes, la méthode A avec l'utilisation d'un tube de gel de silice imprégné de DNPH est classée en catégorie 1B pour le contrôle technique réglementaire de la VLEP-8h et de la VLCT-15 min et pour le suivi des expositions court terme. La plupart des données de validation sont en effet disponibles et satisfont aux exigences de la norme NF EN 482 mais la méthode doit être adaptée pour pouvoir mesurer sur 8 heures une concentration égale à 2 fois la VLEP-8h. L'élément limitant est, en effet, la quantité de DNPH disponible dans le tube de prélèvement pour piéger l'acétaldéhyde. Pour le suivi de la VLEP-8h, deux prélèvements consécutifs de 4 heures sont préconisés, la capacité des différents tubes étudiés étant légèrement insuffisante pour évaluer 2 fois la valeur de la VLEP-8h avec une sécurité sur la capacité de 75 % en un unique prélèvement de 8 heures. Deux prélèvements de 4 heures permettent d'évaluer des niveaux d'exposition jusqu'à 4 fois la valeur de la VLEP-8h tout en conservant une limite de quantification inférieure au dixième de la valeur limite. Toutefois les débits préconisés dans le tableau 2, prenant en compte la quantité de DNPH disponible pour le piégeage, doivent être respectés.

La méthode A mettant en œuvre le piégeage de l'acétaldéhyde sur un ou plusieurs filtres imprégnés de DNPH est quant à elle classée en catégorie 2 pour le contrôle technique réglementaire de la VLEP-8h et de la VLCT-15 min, ainsi que pour le suivi des expositions court-terme, du fait de l'incertitude sur la quantité réelle de DNPH disponible pour réagir avec l'acétaldéhyde et de l'efficacité de piégeage qui semble plus faible et variable que sur les tubes et cartouches. Les performances de ce mode de prélèvement mériteraient d'être précisées par des essais complémentaires avec un, deux, voire trois filtres placés dans la cassette de prélèvement.

La méthode B est également classée en catégorie 2 pour le contrôle technique réglementaire de la VLEP-8h et pour le suivi des expositions court-terme, et en catégorie 3 pour le contrôle technique réglementaire de la VLCT-15 min. Pour cette méthode, la plupart des données de validation sont disponibles et répondent aux exigences de la norme NF EN 482 mais ont été déterminées sur un domaine de concentration très largement supérieur à celui visé par la présente évaluation. Il est toutefois possible d'adapter la méthode en prélevant 24 litres au débit de 0,05 $L.min^{-1}$. Ce volume permet de couvrir une durée de 8 heures sans risque de dépasser la capacité de piégeage du tube. Pour une mesure de l'exposition professionnelle sur 15 minutes, l'adaptation du volume injecté dans l'analyseur, 2 µL en place de 1 µL, permet d'atteindre une sensibilité suffisante pour évaluer 0,5*VLCT-15 min sans toutefois parvenir au dixième.

Enfin, la méthode C est classée en catégorie 3 pour le contrôle technique réglementaire de la VLEP-8h et de la VLCT-15 min, ainsi que pour le suivi des expositions court terme. Les données des rapports du Laboratoire central de surveillance de la qualité de l'air (LCSQA) ne permettent pas de valider la méthode de prélèvement passif sur badge renfermant un support imprégné de DNPH, des critères de validation n'étant pas renseignés tels la conservation et la capacité des supports de prélèvement. De plus, pour un prélèvement de 15 minutes, la variation du débit de diffusion est trop importante pour envisager une mesure répondant aux critères de qualité énoncés dans l'EN 482.

Le CES recommande donc pour la mesure de l'acétaldéhyde dans l'air des lieux de travail, au regard des VLEP recommandées, la mise en œuvre de la méthode A, avec l'utilisation d'un tube de gel de silice imprégné de DNPH et en respectant les conditions d'utilisation précisées dans le tableau suivant.

Tableau 2 : Méthode de mesure recommandée pour la mesure de l'acétaldéhyde dans l'air des lieux de travail, au regard des VLEP recommandées

Méthode	**Prélèvement actif sur tube de gel de silice imprégné de DNPH – désorption à l'acétonitrile – analyse par chromatographie liquide détection UV/visible**	
Protocoles	NF ISO 16000-3 (2011) ; NF X 43 264 2011) ; INRS M-66 (2016) ; US EPA TO 11A (1999) ; NIOSH 2018 (2013) ; HSE MDHS 102 (2010), DFG Method 2 (1995)	
Pour le contrôle de la VLEP-8h	**Catégorie**	**1B**
	Conditions d'utilisation	Gel de silice imprégné au minimum de 1 mg de silice par tube Débit maximal recommandé en fonction de la quantité de DNPH : 0,5 $L.min^{-1}$ pour 5 mg 0,2 $L.min^{-1}$ pour 2 mg 0,1 $L.min^{-1}$ pour 1 mg Durée de prélèvement : 2x4 heures
Pour le contrôle de la VLCT-15min et le suivi des expositions court terme	**Catégorie**	**1B**
	Conditions d'utilisation	Gel de silice imprégné au minimum de 1 mg de silice par tube Débit maximal recommandé en fonction de la quantité de DNPH : 1 $L.min^{-1}$ pour 5 mg 0,8 $L.min^{-1}$ pour 2 mg 0,4 $L.min^{-1}$ pour 1 mg

4. CONCLUSIONS ET RECOMMANDATIONS DE L'AGENCE

Conformément aux conclusions de son Comité d'Experts Spécialisés (CES) « Valeurs Sanitaires de Référence », l'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail recommande la fixation d'une VLEP-8h de 1,8 $mg.m^{-3}$ (1 ppm) à 25°C et d'une VLCT-15min de 14,2 $mg.m^{-3}$ (7,9 ppm) à 25°C. En l'état des données disponibles, ni la mention « peau », ni la mention « bruit » ne sont recommandées.

Au regard de l'évaluation des méthodes de mesure de l'acétaldéhyde dans l'air des lieux de travail, l'Anses recommande la méthode consistant à effectuer un prélèvement actif sur un tube de gel de silice imprégné de DNPH, puis une désorption avec de l'acétonitrile et une analyse par chromatographie liquide avec détection UV/visible.

Cette méthode est validée et classée en catégorie 1B pour le contrôle réglementaire de la VLEP-8h, de la VLCT-15min et le suivi des expositions court-terme, à condition de respecter les conditions d'utilisation précisées dans le tableau 2.

L'Anses complète cette proposition de valeurs de référence en rappelant qu'au niveau européen, l'acétaldéhyde dispose d'une classification harmonisée comme cancérogène de catégorie 1B, c'est-à-dire dont le potentiel cancérogène pour l'être humain est supposé (ATP 13 – règlement (CE) n°1272/2008). A cet égard, des dispositions particulières prévues au

Code du travail s'appliquent aux agents chimiques cancérogènes, mutagènes et toxiques pour la reproduction. Parmi celles-ci, l'agence rappelle l'obligation « *de [réduire] l'utilisation de cet agent sur le lieu de travail, notamment en le remplaçant, dans la mesure où cela est techniquement possible…* », qui constitue une démarche prioritaire. Lorsque la substitution n'est pas possible, les dispositions, dont la limitation et le contrôle des expositions, ainsi que la surveillance médicale des personnes exposées doivent être mises en place.

Pr Benoit Vallet

MOTS-CLÉS

VLEP, valeurs limites, niveaux d'exposition, milieu professionnel, agents chimiques, effets sur la santé, métrologie, méthodes de mesure, lieux de travail, valeur de référence, acétaldéhyde

KEY WORDS

OEL, limit values, exposure levels, occupational, chemical agents, health effects, metrology, measurement methods, workplaces, reference value, acetaldehyde

CITATION SUGGÉRÉE

Anses. (2025). Avis de l'agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail relatif à l'expertise en vue de la fixation de valeurs limites d'exposition à des agents chimiques en milieu professionnel - Évaluation des effets sur la santé et des méthodes de mesure des niveaux d'exposition sur le lieu de travail de l'acétaldéhyde. Maisons-Alfort : Anses, 24 p.

Annexe 1 : Détails des protocoles de mesure identifiés et classement des différentes méthodes de mesure de l'acétaldéhyde dans l'air des lieux de travail au regard des VLEP recommandées

	Méthode	Protocoles	VLEP-8h	VLCT-15 min
			Contrôle technique réglementaire	Contrôle technique réglementaire
A	**Prélèvement actif sur tube de gel de silice ou filtre imprégné de DNPH – désorption acétonitrile – analyse par chromatographie liquide détection UV/visible**	NF ISO 16000-3 (2011) ; NF X 43 264 (2011) ; INRS M-66 (2016) ; US EPA TO 11A (1999) ; NIOSH 2018 (2013) ; MDHS 102 (2010) ; DFG Method 2 (1995)	**1B** **Gel de silice imprégné de DNPH, 2x4h**	**1B** **Gel de silice imprégné de DNPH**
		;DFG method 1 (1990) DFG method 2 (1995)	**2** **Filtre imprégné de DNPH**	**2** **Filtre imprégné de DNPH**
B	**Prélèvement actif sur résine polymèrique XAD-2® imprégnée de 2-(hydroxyméthyl)pipéridine (HMP), désorption solvant toluène, analyse par chromatographie en phase gazeuse et détection par détection spécifique de l'azote (NPD) ou ionisation de flamme (FID).**	OSHA 68 (1988), NIOSH 2538 (1994), IRSST 385	**2**	**3** **(2 suivi des expositions court terme)**
C	**Prélèvement passif sur support imprégné de DNPH, désorption solvant acétonitrile, analyse par chromatographie en phase liquide et détection par spectrométrie UV.**	LCSQ (2007); LCSQA (2013), Radiello®	**3**	**3**

Expert appraisal on recommending occupational exposure limits for chemical agents

Assessment of health effects and methods for the measurement of exposure levels in workplace atmospheres for acetaldehyde (CAS N° 75-07-0)

OEL Permanent Mission
Request n°2022-MPEX-0178

Collective expert appraisal

REPORT

Expert Committee on "health reference values"

Working group on « metrology »

December 2024

AGENCE NATIONALE DE SÉCURITÉ SANITAIRE de l'alimentation, de l'environnement et du travail
14 rue Pierre et Marie Curie 94701 Maisons-Alfort Cedex
Tél : +33 (0)1 49 77 13 50 — www.Anses.fr — @Anses_fr

Suggested citation

Anses. (2024). Expert appraisal on recommending occupational exposure limits for chemical agents. Assessment of health effects and methods for the measurement of exposure levels in workplace atmospheres for acetaldehyde (CAS N° 75-07-0) (Saisine 2022-MPEX-0178). Maisons-Alfort: Anses, 130 p.

Key words

OEL, limit values, exposure levels, occupational, chemical agents, health effects, metrology, measurement methods, workplaces, reference value, acetaldehyde

Presentation of participants

PREAMBLE : The expert members of the Expert Committees and Working Groups or designated rapporteurs are all appointed in a personal capacity, intuitu personae, and do not represent their parent organisations.

EXPERT COMMITTEE (CES)

The work carried out as part of this report was adopted by:

- The “Health Reference Values” Committee (2024-2028)

Chair

Mr Jérôme THIREAU – Standard Grade Researcher, French National Centre for Scientific Research (CNRS) – Doctor of science (PhD) - Expertise: animal physiology, electrophysiology, cell biology, cardiotoxicity.

Vice-Chair

Mrs Maylis TELLE-LAMBERTON – Epidemiologist, statistician at ORS Ile de France – Expertise: epidemiology, occupational risk, statistics

Members

Mr Marc BARIL – Assistant Professor University of Montreal – Expertise: chemist, toxicologist, industrial hygiene

Mrs Michèle BISSON – Study director, French National Institute for Industrial Environment and Risks (INERIS) – Expertise: pharmacist-toxicologist, reference toxicological values, general toxicology, risk assessment.

Mr. Nicolas CHEVALIER – Hospital doctor (PU-PH) Nice University Hospital – Expertise: medicine, endocrinology; thyroid; metabolism; epidemiology; diabetes

Mr. Mihai Ciprian CIRTIU – Specialist scientific advisor, INSPQ and associate professor University of Laval and University of Québec at Trois-Rivières – Expertise: toxicology, biometrology, chemistry, method development and validation, nanomaterials

Mrs Fatiha EL-GHISSASSI – Retired from International Agency for Research on Cancer – Expertise: biochemistry, cancerogenicity, genotoxicity.

Mr Claude EMOND – Assistant clinical professor, University of Montreal, Canada - Department of environmental and occupational health – Expertise: toxicology, physiologically based pharmacokinetic (PBPK) modelling, toxicokinetics, nanotoxicology, endocrine disruptors.

Mr Robert GARNIER – medicine and toxicologist, Paris - Expertise: medical toxicology, occupational medicine, environmental health.

Mr Kevin HOGEVEEN – Toxicologist, Anses – Fougères, Toxicology of contaminants – Expertise: toxicology, genotoxicity, hepatotoxicity, *in vitro* toxicology.

Mrs Yuriko IWATSUBO – Retired from French Public Health Agency (SPF) – Expertise: occupational risks epidemiology.

Mr Jérôme LANGRAND – Hospital doctor (PU-PH), Head of department of Paris Poison control center, AP-HP Fernand-Widal hospital, Paris Poison control center – Expertise: toxicology, medicine, occupational toxicology, environmental and occupational pathologies, toxins.

Mr Fabrice MICHIELS – Occupational physician-toxicologist, Intercompany association for occupational health, Corrèze and de Dordogne (SPST 19-24) – Expertise: occupational medicine, occupational and environmental toxicology.

Mrs Gladys MIREY – Research Director in toxicology, Head of the Genotoxicity & Signaling team, INRAE UMR TOXALIM – Expertise: cellular toxicology, genotoxicity, mechanisms of action, contaminants, study models/alternative methods, effects of mixtures.

Mrs Christelle MONTEIL – Professor of Toxicology, University of Rouen– Expertise: health impact, experimental toxicology, cardiorespiratory toxicology

Mr Johnny MORETTO – Lecturer in Physiology, University of Franche-Comté – Expertise: pharmacokinetic, physiology, pharmacology, biochemistry

Mr Luc MULTIGNER – Research Director, INSERM U1085 – Research Institute for Environmental and occupational Health (IRSET) – Expertise: epidemiology, endocrine disruptors, pathologies of reproductive functions and organs.

Mrs Nadia NIKOLOVA-PAVAGEAU – Medical advisor at the French National Research and Safety Institute for Prevention of Occupational accident and disease (INRS) – Expertise: occupational medecine, medical toxicology, biomarkers of exposure.

Mrs Magali OLIVA-LABADIE – Hospital doctor (PU-PH), Head of department, Bordeaux CHU, Pellegrin hospital, Nouvelle Aquitaine Poison control center – Expertise: toxicology, medicine, environmental toxicology, toxins.

Mr Stéphane PERSONNE – Pharmacovigilance assessor, ANSM – Expertise: general toxicology, experimental toxicology, toxicokinetic, PBPK

Mr Renaud PERSOONS – Hospital doctor, Grenoble and Teacher University of Grenoble Alpes – Expertise: biological monitoring, toxicology, analysis of toxics, metrology

Mr Julien ROUSSEL – Lecturer, University of Montpellier – pharmacology, physiopathology, neurobiology, electrophysiology, metabolism

Mr Rachid SOULIMANI – University Professor and head of Neurotox site, University of Lorraine – Expertise: neurotoxicology, exposome, multi-exposure, health risk

Mr Antoine VILLA – Hospital doctor (PU-PH), Occupational physician, La Timone hospital, Marseille – Expertise: occupational pathologies, toxicology, medicine, expology, biomonitoring, asbestos, cytotoxics.

Mrs Maeva WENDREMAIRE – Lecturer, University of Bourgogne – Expertise: toxicology, reprotoxicity, pharmacology, analytical toxicology.

- The “Health Reference Values” Committee (2021-2024)

Chair

Mr Fabrice MICHIELS – Occupational physician-toxicologist, Intercompany association for occupational health, Corrèze

Vice-Chair

Mr Jérôme THIREAU – Standard Grade Researcher, French National Centre for Scientific Research (CNRS) – Doctor of science (PhD) – Expertise: Animal physiology, cell biology, cardiotoxicity – April 2023

Members

Mr Benoît ATGE – Occupational physician-toxicologist, AHI33 – Expertise: Toxicology, Medicine, Occupational medicine, Biomonitoring, Cytotoxics, Exposure assessment, Surface contamination

Mr Luc BELZUNCES – Research Director and Director of the Environmental Toxicology Laboratory at INRAE – Expertise: toxicology, neurotoxicity, ecotoxicology, analytical chemistry, risk assessment

Mrs Michèle BISSON – Study director, French National Institute for Industrial Environment and Risks (INERIS) – Expertise: Pharmacist-toxicologist, reference toxicological values, general toxicology, risk assessment

Mrs Céline BOTINEAU - Chemical risk prevention engineer at CEA – Expertise: Industrial hygiene, chemistry, risk assessment. Resignation in November 2022.

Mrs Anne CHEVALIER – Retired epidemiologist, French Institute for Public Health Surveillance (InVS) - Expertise: epidemiology

Mr François CLINARD - Epidemiologist at the French Public Health Agency (SPF) – Expertise: Pharmacist-toxicologist, Epidemiology, risk assessment. Resignation in March 2023.

Mrs Fatiha EL-GHISSASSI – Scientist, IARC Monographs Section (IMO) International Agency for Research on Cancer – Expertise: biochemistry, cancerogenicity, genotoxicity

Mr Claude EMOND – Assistant clinical professor, University of Montréal, Canada - Department of environmental and occupational health – Expertise: toxicology, physiologically based pharmacokinetic (PBPK) modelling, toxicokinetics, nanotoxicology, endocrine disruptors

Mr Robert GARNIER – Medical toxicologist, Paris - Expertise: medical toxicology, occupational medicine, environmental health

Mrs Perrine HOET – Professor, Université catholique de Louvain. Institute of Experimental and Clinical Research – Expertise: Medicine, occupational and environmental toxicology. Resignation in March 2023.

Mr Kevin HOGEVEEN – Toxicologist, Anses – Fougères, Toxicology of contaminants – Expertise: toxicology, genotoxicity, hepatotoxicity, *in vitro* toxicology

Mrs Yuriko IWATSUBO – Epidemiologist physician, French Public Health Agency (SPF) – Expertise: occupational risks epidemiology

Mr Jérôme LANGRAND – Hospital doctor (PU-PH), Head of department of Paris Poison control centre, AP-HP Fernand-Widal hospital, Paris Poison control centre – Expertise: Toxicology, Medicine, Occupational toxicology, Environmental and occupational Pathologies, Toxins

Mr Frédéric LIRUSSI – Lecturer – Hospital doctor (PU-PH), Health Sciences & Dijon University Hospital UFR – Expertise: Clinical toxicology, analytical toxicology, innate immunity, reprotoxicity. Resignation in March 2023.

Mrs Gladys MIREY – Research Director in toxicology, Head of the Genotoxicity & Signalling team, INRAE UMR TOXALIM – Expertise: Cellular Toxicology, Genotoxicity, Mechanisms of action, Contaminants, Study models/alternative methods, Effects of mixtures

Mr Luc MULTIGNER – Research Director, INSERM U1085 – Research Institute for Environmental and occupational Health (IRSET) – Expertise: epidemiology, endocrine disruptors, pathologies of reproductive functions and organs

Mrs Nadia NIKOLOVA-PAVAGEAU Medical advisor at the French National Research and Safety Institute for Prevention of Occupational accident and disease (INRS) – Expertise: occupational medicine, medical toxicology, biomarkers of exposure

Mrs Magali OLIVA-LABADIE – Hospital doctor (PU-PH), Head of department, Bordeaux CHU, Pellegrin hospital, Nouvelle Aquitaine Poison control centre – Expertise: Toxicology, Medicine, Environmental toxicology, Toxins

Mr Benoît OURY – Retired from the French National Research and Safety Institute for Prevention of Occupational accident and disease (INRS) – Expertise: atmospheric metrology, workplace atmosphere, occupational exposure assessment

Mr Henri SCHROEDER – Professor at the Faculty of Science and Technology of the University of Lorraine, Department of Neuroscience and Animal Biology and INSERM unit U1256 Nutrition, Genetics and Exposure to Environmental Risks - Pharmacist neurobiologist - Expertise: neurotoxicity, environmental pollutants, animal behaviour, cerebral development, perinatal exposure

Mr Olivier SORG – Head of research group, University of Geneva, Switzerland - Expertise: biochemistry, experimental toxicology, dermatotoxicology

Mr Antoine VILLA – Hospital doctor (PU-PH), Occupational physician, La Timone hospital, Marseille – Expertise: occupational pathologies, toxicology, medicine, expology, biomonitoring, asbestos, cytotoxic

Mrs Maeva WENDREMAIRE – Lecturer, University of Bourgogne – Expertise: toxicology, reprotoxicity, pharmacology, analytical toxicology

WORKING GROUP METROLOGY

- The working group “metrology” (2024-2028)

Chairman

Mr Benoît OURY – Retired from the French National Research and Safety Institute for Prevention of Occupational accident and disease (INRS) – Expertise: atmospheric metrology, workplace atmosphere, occupational exposure assessment

Vice-Chairman

Mr Olivier RAMALHO – Multi-exposure project manager at the Centre Scientifique et Technique du Bâtiment (CSTB) and metrology manager at the Observatoire de la Qualité de l'Air Intérieur (OQAI) - Skills: indoor air quality, metrology

Members

Mr Fabrice ALLIOT - Research Engineer in Chemical Analysis (Ecole Pratique des Hautes Etudes) – Expertise: Air quality, chemical analysis, endocrine disruptors, air sampling

Mr Christophe DEBERT - Metrology and Innovation Manager (AirParif) – Expertise: Air quality, flow measurment, aerosol, gas, reference materials

Mrs Nadine FOURRIER - Divisional Engineer (Ville de Paris) – Expertise: metrology, air quality, analysis

Mrs Tatiana MACE – Head of department (Laboratoire National de métrologie et d'Essais (LNE) – Expertise: Metrology, Traceability, Uncertainty, standards, calibration

Mr Fabien MERCIER - R&D Manager for Organic Micropollutants (Ecole des hautes études en santé publique (EHESP)) – Expertise: *analytical chemistry, development, exposure, health, environment, biomonitoring*

Mr Gregory PLATEEL - Laboratory manager (Centre universitaire romand de médecine légale (CURML)) – Expertise: occupational and environmental exposure, Indoor air quality, biomonitoring

Mrs Caroline RIO – Laboratory manager (ATMO Grand Est) - Expertise: ambient and indoor air quality, physical chemistry, organic aerosol, metrology.

Mrs Dominique SAURAT - Head of the dosimetry division (Ministère des armées) – Expertise: analytical chemistry, sampling, indoor air, exposomics, radionuclides

Mrs Sophie SOBANSKA – Research Officer (Centre national de la recherche scientifique (CNRS)) - Expertise: Air quality, biochemistry, particles, metals.

Mr Guénaël THIAULT – Section Head (CPPL) - Expertise: indoor and workplace air quality, metrology, chemistry.

ANSES PARTICIPATION

Scientific Coordination

Ms Isabelle MANIERE GUERRERO

Mr François POUZAUD

Scientific Contribution

Ms Dominique BRUNET

Ms Emmanuelle DURAND

Ms Isabelle MANIERE GUERRERO

Ms Aurélie MATHIEU-HUART

Ms Amandine PAILLAT

Mr François POUZAUD

Ms Fatoumata SISSOKO

Administration

Ms Patricia RAHYR

Ms Agnès BRION

Table of contents

Acronyms and abbreviations

ALDH	Aldehyde dehydrogenase
ACGIH	American Conference of Governmental Industrial Hygienists
Anses	Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail (French agency for food, environmental and occupational health and safety)
CCET	Cumulative Contact Enhancement Test
CES	Comité d'Experts Spécialisé (Expert Committee)
CLP	Classification Labelling Packaging
DEDuCT	Database of Endocrine Disrupting Chemicals and their Toxicity profiles
DFG	Deutsche Forschungsgemeinschaft (German research foundation)
ECB	European Chemicals Bureau
ECHA	European Chemicals Agency
FDH	Formaldehyde dehydrogenase
GIT	Gastro intestinal tract
GPx	Glutathione peroxidase
GST	Glutathione *S*-transferase
IARC	International Agency for Research on Cancer
INERIS	Institut National de l'Environnement Industriel et des Risques (French National Institute for Industrial Environment and Risks)
INRS	Institut National de Recherche et de Sécurité (Reference body for occupational risk prevention in France)
IPCS	International Programme on Chemical Safety
JSOH	Japan Society for Occupational Health
LOAEL/C	Lowest Observed Adverse Effect Level/Concentration
MAK	Maximale Arbeitsplatz-Konzentration (maximum workplace concentration)
MLA	Mouse Lymphoma Assay
NOAEL/C	No Observed Adverse Effect Level/Concentration
OEHHA	Office of Environmental Health Hazard Assessment
RAC	Risk Assessment Committee
REL	Reference Exposure Level
RGDR	Regional Gas Dose Ratio
RR	Relative risk
SCE	Sister Chromatid Exchange
SCOEL	Scientific Committee on Occupational Exposure Limits
TWA	Time-weighted average
US EPA	United States Environmental Protection Agency
WHO	World Health Organization
8h-OEL	8 hours occupational exposure limit
15min-STEL	15 minutes short term exposure limit
UF	Uncertainty factor

List of tables

List of figures

1 Background, purpose and procedure for carrying out the expert appraisal

Scientific background

The French system for establishing Occupational Exposure Limits OELs has three clearly distinct phases:

- independent scientific expertise (the phase entrusted to ANSES);
- proposal by the Ministry of Labour of a draft regulation for the establishment of limit values, which may be binding or indicative;
- stakeholder consultation during the presentation of the draft regulation to the French Steering Committee on Working Conditions (COCT). The aim of this phase is to discuss the effectiveness of the limit values and if necessary, to determine a possible implementation timetable, depending on any technical and economic feasibility.

The organisation of the scientific expertise phase required for the establishment of Occupational Exposure Limits (OELs) was entrusted to the agency in the framework of the French 2005-2009 Occupational Health Plan.

Purpose of the request

In the scope of the memorandum of understanding between ANSES and the Ministry of Labour for the implementation of the scientific expertise work programme on atmospheric and biological limit values for occupational exposure established in July 2018, the Labour General Directorate (DGT) requested ANSES to carry out the necessary assessment for setting occupational exposure limits (OELs) for acetaldehyde.

France currently has a maximal exposure value of 180 $mg.m^{-3}$ over 8 hours for acetaldehyde. It was set by the Circular of the Ministry of Labour of 13 May 1987.

Procedure: means implemented and organization

ANSES entrusted examination of this request to the Expert Committee on Health Reference Values (HRV Committee). The « metrology » working group was mandated for the assessment of the measurement methods in workplace air.

The methodological and scientific aspects of the expert appraisal work were regularly submitted to the HRV Committee.

The report produced takes into account the comments and additional information provided by the members of the Committees.

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”.

This collective expert appraisal work and its conclusions and recommendations were adopted by the HRV Committee on12 December 2024. One expert abstained. His position is laid out in annexe 5.

Prevention of risks of conflicts of interest

ANSES analyses links of interest declared by the experts before they are appointed and throughout their work in order to prevent potential conflicts of interest in relation to the points addressed in expert appraisals.

The experts' declarations of interests are published on the website https://dpi.sante.gouv.fr/

2 Method

The OELs, as proposed by the “Health Reference Values” Committee (HRV Committee), are concentration levels of pollutants in workplace atmospheres that should not be exceeded over a determined reference period and below which the risk of impaired health is considered as negligible. Although reversible physiological changes are sometimes tolerated, no organic or functional damage of an irreversible or prolonged nature is accepted at this level of exposure for the large majority of workers. These concentration levels are determined by considering that the exposed population (the workers) is one that excludes both children and the elderly.

The Committee recommends the use of three types of values:

- 8-hour occupational exposure limit (8h-OEL): this corresponds to the limit of the time-weighted average (TWA) of the concentration of a chemical in the worker's breathing zone over the course of an 8-hour work shift. In the current state of scientific knowledge (toxicology, medicine and epidemiology), the 8h-OEL is designed to protect workers exposed regularly and for the duration of their working life from the medium- and long-term health effects of the chemical in question;
- Short-term exposure limit (STEL): this corresponds to the limit of the time-weighted average (TWA) of the concentration of a chemical in the worker's breathing zone over a 15-minute reference period during the peak of exposure, irrespective of its duration. It aims to protect workers from adverse health effects (immediate or short-term toxic effects such as irritation phenomena) due to peaks of exposure;
- Ceiling value: this is the limit of the concentration of a chemical in the worker's breathing zone that should not be exceeded at any time during the working period. This value is recommended for substances known to be highly irritating or corrosive or likely to cause serious, potentially irreversible effects after a very short period of exposure.

The 8h-OEL may be exceeded for short periods during the working day provided that:

- the weighted average of levels calculated over the entire working day is not exceeded;
- the short-term exposure limit value (STEL), when one exists, is not exceeded.

Depending on the corpus of data and knowledge available on the biological mechanism(s) of action of the chemical of interest, two main types of 8h-OEL can be derived:

- Threshold dose OELs are estimates of the maximum quantity or concentration of the chemical to which an individual or a population can theoretically be exposed, without risk of adverse health effects, over a given period of time and on the basis of all the information available at the time the OEL was drawn up. They are designed for chemicals which, above a certain dose, cause effects whose severity increases with the absorbed dose;
- OELs without threshold are designed for chemicals for which the adverse effect may appear regardless of the dose received. The probability of occurrence of adverse effects increases with the dose. These are essentially carcinogenic effects resulting from a direct genotoxic mechanism. OELs without threshold correspond either to the additional probability, per unit dose of exposure to the chemical agent (unit excess risk: UER), of developing the critical effect for an individual or population exposed over an occupational lifetime, or to concentrations/doses corresponding to a given level of risk (usually 10^{-4}, 10^{-5} and 10^{-6}).

The derivation of OELs is based on the methodological guidance document of ANSES (Anses, upcoming).

Before the elaboration of OELs, a collection of data useful for characterizing the chemical agent is carried out (identification, physicochemical properties, classifications), as well as general information on uses, sources and exposures.

A toxicological profile is also systematically drawn up to define the effects, observed in humans and animals, associated with different types of exposure to a chemical agent, characterized by their duration and route of exposure (oral, respiratory, dermal), as well as the mechanisms of action and sensitive populations. Any beneficial effects of chemical agents are not described in the toxicological profiles.

The summary of toxicity data by inhalation route for the chemical substance names was drawn up on the basis of summary reports produced by nationally, European and internationally recognized organizations (ANSES, 2014, DFG 2013, ACGIH® 2014, INRS 2022) supplemented by a literature review. This was carried out using the Pubmed and Scopus databases in October 2023. This bibliographic search identified 72 references, 56 studies were retained and 4 additional references were then added. Details of the bibliographic search are presented in Annex 1.

The development of OELs follows a structured and demanding approach involving collective assessments by groups of specialists. The construction of OELs differs according to the knowledge and hypotheses formulated on the mechanisms of action of the substances. At present, the default assumption is to consider a monotonic relationship between exposure, or dose, and effect, or response. In the current state of knowledge and by default, it is generally considered that, for non-carcinogenic effects, toxicity is only expressed above a dose threshold (Anses, to be released).

In practice, the construction of an OELs involves the following steps (Figure 1):

- identify the target organ(s) and the critical effect(s) on the basis of the toxicological profile;
- identify the establishment assumption, with or without a dose threshold, depending on the substance's mode of action;
- select one (or more) key study(s) of good scientific quality, the most relevant among the epidemiological or toxicological studies enabling the establishment of a dose-response relationship;
- define a starting point (PoD) in humans or animals on the basis of this (these) study(s), and adjust it to humans if necessary in the case of a PoD obtained in animals;
- for a threshold OEL, apply uncertainty factors to this PoD;
- for an OEL without threshold, determine a slope and/or concentrations/doses associated with several risk levels.

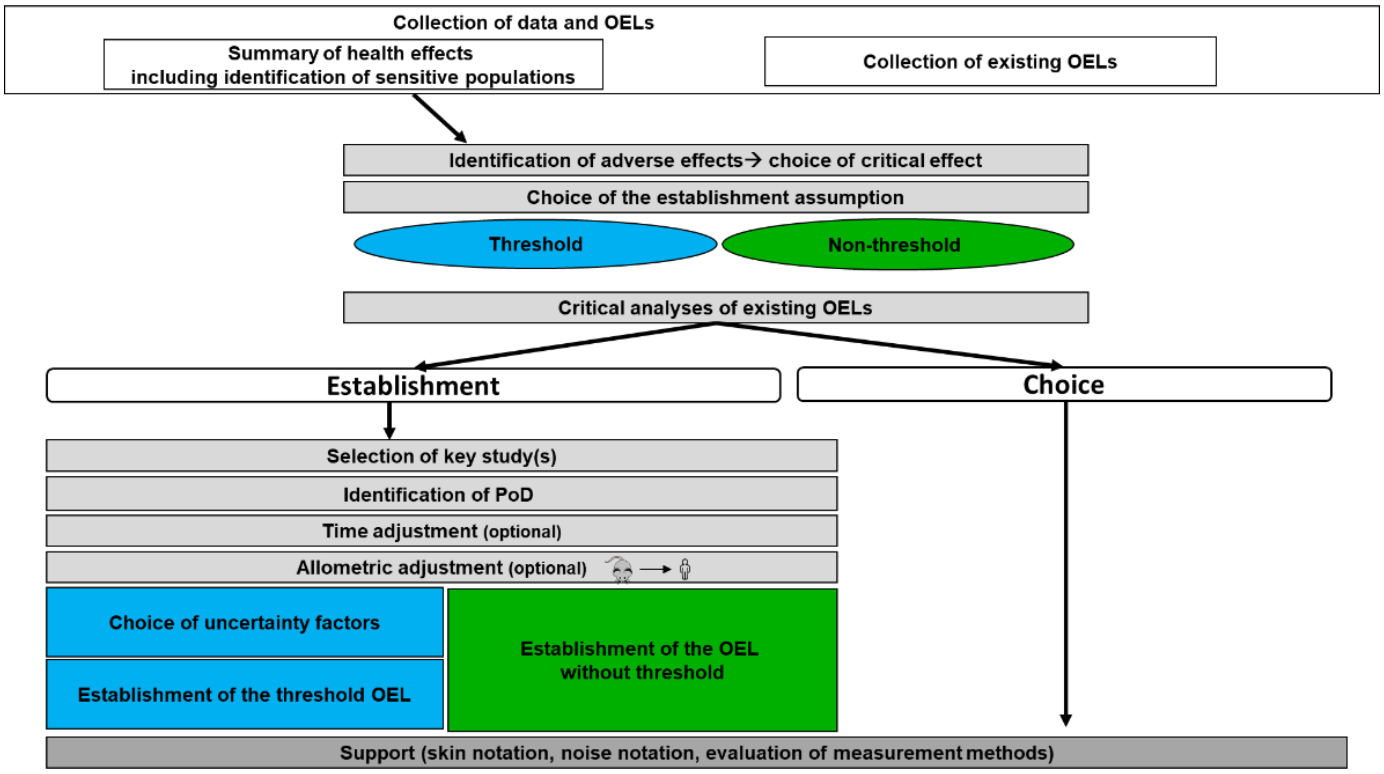


Figure 1 : Steps for proposing an OEL

In addition to OELs, the HRV Committee assesses the need to assign a “skin” notation, when significant skin penetration is possible. This notation indicates the need to consider the dermal route of exposure in the exposure assessment and, where necessary, to implement appropriate preventive measures (such as wearing protective gloves). Skin penetration of substances is not considered when determining the atmospheric limit levels, although it can potentially cause health effects even when the atmospheric levels are respected.

The HRV Committee also assesses the need to assign a “noise” notation indicating a risk of hearing impairment in the event of co-exposure to noise and the substance below the recommended OELs, to enable OSH experts to implement appropriate measures (collective, individual and/or medical) (Anses 2017).

The OEL Committee also assesses the applicable reference methods for the measurement of exposure levels in the workplace. The quality of these methods and their applicability to the measurement of exposure levels for comparison with an OEL are assessed, particularly with regards to their compliance with the performance requirements in the NF-EN 482 Standard and their level of validation[1]. Once they have been assessed, these methods can be classified into one of the following categories:

- Category 1A: the method has been recognized and validated (all of the performance criteria in the NF-EN 482 Standard are met);

[1] NF EN 482: "Workplace atmospheres - General requirements for the performance of procedures for the measurement of chemical agents"

- Category 1B: the method has been partially validated (the essential performance criteria in the NF-EN 482 Standard are met);
- Category 2: the method is indicative (essential criteria for validation are not clear enough);
- Category 3: the method is not recommended (essential criteria for validation are lacking or inappropriate) (Anses 2017)

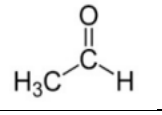
Part A – Report on assessment of health effects

1 General information

1.1 Substance identification

Acetaldehyde is a saturated aldehyde.

Table 1 : Substance identity and structural formula of the substance

Name	Acetaldehyde
Chemical family	Aldehydes
CAS number	75-07-0
EC number	200-836-8
Index number	605-003-00-6
Molecular formula	C_2H_4O
Structural formula	
Synonyms	Acetic aldehyde, Ethanal

1.2 Physical and chemical properties

Physical and chemical properties of acetaldehyde are described in Table 2.

Table 2 : Physical and chemical properties of acetaldehyde

	Value
State of the substance at 20°C, 101.3 kPa	Colourless highly volatile liquid with a fruity pungent odour
Molecular weight	44.05 $g.mol^{-1}$ (INRS 2022)
Melting point	-123.5°C (INRS 2022)
Boiling point	20.1°C at atmospheric pressure (INRS 2022)
Vapour pressure	4 kPa at 0°C; 279.4 kPa at 50°C; 1014 kPa at 100 °C (INRS, 2017) 100.6 kPa at 20 °C (IUCLID 2000 ; K. Othmer, 2004 quoted by INERIS 2018)
Density	0.778 (INRS 2022)
Flash Point	-38°C (closed system) (INRS 2022)
Conversion factors at 25°C and at 20°C 101.3 kPa	1 ppm ($mL.m^{-3}$) = 1.8 $mg.m^{-3}$ at 25°C 1 ppm ($mL.m^{-3}$) = 2 $mg.m^{-3}$ at 20°C (WHO 1995 quoted by INERIS 2018) (INRS 2022)

Solubility	Soluble in water (10^6 mg.L^{-1} at 25°C (HSDB 2005 quoted by INERIS 2018)
Partition coefficient n-octanol/water (Log Kow)	-0.53 to 0.52 (Santé Canada 2000), (IUCLID 2000 quoted by INERIS 2018)
Vapour density relative to air	1.52 (INRS 2022)
Vapour index	49.1 (INRS 2022)
Olfactive perception level	Sweet, fruity smell at low concentrations (Ruth *et al*., 1986) then pungent and suffocating at high concentrations (NTP, 2011; Santé Canada 2000 ;SCCS 2012; WHO 1995; INRS 2022) 0.04 ppm (WHO 1995 quoted by INERIS 2018) 0.09 mg.m^{-3} (0.05 ppm) (Amoore and Hautala, 1983 quoted by WHO 1995) 0.025 mg.m^{-3} (Devos et al. 1990 quoted by Anses 2014) 0.005 to 1800 mg.m^{-3} (AIHA 1989 quoted by Anses 2014) 0.12 mg.m^{-3} (New Jersey Department of Health and Senior Services 2010, revised 2016)

According to the updated version of the methodological guidance document on reference values including OELs (Anses, to be released), the conversions of concentrations from ppm to mg.m^{-3} are carried out in the report using the conversion factor at 25 °C indicated in Table 2.

1.3 Classification

Acetaldehyde is registered under Regulation (EC) n°1907/2006 (REACH regulation) and is not subjected to any restrictions. Between 1 and 10 tons of this substance are produced or imported into the European Union every year.

Acetaldehyde is classified under Regulation (EC) no. 1272/2008 on the classification, labelling and packaging of substances and mixtures (CLP Regulation) (ECHA 2023).

Table 3 : CLP classification of acetaldehyde

INDEX number of acetaldehyde: 200-836-8			
ATP of CLP	Hazard class, and category code(s)	Hazard statement	Pictograms, Signal word code
CLP00/ATP13	Carc. 1 B – carcinogenicity category 1 and subcategory 1B Muta. 2 - mutagen on germ cell category 2	H350 – may cause cancer H341 – suspected of causing genetic defects	 *Danger!*

	Flam. Liq. 1 - flammable liquid category 1 Eye Irrit. 2 – eye damage/eye irritation category 2 STOT SE 3 – Specific toxicity on certain target organs (single exposure)	H224 – extremely flammable liquid and vapour H319 – causes serious eye irritation H335 – may cause respiratory irritation	GHS08  GHS02  GHS07 Dgr

Acetaldehyde is classified for its carcinogenic effects by:

- IARC : group 2B (possibly carcinogenic to humans) (IARC, 1999) and, for acetaldehyde associated with consumption of alcoholic beverages, group 1 (carcinogenic to humans) (IARC 2012),
- European Union : category 1B (presumed to have carcinogenic potential for humans) (ECHA 2016),US EPA: category B2 (probable human carcinogen) based on sufficient evidence of carcinogenicity effects in animals (US EPA 1991).
- Japan Society for Occupational Health (JSOH): group 2B (possibly carcinogenic to humans) in 1991 (Nomiyama 2021),
- NTP: acetaldehyde is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in experimental animals (NTP 2014),
- DFG: category 3B (substance for which in vitro or animal studies have yielded evidence of carcinogenic effects that is not sufficient for classification of the substance in one of the other categories) (DFG 2013b),
- ACGIH: category A2 (suspected human carcinogen) based on the combination of positive inhalation bioassays in 2 species showing an excess of nasal and laryngeal carcinomas, positive in vitro and in vivo results for genotoxicity, the ability to form DNA adducts, DNA breaks, and micronuclei in human cell lines, plus upper respiratory tract irritation observed in human (ACGIH® 2014).

Acetaldehyde is classified for endocrine disruptor effects in category III (effects are reported only *in vivo* in rodents, and data on mode of action are lacking) in the DEDuCT database (Database of Endocrine Disrupting Chemicals and their Toxicity profiles) in 2024.

1.4 Sources and major uses

Acetaldehyde is produced on a large industrial scale for many purposes and uses (INRS 2022). For instance, it is used as an intermediate in the production of acetic acid, cellulose acetate, pyridine

derivatives, perfumes, paints (aniline dyes), plastics and synthetic rubber; in leather tanning and silvering mirrors; as a denaturant for alcohol; in fuel mixtures; as a hardener for gelatine fibres; in glue and casein products; as a preservative for fish and fruit; in the paper industry; and as a flavouring agent (ECHA 2016).

Acetaldehyde is an aldehyde occurring widely in nature, as it is produced by the combustion of organic matter: it is present in tobacco smoke (Health Council of the Netherlands 2012) and any smoke including cooking emissions, Acetaldehyde is also formed endogenously in humans in small amounts, for instance through the oxidation of ethanol in the body. It is, furthermore, present in construction, decorative and furnishing materials and in products of everyday consumption (floor cleaners, parquet, laminates, glues, stains, strippers, tiles and flocking, etc.

2 Identification of existing occupational limit values

There is no recommendation for acetaldehyde from the Scientific Committee on Occupational Exposure Limits (SCOEL) or the Risk Assessment Committee (RAC) of the European Chemical Agency (ECHA) available to date**.**

France currently applies a maximal exposure value for acetaldehyde of 180 mg.m^{-3} (100 ppm) over 8 hours, set by the Circular[2] of the Ministry of Labour of 13 May 1987.

In the supplements (supplement to 2000 and 2008 reports) published in 2013, the German DFG ("Deutsche Forschungsgemeinschaft") confirmed the MAK (« Maximale Arbeitsplatz-Konzentration ») value recommended in 1982 at 50 ppm (91 mg.m^{-3} at 25°C)[3] (DFG 2013a and b). In analogy to formaldehyde, it was assumed that chronic local tissue damage is a precondition for the development of tumours in the nasal mucosa of rats, also in the case of acetaldehyde. According to DFG, observance of the MAK value of 50 ppm, at which no irritation and no local tissue damage in the nasal mucosa were observed in animal studies, should thus also protect against the formation of tumours. In the 2000 report, DFG recommended a momentary value of 100 ppm (the momentary value is a value which should not be exceeded at any time), which was confirmed in the 2008 supplement. There is also a Peak limitation, category I, excursion factor of 1, which, when multiplied with the MAK value, gives the maximum permissible peak concentration as average of a sampling period of 15 minutes.

The American Conference of Governmental Industrial Hygienists (ACGIH) has recommended since 1993 (confirmed in 2014) a threshold limit value – ceiling (TLV-ceiling)[4] of 25 ppm (45 mg.m^{-3} at 25°C) in order to prevent ocular and upper respiratory tract irritation which occurs from 25-50 ppm (ACGIH.® 2018). ACGIH considered that the prevention of these effects by the ceiling value would protect against an increase of carcinogenic effects. ACGIH did not recommend specific notations.

The Japan Society for Occupational Health (JSOH) recommended in 2021 (confirmed in 2022) an occupational exposure limit-ceiling (OEL-C) of 10 ppm (18 mg.m^{-3} at 25°C) for acetaldehyde (JSOH 2021). This value is based on eye irritation observed in humans at concentrations of 50 ppm for 15 minutes (Silverman et al. 1946) and 4 hours (Muttray et al. 2009) and the possibility of slight impairment of mucociliary transport in upper respiratory tract in rats exposed at 150 ppm for 6 hours/day, 5 days/week for up to 65 exposure days in the study of Dorman et al. (2008) (Nomiyama 2021). This OEL-C is expected to prevent the effects observed on the eyes and upper respiratory tract considering the higher sensitivity due to the lower metabolic rate of acetaldehyde in about 40% of the Japanese population, which has the ALDH2*2 genotype.

[2] Circular of 13 May 1987 supplementing the appendix of the Circular of 19 July 1982 on the acceptable values for concentrations of certain hazardous substances in workplace atmosphere (not published in the Official Journal).

[3] The MAK value of 50 ppm was originally proposed in 1982.

[4] Concentration that should not be exceeded during any part of the working exposure.

The gathered OELs values elaborated by the different scientific organisations following inhalation are presented in Table 4.

Table 4 : Summary table of threshold OELs

Type of OEL		Ceiling value	8h-OEL	Ceiling value
RV	**Organisation**	ACGIH	DFG	JSOH
	Year	1993	1982 value confirmed in 2008	2021
	Name	TLV-ceiling = 25 ppm	MAK value = 50 ppm (≅ 91 mg.m^{-3}) Plus momentary value of 100 ppm which may not be exceeded at any time Peak limitation, category I, excursion factor of 1 multiplied with the MAK value, gives the maximum permissible peak concentration as average of a sampling period of 15 minutes.	OEL-ceiling = 10 ppm (18 mg.m^{-3})
	Value			
Target population		Workers	Workers	Workers
Critical Effect		Eye and upper respiratory tract irritation	Irritant effects in the nasal mucosa (the avoidance of these effects shall also prevent chronic local tissue damage, caused by cytotoxic effects, which is a precondition for the development of tumours in the nasal mucosa of rats, by analogy with formaldehyde).	Eye irritation in humans and a slight impairment of mucociliary transport in upper respiratory tract in rats
Key study	**Reference**	Based on weight of evidence from different studies in rodents and in humans to prevent acute ocular and respiratory tract irritation that occurs at levels between 25-50 ppm: (Silverman et al. 1946; Sim and Pattle 1957; Muttray et al. 2009; Stanek et al. 2001; Dorman et al. 2008 ; Woutersen et al. 1986 ; Feron et al. 1982)	Appelman et al.1986	Silverman et al. 1946, Muttray et al. 2009 (in humans), Dorman et al. 2008 (in rats)
	Population of the study or species	Humans, rats, hamsters	Rats	Human and rats
	Exposure (time, route)	see above references Human controlled exposure studies: - at 25 ppm and 200 pm for 15 minutes (Silverman et al. 1946) -135 ppm for 30 minutes (Sim and Pattle1957)	150 and 500 ppm 6 hours/day, 5 days/week during 4 weeks	-15 minutes and 4 hours at 50 ppm, inhalation (humans) -0, 50, 150, 500, or 1500 ppm for 6 hours/day, 5 days/week during 13 weeks (rats)

		-50 ppm for 4 hours (Muttray et al. 2009) Rodent studies -25 ppm and above for 30 minutes (Stanek et al. 2001) -50 ppm for 65 days, whole body exposure (Dorman et al., 2008) -750 ppm and above in rats (Woutersen et al. 1986) and at 1650 ppm in hamster, 52 weeks, 7 hours per day, 5 days per week, whole-body exposure (Feron et al. 1982)		
Point of departure (POD)*		Not specified	NOAEC: 150 ppm	LOAEC: 50 ppm (eye irritation) in humans NOAEC: 50 ppm (slight impairment of mucociliary transport in upper respiratory tract) in rats
Time Adjustment		/	no increase in the effects over time by analogy with formaldehyde)	/
Dosimetry Adjustment		/	/	
Uncertainty Factors (UF)			3 (UF_A)*	Applied UF are not specified
Notations (skin, noise)		No skin notation (insufficient data)	No « H »** designation	Not mentioned
Remarks				The OEL-C is expected to prevent effects on eyes and upper respiratory tract considering the high sensitivity due to the metabolic rate for acetaldehyde on approximately 40% of the Japanese population, which has the ALDH2*2 genotype

* For formaldehyde, the ratio between the NOAEC for sensory irritation obtained in animal studies and the MAK value of 50 ppm derived from results in humans is thus a factor of 3. The situation should be similar also with acetaldehyde.

** Substances are designated with an “H” if through dermal exposure the observance of the MAK value on its own no longer guarantees the prevention of important adverse effects on health which were considered for establishment of the threshold value (mbwl_2022_eng.pdf, consulted in October 2023).

3 Toxicity data

Inhalation and dermal exposure to acetaldehyde studies are mainly described in the present report. Some oral and intraperitoneal studies have also been described. The conversions of concentrations tested in the studies from ppm to $mg.m^{-3}$ were done at 25°C.

3.1 Toxicokinetics

3.1.1 Absorption

Toxicokinetic studies conducted in humans and animals (rats) show that the absorption of acetaldehyde and its passage through the systemic circulation are limited (Anses 2014).

Available data show that a large part of the inhaled acetaldehyde is retained at the contact site becoming rapidly and irreversibly bound to free proteins and thiol molecules (e.g. cysteine, glutathione) (Anses 2014; Serio and Gudas 2020).

In a human study, the retention of acetaldehyde in the respiratory tract, the site of first contact, varied from 45% to 70% in volunteers exposed by inhalation at concentrations between 100 and 800 $mg.m^{-3}$ (Egle 1970).

Numerous agency reports mentioned that oral exposure to aqueous solutions of acetaldehyde results in its absorption in the gastrointestinal tract, but specific literature and quantitative data are not cited (CEPA 1993).

Due to its high reactivity at the contact site, it is expected that acetaldehyde

- is poorly absorbed through the digestive tract;
- binds to compounds in epidermis and does not reach the dermis. Limited data are available on dermal or percutaneous absorption (ECHA 2016).

An *ex vivo* study using a human skin model provided experimental data on the skin absorption properties of common aldehydes used in industry, namely acetaldehyde, acrolein, benzaldehyde and formaldehyde, in gaseous or vapour form (Thredgold et al. 2020). This study used an adapted *ex vivo* technique where human epidermal skin was exposed using Franz diffusion cells to dynamic aldehyde vapour atmospheres over short-term exposure times (10, 20 and 30 minutes), at 996 ppm for acetaldehyde.
According to the authors, 2 of the 4 tested aldehydes were found to penetrate the skin in "appreciable" amounts following a 30-minute exposure: acetaldehyde (5.29 ± 3.24 $\mu g.cm^{-2}$) and formaldehyde (3.45 ± 2.58 $\mu g.cm^{-2}$). Skin penetration by acrolein (0.480 ± 0.417 $\mu g.cm^{-2}$) and benzaldehyde (1.46 ± 0.393 $\mu g.cm^{-2}$) was much lower. However, this study presents some limitations including the short sampling periods (10, 20, 30 minutes instead of 24 hours), no simultaneous test with a reference substance whose transcutaneous passage is known; viability and thickness of the skin were not reported, skin integrity was not verified before and after the test; the protocol used is validated for transcutaneous passage of liquid or solid agents in direct contact with the skin but not for aerosols. Additionally, the only concentration used was very high (1000 ppm) leading to non-specific penetration as a result of skin lesions. Therefore, this study does not allow an estimation of the contribution of the dermal route to overall exposure.

3.1.2 Distribution

No data were found in the literature concerning acetaldehyde distribution following exposure in humans.

Acetaldehyde was found to be increased in various tissues of male Sprague-Dawley rats (n=3), exposed to acetaldehyde gas at a flow rate of 1 litre.min^{-1} for 1 hour by passing air through 400 mL of a 2.5% aqueous acetaldehyde solution in a sealed box, compared to unexposed control animals (Hobara et al. 1985). In this study, the authors reported that acetaldehyde was distributed into the blood, liver, kidney, spleen, heart, and skeletal muscles (only organs studied).

Acetaldehyde appears to easily cross the blood-brain barrier (Deng and Deitrich 2009; Shiohara, Tsukada and Chiba 2007; Kharchenko 1998; Latge et al. 1987). Acetaldehyde was detectable in brains of male Wistar rats 120 hours after a single intraperitoneal injection of acetaldehyde (5 mmol.kg^{-1} or 0.22 mg.kg^{-1}) (Heap et al. 1995). In other studies, acetaldehyde was detected in the blood and brain of rats, after intragastric administration or intraperitoneal injection (WHO 1995).

Limited data obtained from animal experiments suggest that acetaldehyde can be partially transferred from maternal to fetal blood (CEPA 1993).The transplacental passage, as demonstrated in mice, is rapid. Five minutes after intraperitoneal injection (200 mg.kg^{-1} on day 10 of gestation), acetaldehyde reached its maximum concentration in maternal blood and liver, as well as in the embryo and in the yolk sac. It became undetectable 2 hours after treatment (Blakley and Scott 1984). Zorzano and Herrera found that acetaldehyde freely crossed the placenta in Wistar rats at high concentrations (4.4 mg.L^{-1}or 100 µM). At all times following an intravenous injection of 10 mg.kg^{-1} acetaldehyde to pregnant rats on gestation day 21, acetaldehyde concentrations in maternal and foetal blood as well as in amniotic fluid were similar and reached an equilibrium within 2 minutes after dosing (Zorzano and Herrera 1989) quoted by (CEPA 1993).

3.1.3 Metabolism

The main metabolic pathways involve conjugation to thiol groups at the site of contact (cysteine, glutathione, etc.) and oxidation by NAD-dependent aldehyde dehydrogenase (ALDH) which rapidly metabolises acetaldehyde into acetate, mainly in the liver, the main site of ALDH activity. ALDH are present in cells of most tissues, including liver, respiratory tract mucous membranes, and the testes. Acetate is further degraded into carbon dioxide and water through the citric acid cycle (Anses 2014; Serio and Gudas 2020; WHO 1995).

Globally, available data indicate a highly effective acetaldehyde metabolism, with half-life values of 3 minutes for acetaldehyde in rats (after repeated exposure by inhalation) and mice (single intraperitoneal injection) (Health Council of the Netherlands 2014; ECHA 2016). There are no reliable data available for the half-life of acetaldehyde in humans (Health Council of the Netherlands 2014; ECHA 2016).

Several ALDH-isozymes exist, and have different kinetics and binding affinities that influence acetaldehyde oxidation rates (Goedde and Agarwal 1987; Klyosov et al. 1996), including a mitochondrial (ALDH2) and a cytosolic form (ALDH1). The kinetic characteristics of the enzymatic reaction of liver mitochondrial ALDH are similar among humans, rats and Syrian hamsters. The Km value of human cytosolic ALDH1 was approximately 180 µM, but those of rats and Syrian hamsters were 15 and 12 µM, respectively (Klyosov et al. 1996). In human liver, mitochondrial ALDH2 alone oxidises acetaldehyde at physiological concentrations, but in rodent liver, both mitochondrial and cytosolic ALDHs have a role in acetaldehyde metabolism (Klyosov et al. 1996).

As a result of its high affinity for acetaldehyde, the mitochondrial NAD-dependent ALDH2 oxidises at least 90% of the absorbed acetaldehyde. In humans, acetaldehyde dehydrogenases show genetic polymorphism. The *ALDH2*2* allele, encodes an inactive enzyme. This allele is common in East-

Asian populations with about 5-10 % homozygotes and 30-40 % heterozygotes (IARC 2012,1999). As a consequence, approximately 40-50 % of East-Asian people have a mitochondrial ALDH2 with a null or low activity, which is associated with alcohol intolerance.

At least 2 ALDH isozymes were found in nasal mucosa homogenates isolated from F344 rats (Lam Casanova and Heck 1986). The specific activity of ALDH1 isozyme in the respiratory mucosa was approximately 5 to 8 times greater than that in the olfactive mucosa whereas the specific activities of ALDH2 isozymes were similar in both tissues. In the homogenates of olfactive and respiratory epithelium from F344 rats, an ALDH activity of 1.2 µg.min^{-1} was determined for acetaldehyde (2 mM) oxidation (Morris and Blanchard 1992). In another study from the same research group, uptake of inspired acetaldehyde was measured at an inspiratory flow rate that approximated the minute ventilation rate in the surgically isolated nasal cavity of F344 rats pre-treated with either saline (control) or the ALDH inhibitor, cyanamide (10 mg.kg^{-1}sc). In saline-pre-treated rats, the ALDH activities were determined to be 210 and 160 nmol.min^{-1} in the olfactive and the respiratory tissues, respectively, at acetaldehyde concentrations of 60 mM. In the control group, total activity was 370 nmol.min^{-1}, compared to 60 and 80 nmol.min^{-1} in the respiratory and olfactive tissues, respectively in cyanamide-pretreated[5] rats ($p < 0.05$) for 1 or 7 h prior to sacrifice, indicating that approximately 60% inhibition was obtained (Stanek and Morris 1999). Nasal uptake was measured at 3 inspired concentrations: 10, 300, and 1500 ppm corresponding to delivered dosage rates of (210, 420, and 1990 nmol.min^{-1}, respectively). These results suggest that inspired acetaldehyde is metabolised in situ by ALDH, but at exposure concentrations of 300 ppm or higher, the delivered dosage rate may equal or exceed the capacity of this enzyme.

These results are consistent with ECHA's conclusion: "*Saturation of metabolism of acetaldehyde by ALDH indicating limited enzyme capacity is suggested to occur at acetaldehyde concentrations of 300 ppm (Stanek and Morris 1999)" (ECHA 2016)*. Capacity limitation of nasal metabolism occurs when the rate of acetaldehyde delivered to the nasal tissues exceeds the total metabolic capacity of the tissues (Morris 1997) or such protective mechanisms as binding to intracellular thiols (Santé Canada 2000).

ALDH activity was also characterized in the renal cortex and renal tubules in dogs, rats, guinea pigs and baboons, and in the testes of mice (WHO 1995).

In addition to the ALDH-catalysed reactions, cytochrome P450 monooxygenases, especially CYP2E1, may also metabolise acetaldehyde to a minor extent (Dey et al. 2005; Heit et al. 2013; Terelius et al. 1991).

From *in vitro* and *in vivo* investigations in rats, Otsuka et al. concluded that there is a minor detoxifying metabolic pathway of acetaldehyde via 3-hydroxy-2-butanone (acetoin) formation, the latter being further reduced into 2,3-butane diol, subsequently conjugated with uridine diphosphate glucuronide to 2,3-butanediol-β-glucuronide. Meanwhile, the same team reported the presence and the identification of this glucuronide in the urine of rats and humans orally exposed to 2,3-butane diol (Otsuka et al. 1996).

Due to its chemical reactivity in all tissues, acetaldehyde spontaneously binds to a variety of nucleophilic constituents, amino-acids, nucleic acids, peptides and proteins being the most common targets.

Metabolic pathways of ethanol and acetaldehyde are summarised in Figure 2.

[5] Cyanamide is a strong ALDH inhibitor.

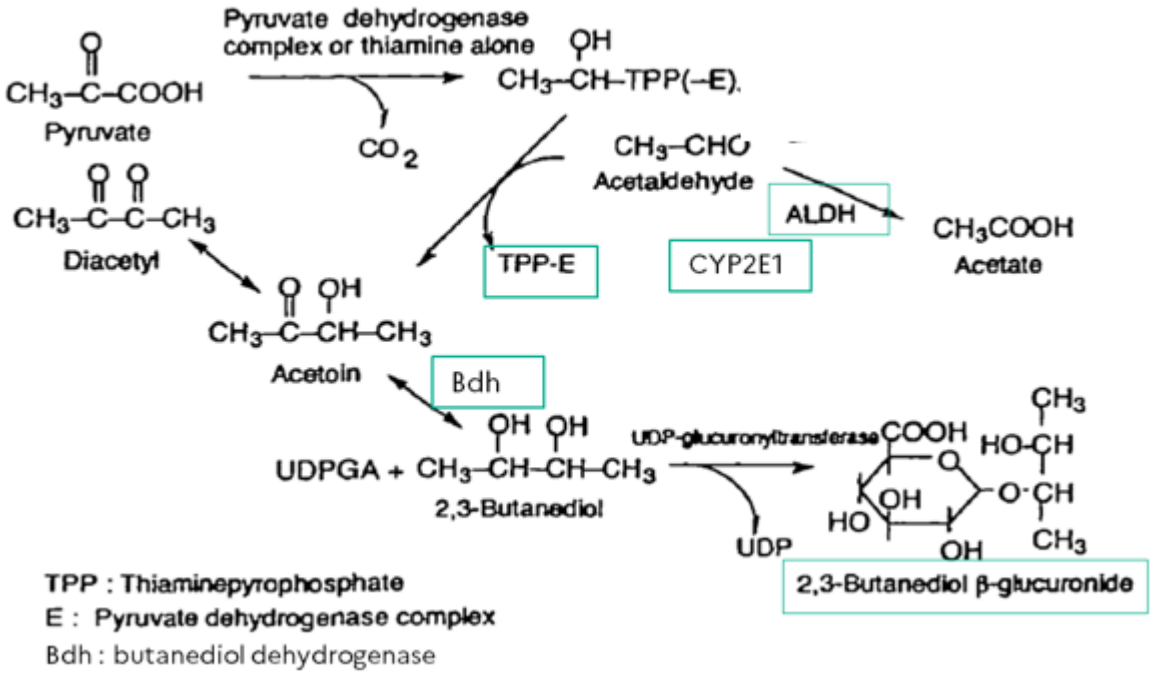


Figure 2 : Metabolic pathways of acetaldehyde (from Otsuka et al. 1996)

3.1.4 Elimination

There is no human data referring to the elimination of acetaldehyde and animal data are very limited.

Acetaldehyde is eliminated mostly in the form of acetate in the urine (ECHA 2016). In one study in dogs exposed by a single administration of acetaldehyde via a stomach tube, the parent molecule was present as a trace in the urine, although in most dogs no urinary acetaldehyde could be detected, due to the rapid metabolism in this species (Booze and Oehme 1986). Acetate is also partly broken down into water and carbon dioxide, both further rapidly eliminated in urine and exhaled air, respectively.

Studies in rabbits and rats also showed a rapid elimination of metabolites such as acetate, 2,3-butane diol glucuronide and pyruvate, in the urine after intravenous administration of acetaldehyde (ECHA 2016).

3.1.5 PBPK Modelling

A physiologically based pharmacokinetic model (PBPK) developed in 2008 was proposed by Teeguarden *et al.* to describe the uptake and distribution of acetaldehyde in the human and rat nasal cavity (Teeguarden et al. 2008). In rats, the PBPK model used is a 5-compartment model: dorsal respiratory tissue, dorsal olfactive tissue (anterior and posterior), ventral respiratory tissue (anterior and posterior). For humans, the model proposed is reduced to 4 compartments, the dorsal olfactive tissue being considered in its entirety (no distinction between the anterior and posterior zone unlike the rat). According to Anses, confidence in this model is limited (model not validated in humans and lack of sensitivity analysis) (see annex 3, Anses 2014).

Corley et al. published what they called a “Dynamics/ physiologically based pharmacokinetic model” for rats and humans describing the respiratory exchange of acetaldehyde in the different regions of respiratory tract (Corley et al. 2015).

A model was also developed for acetaldehyde systemic kinetics by Umulis et al. It used 3 compartments (liver, central and fat/muscle compartments) and 2 pseudo compartments (gastrointestinal tract and stomach). In this model, the authors described reversible enzyme kinetics of acetaldehyde that accurately predicts simultaneously the concentrations of both ethanol and acetaldehyde in the blood as a function of time (when systemic acetaldehyde results from ethanol metabolism). The authors reported a very good-fit with the experimentally observed results for healthy volunteers exposed orally to ethanol (Umulis et al. 2005).

Another recent publication from Zhu et al. combined a PBPK model with a genome scale model. This model consisted of 14 compartments (including lung, liver, stomach, small intestine, large intestine, pancreas, spleen, kidney, skin, muscle, adipose, brain, heart, and blood) and 3 luminal compartments (consisting of the stomach, small intestine, and large intestine) (Zhu et al. 2021). This model describes relatively well the concentration of ethanol and acetaldehyde in human blood. The improvement of this whole-body physiologically based pharmacokinetic (WB-PBPK) model compared to that of Umulis is the comprehensive information on liver metabolism, which could simulate the effect of the different levels of enzyme expression on elimination by changing the upper bounds of both the liver alcohol metabolism reaction and acetaldehyde reactions.

No other PBPK model related to acetaldehyde was found in the literature. These models provide information on distribution and metabolism of acetaldehyde, especially when originating from ethanol metabolism.

3.2 Acute toxicity

3.2.1 Human data

The main effects observed in humans after exposure to acetaldehyde vapours are respiratory, eye and skin irritations, with bronchoconstriction in asthmatics.

- **Respiratory and eye irritation**

Several studies in healthy or asthmatic volunteers were conducted over relatively short exposure periods (2-4 minutes) in the years 1990-2000 (Table 5). Studies based on longer exposure periods (15-30 minutes) are also available, but these studies are older (1946, 1957), with limited methodological description (Silverman, Schuttle and First 1946; Sim and Pattle 1957) and low robustness of the acquired data.

Twelve healthy subjects were exposed to 25, 50 or 200 ppm acetaldehyde (45, 90 or 360 $mg.m^{-3}$) for short periods of 15 minutes. Exposure to acetaldehyde vapours at 50 ppm (90 $mg.m^{-3}$) for 15 minutes induced moderate subjective eye irritation in the 12 volunteers. In several subjects, eye irritation was reported at concentrations as low as 25 ppm (45 $mg.m^{-3}$) (Silverman, Schuttle and First 1946).

In another controlled exposure study, 14 healthy male volunteers (18-45 years old) were exposed to 241 $mg.m^{-3}$ for 30 minutes and reported mild upper respiratory tract irritation. No eye irritation was reported (Sim and Pattle 1957 quoted by CERI 2007).

Proctor and Hughes reported transient conjunctivitis in humans exposed to acetaldehyde at a concentration of 200 ppm (360 $mg.m^{-3}$) for 15 minutes (Proctor et al. 1978 quoted by CERI 2007).

In several studies in asthmatic volunteers, inhaled acetaldehyde aerosol has been tested for its bronchoconstrictive effect: firstly in three studies in Japanese subjects, (Myou et al. 1993; Myou et al. 1994; Fujimura et al. 1999) and subsequently in several studies in Caucasian subjects (Prieto et al. 2000; Prieto et al. 2002a; Prieto et al. 2002b). In these studies, subjects inhaled aerosolised acetaldehyde aqueous solutions for very short periods (2-4 minutes).

In the study by Myou *et al.*, 9 asthmatic volunteers (mean age = 39.2 ± 5.4 years) and 9 healthy volunteers, matched by sex and age, were exposed by inhalation to an acetaldehyde aerosol (140, 280, 560 or 1120 $mg.m^{-3}$) for 2 minutes. A significant reduction in forced expiratory volume in one second (FEV1) was observed in relation to acetaldehyde exposure at all concentrations tested in asthmatic volunteers, while no significant effect was observed in healthy volunteers (Myou et al. 1993).

In another study by Myou *et al.* (1994), 9 Asian asthmatic volunteers (mean age = 46.1 ± 6.7 years) received three pre-treatments on three occasions separated by two weeks from each other:

1) an oral placebo (saline) followed by an exposure for 4 minutes to an acetaldehyde aerosol at a concentration of 0.8 $mg.mL^{-1}$ (i.e. an air concentration of 22.4 $mg.m^{-3}$);
2) an oral placebo followed by exposure for 4 minutes to an aerosol of isotonic saline solution;
3) terfenadine (histamine H1 receptor antagonist) administered orally in saline solution followed by exposure for 4 minutes to an acetaldehyde aerosol at a concentration of 0.8 $mg.mL^{-1}$.

These three pre-treatments were then followed by a methacholine challenge test. Two objectives were followed 1) to determine whether the bronchial reaction to methacholine administration was increased by the inhalation of a low concentration of acetaldehyde (concentration identified as not inducing bronchoconstriction), and 2) to determine whether the increase of bronchial reactivity induced by acetaldehyde was a direct effect or induced by a release of histamine. The results showed that acetaldehyde aerosol at 0.8 $mg.mL^{-1}$ in solution (22.4 $mg.m^{-3}$ in air) induced a bronchial hyper reactivity to the methacholine challenge test, in asthmatic volunteers. These results also showed that this reaction was not mediated by histamine receptors, as it was observed after both n° 1 and n°3 experiments (Myou et al. 1994).

Fujimura *et al*. exposed Japanese volunteers (10 alcohol-sensitive asthmatics and 16 alcohol-insensitive asthmatics) to increasing concentrations of an acetaldehyde aerosol (0.04 to 80 $mg.mL^{-1}$ solution = 1.12 to 2,240 $mg.m^{-3}$ in air) for 2 minutes. FEV1 was measured in each subject, immediately after the exposure period. The results showed that in subjects with bronchoconstriction (≥20% decrease in FEV1) after alcohol consumption (alcohol-sensitive subjects), acetaldehyde increased bronchial reactivity as measured by the methacholine challenge test (Fujimura et al. 1999).

Prieto *et al.* exposed 61 subjects with moderate asthma and 20 healthy control subjects to acetaldehyde aqueous solution aerosol (5 to 40 $mg.mL^{-1}$ solution) for 2 minutes. Aqueous solutions containing acetaldehyde were nebulised in a Hudson 1720 nebuliser operated by compressed air at 6 litres/minute with a nebuliser output of 0.18 $mL.min^{-1}$. At this rate, inhalation of acetaldehyde solutions of 5 to 40 $mg.mL^{-1}$ corresponded to air concentrations of approximately 150 to 1200 $mg.m^{-3}$ acetaldehyde[6]. The mean acetaldehyde concentration inducing a 20% decrease in FEV1 (PC20) in asthmatic subjects ranged from 1.96 to 40 $mg.mL^{-1}$ (geometric mean = 17.55 $mg.mL^{-1}$ corresponding to an atmospheric concentration of 526.5 $mg.m^{-3}$; CI95 = 4.72-38.3 $mg.mL^{-1}$). PC20 was determined for some subjects using back extrapolation which was performed by constructing a straight line from the diluent point to the value obtained with the first administered concentration; then the PC20 was determined as the point where that line intersected a horizontal line representing a 20% decrease in

[6] 4.72 $mg.mL^{-1}$ * [(0.18 $mL.min^{-1}$ / 0.006 $m^{-3}.min^{-1}$)] = 4.72 $mg.mL^{-3}$*30 $mL.m^{-3}$ =142 $mg.m^{-3}$

FEV1. In addition, the results indicate that among the 61 subjects, 56 subjects showed bronchoconstriction while in healthy volunteers, none of the 20 subjects presented bronchoconstriction (Prieto et al. 2000).

In a follow-up study, Prieto et al. exposed mildly asthmatic subjects (aged 18-58 years) to acetaldehyde concentrations of 2.5 to 80 $mg.m^{-3}$, using a Hudson 1720 nebuliser with an output of 5 litres/minute. The nebuliser was operated at 5 litres $air.min^{-1}$ for 2 minutes with an acetaldehyde solution output of 0.16 $mL.min^{-1}$. At this rate, inhalation of acetaldehyde solutions of 2.5 to 80 $mg.mL^{-1}$ corresponded to concentrations in air of approximately 80 to 2560 $mg.m^{-3}$. In the first group, 16 subjects were measured for their response to acetaldehyde which was compared to that of methacholine and adenosine-5'-monophosphate (two bronchoconstrictive agents of known potency). In the second group of 14 subjects, repeatability and side effects of acetaldehyde inhalation were examined. For acetaldehyde, the PC20 ranged from 8.4 to 80 $mg.mL^{-1}$ with a geometric mean of 38.9 $mg.mL^{-1}$ (geometric mean values for methacholine and adenosine-5'-monophosphate were 0.6 and 17.4 $mg.mL^{-1}$, respectively), which corresponds approximatively to 1245 $mg.m^{-3}$ (692 ppm). The response to acetaldehyde was moderately repeatable. For the group in which repeatability was examined, for acetaldehyde concentrations producing a >20% decrease in FEV1, most subjects had cough (64%), dyspnea (57%) or throat irritation (43%) (Prieto et al. 2002a).

In a third volunteer study, Prieto et al. compared airway responsiveness to acetaldehyde (2.5 mg to 80 $mg.mL^{-1}$ = 80 $mg.m^{-3}$ to 2560 $mg.m^{-3}$)[7] in subjects with allergic rhinitis (n=43), asthmatics (n=16) and healthy subjects (n=19). The number of subjects with a decrease in FEV1 >20%, after acetaldehyde exposure was 8/43 in the group with allergic rhinitis, 13/16 in the asthmatic group and 0/19 in the healthy subjects group. PC20 values in the group with allergic rhinitis ranged from 15.5 to 80.0 $mg.mL^{-1}$ with a geometric mean of 67.7 $mg.mL^{-1}$, corresponding to approximately 2166 $mg.m^{-3}$ (1203 ppm), whereas in the asthmatic group PC20 ranged from 8.4 to 80.0 $mg.mL^{-1}$ with a geometric mean of 35.5 $mg.mL^{-1}$, ($p < 0.001$) corresponding to approximately 1136 $mg.m^{-3}$ (631 ppm). PC20 values in the allergic rhinitis group were also significantly lower than in the healthy control group in which acetaldehyde PC20 geometric mean was 80.0 $mg.mL^{-1}$ corresponding to approximately 2560 $mg.m^{-3}$) ($p = 0.04$) (Prieto et al. 2002b).

The studies have shown that people with pre-existing bronchial hyperreactivity like asthmatics or individuals with rhinitis are more sensitive to the irritative properties of inhaled aerosolised acetaldehyde solutions, which significantly decreased their FEV1 by more than 20% for lower exposure than in healthy people (Myou et al. 1993; Myou et al. 1994; Fujimura et al. 1999; Prieto et al. 2002a; Prieto et al. 2002b).

Twenty healthy young men were exposed in a chamber to 50 ppm (90 $mg.m^{-3}$) acetaldehyde for 4 hours. Subjective symptoms were assessed using a questionnaire. The following parameters were measured: mucociliary transport time, olfactive threshold for n-butanol, concentrations of interleukin 1 and interleukin 8 in nasal secretions, and mRNA levels of inflammatory factors[8] in nasal epithelial cells after exposure. Acetaldehyde exposure did not cause any subjective irritative symptoms. A

[7] The nebuliser was operated at 5 liters air/minute for 2 minutes with an acetaldehyde solution output of 0.16 $mL.min^{-1}$. Thus, inhalation of acetaldehyde solutions (2.5 to 80 $mg.mL^{-1}$) corresponds to concentrations in air of 80 to 2560 $mg.m^{-3}$ (Prieto et al. 2002b).

[8] Interleukins 1, 6 and 8, tumour necrosis factor, granulocyte–macrophage colony-stimulating factor, monocyte chemotactic protein 1 and cyclooxygenases 1 and 2.

slight increase (not significant according the authors) in mucociliary transport rate at 25°C was reported. N-butanol olfactive threshold did not change. Concentrations of interleukins in nasal secretions or mRNA levels of inflammatory factors were not higher after exposure. Pulmonary function was not tested in this study (Muttray et al. 2009).

Table 5 : Summary of effects after acute inhalation exposure to acetaldehyde in adult volunteers

Volunteers (number)	Exposure	Response	Reference
Healthy volunteers (n = 12 of both sexes)	45, 90, 360 mg.m^{-3} (25, 50, 200 ppm) 15 minutes	Subjective eye irritation at 90 mg.m^{-3} (50 ppm) and for several subjects below 45 mg.m^{-3} (25 ppm)	Silverman, Schuttle, and First 1946)
Healthy volunteers (n = 14)	241 mg.m^{-3} (134 ppm) 30 minutes	Slight subjective irritation of the upper respiratory tract	Sim and Pattle 1957
Healthy volunteers (n = 20)	45, 90, 360 mg.m^{-3} (25, 50, 200 ppm) 4 hours	No effect on nasal mucosa at 50 ppm	Muttray et al. 2009
Japanese asthmatic volunteers (n = 9 asthmatic volunteers vs 9 controls)	140, 280, 560, 1120 mg.m^{-3} (77.8, 155.6, 311.2, 622.4 ppm) 2 minutes	PC20*: 565 mg.m^{-3} (314 ppm)	Myou et al. 1993
Japanese asthmatic volunteers (n=9)	140, 280, 560, 1120 mg.m^{-3} (77.8, 155.6, 311.2, 622.4 ppm) 2 minutes	PC20*: 651 mg.m^{-3} (362 ppm)	Myou et al. 1994
Japanese asthmatic volunteers sensitive to ethanol (n = 10)	1.12, 2.24, 4.48, 8.68, 17.64, 35, 70, 140, 280, 1120, 2240 mg.m^{-3} (0.62, 1.24, 2.48, 4.82, 9.80, 19.44, 38.89, 77,78, 155.56, 622.22, 1,244.44 ppm) 2 minutes	PC20*: 588 mg.m^{-3} (327 ppm)	Fujimura et al. 1999
Japanese asthmatic volunteers tolerant to ethanol (n = 16)		PC20*: 900 mg.m^{-3} (500 ppm)	
Caucasian asthmatic volunteers (n = 61 vs 20 controls)	140, 280, 560, 1120 mg.m^{-3} (77.8, 155.6, 311.2, 622.4 ppm) 2 minutes	PC20*: 142 mg.m^{-3} (79 ppm)	Prieto et al. 2000
Caucasian asthmatic volunteers (n=16)	80 to 2560 mg.m^{-3} (44 to 1,422 ppm) 2 minutes	PC20*: 692 ppm (1246 mg.m^{-3})	Prieto et al. 2002a
Caucasian asthmatic volunteers (n=16), allergic rhinitis volunteers (n=43), healthy volunteers (n=19)	80 mg.m^{-3} to 2,560 mg.m^{-3} (44 to 1,422 ppm) 2 minutes	PC20*: 2166 mg.m^{-3} (1,203 ppm) in volunteers with rhinitis 1136 mg.m^{-3} (631 ppm) in volunteers with asthma 2560 mg.m^{-3} (1,422 ppm) in healthy volunteers	Prieto et al. 2002b

* PC20 (provocative concentration required to produce a 20% decrease in forced expiratory volume in 1s (FEV1); The indicated values correspond to geometric mean values expressed in mg.mL^{-1} or ppm or in mg.m^{-3} of acetaldehyde in solution converted in concentration of acetaldehyde in air in mg.m^{-3}

The effects described above can be summarised as follows:

- for a 15 minute exposure, concentrations below 25 ppm (45 mg.m^{-3}) induced eye sensory irritation in healthy volunteers (Silverman, Schuttle and First 1946);
- for 2 to 4 minutes exposure:
    - acetaldehyde aerosol at 12.5 ppm (22.4 mg.m^{-3}) potentiated bronchial reactivity to the methacholine challenge test in asthmatic volunteers (Myou et al. 1994);
    - inhalation of an aerosol of acetaldehyde aqueous solution in adults with asthma induced bronchoconstriction from 79 ppm (142 mg.m^{-3}) (Prieto et al. 2000).

- **Skin irritation**

Direct contact with liquid acetaldehyde produced skin irritation (Von Burg and Stout 1991).

Patch tests were performed in volunteers, using a preparation containing 10% acetaldehyde. Erythema reactions were observed in all 12 tested persons (Haddock and Wilkin, 1982, quoted by DFG 2013). In another study, 25 American citizens with oriental ancestry were tested with a 75% solution (v/v) or 22% (v/v) (25 µL for 5 minutes). All subjects presented local erythema less than 300 seconds after skin application (Wilkin and Fortner 1985 quoted by DFG 2013b). These tests presented some limitations (non-occlusive patch-test, vehicle not specified in Haddock and Wilkin (1982); type of patch-test not specified, volunteer participants with medical histories indicating ethanol intolerance (flush) in Wilkin and Fortner (1985).

In a case report, a 55-year old white female employee in a textile factory presented with a history of puriginous eruption on the arms, neck, and face (Shmunes and Kempton 1980). Patch-testing with dimethoxane (2.5% in olive oil) induced, at 48 hours, a bullous reaction on an indurated erythematous area. As dimethoxane is hydrolysed to acetic acid, acetaldehyde, and aldol (3-hydroxybutaraldehyde), a second patch testing using acetaldehyde (1% (v/v) in water), dimethoxane (0.1% in olive oil) and acetic acid (1%(v/v) in water) was performed. The subject developed a positive dermal reaction to the acetaldehyde challenge, but not to dimethoxane (0.1%) or acetic acid (1%). No reactions were observed with acetaldehyde 0.5%, 0.25%, 0.12%, or 0.06%, with acetic acid (1%) or dimethoxane (0.1%). A positive reaction was observed with dimethoxane at 2.5%. The data do not allow to determine if the observed reactions were irritative or allergic.

According to the Scientific Committee on Consumer Safety (SCCS), concentrations of acetaldehyde higher than 1% are likely to be irritating to human skin (SCCS 2012).

3.2.2 Animal data

- **Respiratory irritation**

Data on lethal effects due to acetaldehyde inhalation are limited. However, the acute toxicity of acetaldehyde appears to be low, with LC_{50} values ranging from 24,000 to 31,000 mg.m^{-3} for inhalation exposures from 30 minutes to 4 hours in rodents (rats, Syrian hamsters) (Kruysse, 1970 quoted in OEHHA 2008; Appelman, Woutersen and Feron 1982) (Table 6).

Syrian Golden hamsters were exposed to acetaldehyde vapours for 4 hours at concentrations ranging from 14,450 to 17,600 ppm (26,010 to 31,680 mg.m^{-3}) (Kruysse 1970 quoted in OEHHA 2008). After one to two hours of exposure at all concentrations, the animals showed severe lacrymation, salivation, and nasal discharge. The 4-hour LC50 was determined to be 17,000 ppm (30,600 mg.m^{-3}) in this study. In all exposure groups, the animals that died during exposure had convulsions. Some animals survived at all concentrations, but only after a deep narcosis and apnea (Kruysse 1970 quoted in OEHHA 2008).

Appelman *et al*. exposed Wistar rats for 4 hours to airborne acetaldehyde concentrations of 10,436 to 16,801 ppm (18,831 to 30,317 $mg.m^{-3}$). During the first 30 minutes of exposure, the animals were stressed with rapid breathing and exhibited closed eyes. After 1 hour of exposure, they showed prostration and respiratory distress. A LC_{50} of 13,300 ppm (24,000 $mg.m^{-3}$) was estimated from this study for rats exposed to acetaldehyde for 4 hours (Appelman, Woutersen and Feron 1982)

The main symptoms observed after acute exposure to acetaldehyde aerosols were: decreased respiratory rate, increased heart rate and blood pressure with proteinuria, and pulmonary oedema. The progression is towards central nervous system depression (Santé Canada 2000).

Table 6 : Summary of acute studies in animals

Effects	Species	Exposure	Response	Reference
Mortality	Golden Syrian Hamster	26,010 to 31,680 $mg.m^{-3}$ (14,450 to 17,600 ppm) for 4 hours	LC_{50} = 30,600 $mg.m^{-3}$ (17,000 ppm)	(Kruysse 1970 in (OEHHA 2008)
	Wistar Rat	18,831 to 30,317 $mg.m^{-3}$ (10,436 to 16,801 ppm) for 4 hours	LC_{50}= 24,000 $mg.m^{-3}$ (3,300 ppm)	(Appelman, Woutersen and Feron 1982)

Some data are available on skin, eye and upper respiratory tract irritation, following short-term inhalation exposure to acetaldehyde. Exposure to vapours or aerosols induced sensory irritation9 in rodents (Steinhagen and Barrow 1984; Babiuk, Steinhagen and Barrow 1985; Cassee, Groten and Feron 1996; Santé Canada 2000).

The RD50 (exposure concentration producing a 50% decrease in respiratory rate) observed in different studies in rats and mice are reported in Table 6. In F344 rats, a RD50 of 3,740 ppm was determined. In the same study, exposure to 200 ppm produced significant vasodilation beyond 3 minutes (Stanek et al. 2001). Similarly, male F-344 rats were exposed in a head-only inhalation chamber using plethysmography approach to acetaldehyde (approximately 800 to 10,000 ppm though exact concentrations from the graph were not provided in the paper) for 10 minutes and experienced sensory irritation as measured by reduction in respiratory rate (Babiuk, Steinhagen and Barrow 1985). The acetaldehyde RD50 was 2,991 ppm (95% CI 2411-3825) in non-formaldehyde-pre-treated rats and 10,601 ppm in pre-treated rats (increased 3.5-fold). Pre-treatment with formaldehyde consisted to an exposure of 15 ppm of formaldehyde, 6 hours per day for 9 days, rats were then challenged on the 10th day with acetaldehyde to decipher about a potential cross tolerance between acetaldehyde and formaldehyde (Babiuk, Steinhagen and Barrow 1985).

9 There is no consensus on the definition of sensory irritation. This can be defined as a chemosensory effect, that is, an interaction between the chemical substance and the nerve endings of the trigeminal nerve. This sensory irritation could be a component of eye and respiratory irritation, the stimulation of the trigeminal nerve leading then to protective phenomena for the individual (reduction of respiratory rate for example) and not necessarily inducing tissue or cell damage. These effects have to be distinguished from olfactive perception. Sensory irritation would also be distinguished from inflammation because it is not accompanied by response such as redness, itching or pain. Sensory irritation could, however, be responsible for other observed effects (discomfort described by subjects, for example) at concentrations equal to or lower than those associated with irritant effects, with cell and tissue lesions. It is difficult to know exactly what this sensory irritation means, as some authors may use it quite broadly to define critical doses (AFSSET 2008; Alarie, 1973 in Anses 2014).

Table 7 : Summary of sensory irritation studies in animals

Species	Exposure	Response	Reference
B6C3F1 Mouse	1,350 to 7,560 mg.m^{-3} (750 to 4,200 ppm) for 10 minutes	RD_{50} = 5,277 mg.m^{-3} (2,932 ppm)	Steinhagen and Barrow 1985
Swiss-Webster Mouse	1,350 to 7,560 mg.m^{-3} (750 to 4,200 ppm) for 10 minutes	RD_{50} = 5,121 mg.m^{-3} (2,845 ppm)	
F-344 Rat	1440 to 18,000 mg.m^{-3} (800 to 10,000 ppm) for 30 minutes	RD_{50} = 5,383 mg.m^{-3} (2,991 ppm)	Babiuk, Steinhagen and Barrow 1985
Wistar Rat	5,040, 8,280 or 11,700 mg.m^{-3} (2,800, 4,600 or 6,500 ppm) for 30 minutes	RD_{50} = 5,500 mg.m^{-3} (3,046 ppm; 95%CI = 997-4,036 ppm)	Cassee, Groten and Feron 1996
F-344 Rat	5, 25, 50, 100, 150, 200, 350 and 3,000 ppm for 3 minutes	RD_{50} = 6733 mg.m^{-3} (3,740 ppm)	Stanek et al. 2001

- **Eye irritation**

The application of 0.04 mg of acetaldehyde on rabbit eyes caused severe irritation, but no detail was reported (CERI 2007).

- **Skin irritation**

Although the details were not available, application of 0.5 mg acetaldehyde on rabbit skin induced moderate irritation.

In a study in rabbits conducted according OECD guideline 404, acetaldehyde was not found to be irritating to the skin. In another study not conducted in accordance with OECD guideline 404, 500 mg acetaldehyde produced slight irritation of the skin in the same specie with no other details (SCCS 2012).

3.3 Sensitisation

The literature does not report investigations on respiratory acetaldehyde sensitisation. Data are available only on skin sensitisation.

In the EU, acetaldehyde is not classified as a human sensitizer.

3.3.1 Human data

In a human repeated insult patch test (HRIPT)[10] for 9 induction applications), four ethanol preparations (concentrations of 6.25%, 12.5%, 25% and 50%) were tested in 93 individuals (77 females and 16 males) exposed for 24 hours. Six of the 93 volunteers presented delayed allergic skin reaction to 50% aqueous preparation (ethanol/water v/v), in the challenge test. The reactions were reproducible in 2 out of 6 volunteers presenting a positive reaction. One subject became strongly sensitised and was further tested for cross-reactivity. A 0.15 mL of 1% aqueous

[10] In 2012, the SCCS considered the HRIPT tests as unethical.

acetaldehyde was applied to a 12 mm Webril patch and, then reactivity to acetaldehyde was observed at 96 hours. In the same study, the author inadvertently sensitised himself to acetaldehyde during a test to find a non-irritant test concentration for acetaldehyde. Aqueous solutions with different acetaldehyde concentrations were successively applied in a single application: for 10% and 5% solutions, at first for 3 hours, followed by single subsequential applications of 0.5% and 1% solution for 24 hours, all within a 8-day period. A subsequent application was performed with the 2% acetaldehyde preparation in water and produced a strong local allergic reaction with also a flare at the 10% application site that was made 20 days earlier (Stotts and Ely 1977 quoted in SCCS 2012).

In a study of intolerance reactions to ethoxylated (fatty) alcohols, six of 528 consecutive patients were also tested with a 1% preparation of acetaldehyde in water (no control). This produced positive reactions (erythema plus papules or vesicles (with oedema)): 6 identified after 48 hours; (3 with oedema) and 1 identified after 72 hours; (1 with oedema). Retests were proposed to positive cases but only a part of them agreed to participate (the number of participants to the second session is not specified). One patient retested with 1% and 0.33% acetaldehyde in water still had positive reactions (Matura et al. 2004).

A maximisation test was performed in 28 healthy male and female volunteers, using 2% acetaldehyde in petrolatum. Application was under occlusion to the same site on the volar forearms of all subjects for 5 alternate-day 48-hour periods. Prior to the induction treatment, the initial patch site was pre-treated for 24 hours under occlusion, with a 2% aqueous solution of sodium lauryl sulphate (SLS). Following a 10- to 14-day rest period, challenge patch tests with acetaldehyde (2% in petrolatum) were applied for 48 hours on the right side of the back, whereas 2% SLS was applied under occlusion on the left side of the back for 30 minutes. Patch-tests were read at 48 and 72 hours. No reactions were observed in any of the 28 participants (RIFM 1976 quoted in SCCS 2012).

3.3.2 Animal data

The skin sensitisation potential of acetaldehyde was evaluated using a non-guideline modified Cumulative Contact Enhancement Test (CCET), in fifteen female Dunkin-Hartley guinea pigs. The induction treatment consisted of the 24-hour occlusive application of 0.2 mL of a 15% aqueous solution of acetaldehyde in saline or of saline alone, on days 0, 2, 7 and 9 and two intradermal injections of 0.1 mL Freund's complete adjuvant (FCA)[11], in the same skin area, on day 7. Fourteen days after the final induction, a 24-hour occlusive challenge test was performed with acetaldehyde at concentrations of 2.5%, 5.0% and 10.0% in water. Reactions were read 48 and 72 hours after the beginning of the exposure. At the 48-hour reading, the challenge test at 2.5% produced 4/15 sensitisation reactions; challenge at 5.0% produced 7/15 sensitisation reactions; challenge at 10.0% produced 13/15 sensitisation reactions. At the 72-hour reading, the numbers of positive reactions were increased: 5/15 at 2.5%, 9/15 at 5.0%, 13/15 at 10.0%. The animals were rechallenged 78 days after the start of the experiment with 0.035 % and 2.5 % acetaldehyde solutions (in water), and no significant reactions were observed. It is unknown whether the reactions initially observed were irritant (false positive) or allergic in nature (Bergh and Karlberg 1999 ; SCCS 2012).

A guinea pig maximisation test according to OECD 406 was carried out in 30 female guinea pigs. The intradermal induction of sensitisation was performed with 5% dilution of acetaldehyde in acetone and in an emulsion of Freund's complete adjuvant/physiological saline. The epidermal induction of sensitisation was conducted under occlusion with acetaldehyde at 50% in acetone. Two weeks after

[11] The adjuvant was administered to enhance the sensitivity of the method.

the epidermal induction application, the challenge was completed by epidermal application of the test item at 25% in acetone under occlusive dressing. The animals of the control group were induced with acetone and Freund's complete adjuvant/physiological saline and challenged similarly to those of the test group. Cutaneous reactions, i.e erythema and eschar, as well as oedema formation were evaluated at 24 and 48 hours after the removal of the dressing. Neither in the test group nor in the control group, erythematous reactions were observed. The positive control group that was treated with the known sensitizer alpha-hexylcinnamaldehyde did show the expected reaction from slight to severe oedemas. Therefore, acetaldehyde was considered not to be a sensitiser under the conditions of the test (Study report unnamed, 1999, ECHA website[12]).

3.3.3 Conclusion

Indeed, the animal and human data are limited and non-conclusive on sensitisation. Acetaldehyde being an irritant; the relevance positive skin sensitisation test is not easy to establish. Therefore, it is necessary to refer to validated methods.

In human, sporadic cases of skin sensitisations are reported. The only study available is a maximisation test which gave negative results (RIFM 1976 quoted in SCCS 2012).

In animals, the only studies available are: a cumulative contact enhancement test (CCET) which gave positive results, but used a non-guideline protocol, and a guinea-pig maximisation test (OECD 46) which gave negative results.

In summary, the sensitisation capacity of acetaldehyde by inhalation has not been investigated. Based on the whole data on skin sensitisation in humans and animals, there is limited evidence for a skin sensitisation potential of acetaldehyde (SCCS 2012).

3.4 Sub chronic and chronic toxicity

3.4.1 Human data

The effects associated with long-term inhalation exposure to acetaldehyde in humans are poorly documented in the literature.

The only epidemiological study identified in the literature showed no association between acetaldehyde exposure and the occurrence of respiratory effects (Billionnet et al. 2011). This study investigated the associations between indoor pollution and allergic and respiratory pathologies using standardised self-questionnaires (n = 1,012, individuals > 15 years, 490 households). This study was part of the first national campaign on air quality at home (2003-2005) performed by the Indoor Air Quality Observatory in the Health & Comfort directorate of the Scientific and Technical Center for Building (IAQO – CSTB) in France, and included the measurement of 30 pollutants in indoor air. The limits of detection and quantification for acetaldehyde air concentration were 0.1 and 0.3 µg.m^{-3},

[12], consulted in October 2023.

respectively. Acetaldehyde was detected in all dwellings. The minimum, median and maximum concentrations were 1.8, 11.0 and 94.6 µg.m^{-3}, respectively. A non-statistically significant association was reported between asthma or rhinitis occurrence and acetaldehyde exposure, after adjusting for the confounders considered (sex, age, smoking, habitat moisture, study period, presence of pets, presence of mould, highest level of education among people living in the same home and sources of outdoor pollution within a radius of 500 meters (motorway, train, airport, industrial facilities, water treatment plant)).

3.4.2 Animal data

Respiratory exposure to acetaldehyde induced non-neoplastic alterations including degeneration and hyperplasia of the respiratory tract epithelia in rats. The tissue in the nasal cavity was the main target after inhalation of acetaldehyde. The olfactive nasal mucosa appears to be more sensitive than the respiratory nasal mucosa to the effects of acetaldehyde (Anses 2014). Studies on subacute, sub chronic and chronic inhalation exposures to acetaldehyde in animals are described below and summarised in Table 8.

In Wistar rats (50 males and 50 females) exposed to acetaldehyde at concentrations of 400, 1,000, 2 200 or 5 000 ppm (720, 1 800, 3 960 or 9 000 mg.m^{-3}), 6 hours per day, 5 days per week for 4 weeks, histopathological changes in the olfactive and respiratory nasal epithelia were observed (rarefaction and disorganisation of epithelial cells, loss of microvilli and sensory cells, focal hyperplasia, stratified squamous metaplasia). Keratinisation of olfactive epithelium was occasionally seen at the 2 highest concentrations (Appelman, Woutersen, and Feron 1982). Tracheal and laryngeal lesions were also observed at the two highest concentrations tested. A lowest observed adverse effect concentration (LOAEC) was identified at 400 ppm (720 mg.m^{-3}), based on observed degeneration of the olfactive epithelium without hyperplasia and metaplasia at this concentration.

The same authors, using a similar methodological approach, exposed Wistar rats (n = 10/concentration) to acetaldehyde at concentrations of 150 and 500 ppm (270 and 900 mg.m^{-3}), 6 hours per day, 5 days per week for 4 weeks (Appelman et al. 1986). The results showed tissue degeneration of the olfactive nasal epithelium at concentration of 500 ppm (900 mg.m^{-3}); no effects were observed in the lowest exposure group. A LOAEC of 500 ppm (900 mg.m^{-3}) and a no adverse effect concentration (NOAEC) of 150 ppm (270 mg.m^{-3}) were identified, based on observed degeneration of the olfactive nasal epithelium at 500 ppm.

Rats (n = 12) exposed by inhalation to an acetaldehyde concentration of 243 ppm (437 mg.m^{-3}), 8 hours per day, 5 days per week for 5 weeks developed an intense inflammatory reaction in the nasal cavity, with hyperplasia of the olfactive epithelium and infiltration of mono- and polynuclear lymphocytes (Saldiva et al. 1985).

In hamsters (10 animals/sex/concentration) exposed to acetaldehyde vapours at concentrations of 0, 390, 1,340 or 4,560 ppm (0, 702, 2,411or 8,206 mg.m^{-3}), 6 hours per day, 5 days per week, for 90 days, histopathological changes attributable to exposure were observed only in the respiratory tract (Kruysse, Feron, and Til 1975). The histopathological changes observed in the nasal cavity, larynx, trachea and bronchi were areas of necrosis, areas of inflammation, hyperplasia and metaplasia of the epithelium from 1,340 ppm (2,411 mg.m^{-3}). At the highest concentration of 4560 ppm (8206 mg.m^{-3}), a significant decrease in the weight of animals was noted, as well as significant increases in the weights of several organs (heart, kidneys, brain, testes and lungs). At 390 ppm (702 mg.m^{-3}), no adverse effects were observed. Thus, a NOAEC of 390 ppm (702 mg.m^{-3}) and a LOAEC of 1,340 ppm (2,411 mg.m^{-3}), based on degeneration of olfactive epithelium, were identified.

In a more recent study (Dorman et al. 2008), 8-week-old male F-344 rats (60 rats/concentration), fed ad libitum, were exposed to 0, 50, 150, 500 and 1,500 ppm of acetaldehyde (0, 90, 270, 900 and 2,700 mg.m^{-3} at 25°C) and then sacrificed at different time intervals (4, 9, 14, 30 and 65 days of exposure), leading to 12 rats per group for observations. There was no mortality, no decrease of

body weight gain, and no significant difference in the weights of rats at the end of the experiment. Several effects were observed in the respiratory and olfactive epithelium in groups of animals exposed to 500 ppm (900 mg.m^{-3}) and 1,500 ppm (2,700 mg.m^{-3}): minimal to mild metaplasia at day 4, in both groups; minimal to mild hyperplasia[13] at day 14 and day 4, respectively[14]. In the larynx, minimal to mild squamous cell metaplasia was observed at the base of the epiglottis, in rats exposed to 1,500 ppm (2,700 mg.m^{-3}), and at the end of the experiment in rats exposed to 900 mg.m^{-3} for 65 days. No apparent treatment-related effects were observed in the lungs or in trachea. In the group with the highest level of exposure (1,500 ppm or 2,700 mg.m^{-3}), there was also evidence of a minimal inflammatory response in the respiratory epithelium after 14 days of exposure and evidence of inflammation, hyperplasia and metaplasia (from the nostrils to the dorsal olfactive tissue) after 65 days of exposure. Histologic evaluation of the nasal cavity showed an increased incidence of olfactive neuronal loss following acute to subchronic exposure to ≥ 150 ppm (≥ 270 mg.m^{-3}) and increased olfactive epithelial cell proliferation following exposure to 1500 ppm (2,700 mg.m^{-3}). The severity of the olfactive neuronal loss demonstrated dose- and temporal-dependent behaviours, with minimal effects noted at 150–500 ppm (90-270 mg.m^{-3}) and moderately severe lesions seen in the highest exposure group, with increased lesion severity and extent as the exposure duration increased. Acetaldehyde exposure was also associated with inflammation, hyperplasia and squamous metaplasia of the respiratory epithelium. Histopathological examination revealed an alteration of the olfactive epithelium, including an increase in the intercellular space with disruption of the olfactive epithelium. The severity and distribution of these lesions increased with exposure duration and concentration. They became moderately severe in the exposure group at the highest concentrations (2,700 mg.m^{-3}) after 14 days, and deeper in the nasal sections after 65 days of exposure. In this study, a NOAEC of 50 ppm (90 mg.m^{-3}) was identified based on degeneration of olfactive epithelium at 150 ppm (270 mg.m^{-3}) (LOAEC).

After exposure between 13 weeks to 2 years at 750 ppm (1,350 mg.m^{-3}), degeneration, with or without hyperplasia and metaplasia, was found in the olfactive epithelium of rats. After 1,500 ppm (2,700 mg.m^{-3}) and above, a degeneration of the respiratory epithelium with metaplasia and hyperkeratosis was observed. A LOAEC of 750 ppm (1,350 mg.m^{-3}) is identified from this study (Woutersen et al. 1984).

Male and female Wistar rats (105 of each sex per group) were exposed to acetaldehyde at concentrations of 0, 735, 1, 412 or 3,000 ppm (0, 1,322, 2,541, or 5,400 mg.m^{-3}), 6 hours per day and 5 days per week, for 15 months. The highest concentration was gradually decreased to 977 ppm (1758 mg.m^{-3}) because of severe growth retardation, loss of body weight and early mortality. Major exposure-related lesions occurred in the nose and the larynx. Nasal changes were observed at each of the exposure levels, including degeneration of the olfactive epithelium. Degenerative changes of the respiratory epithelium were observed only in rats exposed at the highest dose. Hyperplasia and metaplasia of the laryngeal epithelium and mild changes of the tracheal epithelium were also observed, only at the highest dose. Finally, from this study a LOAEC of 750 ppm (1,350 mg.m^{-3}) could be identified (Woutersen et al. 1984).

Syrian golden hamsters were exposed to acetaldehyde vapour, 7 hours per day and 5 days per week for 52 weeks. The acetaldehyde concentrations were 2,500 ppm (4,500 mg.m^{-3}) during the first 9 weeks, 2,250 ppm (4,050 mg.m^{-3}) during weeks 10-20, 2,000 ppm (3,600 mg.m^{-3}) during weeks 21-29, 1,800 ppm (3,240 mg.m^{-3}) during weeks 30-44, and 1,650 ppm (2,970 mg.m^{-3}), during weeks 45-52. This progressive reduction of exposure level was needed to avoid early mortality and severe growth retardation. Control animals were exposed only to air. Compared to control animals, those

[13] Results on cell proliferation were less consistent.

[14] In the deeper nasal cavity, hyperplasia was also observed, but intermittently.

hamsters only exposed to acetaldehyde had lower body weights from week 4 and onwards. Mortality was also slightly higher in acetaldehyde-exposed hamsters. After 52 weeks of exposure, pathological changes attributable to acetaldehyde were found in the nose, larynx and trachea. Nasal changes consisted of rhinitis, degeneration of the olfactive epithelium, hyperplasia and metaplasia of the respiratory epithelium. Both in the larynx and in the trachea, slight to moderate epithelial hyperplasia and metaplasia were also observed in most treated animals (Feron 1979; Feron, Kruysse, and Woutersen 1982; DFG 1982).

In summary:

- a LOAEC between 150 and 750 ppm (270 $mg.m^{-3}$ and 1,350 $mg.m^{-3}$) and a NOAEC between 50 and 150 ppm (90 and 270 $mg.m^{-3}$) could be identified based on degeneration (and hyperplasia) of the olfactive epithelium in rats;
- a LOAEC between 1,340 ppm and 1,650 ppm (2,411 $mg.m^{-3}$ and 2,970 $mg.m^{-3}$) and a NOAEC of 390 ppm (702 $mg.m^{-3}$) could be identified based on degeneration of the olfactive epithelium in hamsters.

Table 8 : Summary of studies for subacute, subchronic and chronic inhalation exposures in rats and hamsters

Species	Exposure	Results	LOAEC/NOAEC	References
Syrian golden hamster (n = 10 ♂/10 ♀ per group)	0; 390; 1,340; 4,560 ppm (0; 702; 2,411 or 8,206 mg.m^{-3}) 6 hours/day, 5 days/week, for 13 weeks whole-body exposure	390 ppm (702 mg.m^{-3}): no adverse effect observed ≥ 1,340 ppm (≥2,411 mg.m^{-3}): histological changes in the nose, larynx and trachea: degeneration of olfactive epithelium, hyperplasic and metaplastic changes in the respiratory epithelia ♂: increased relative kidney weights 4,560 ppm (8,206 mg.m^{-3}): delayed growth, irritation of the eyes and nose, increased relative heart and lung weights; increased alkaline phosphatase in serum; rhinitis, histological changes in the nasal cavity: necrosis and metaplasia in the respiratory and olfactive epithelia, disappearance of sub epithelial glands, thinning of the bones of the nasal concha, keratinisation of the epithelia; bronchi: focal bronchopneumonia, accumulation of macrophages containing pigment and focal irritation in the interstitium; larynx: respiratory epithelium transformed to squamous epithelium, squamous epithelium keratinised; ♀: increased number of erythrocytes, decreased number of leucocytes, increased relative weights of heart, kidneys, lungs, brain ♂: increased relative testis weights	LOAEC = 1,340 ppm (2,411 mg.m^{-3}) Degeneration of olfactive epithelium NOAEC: 390 ppm (702 mg.m^{-3})	Kruysse, Feron and Til 1975
Syrian golden hamster (n = 35 ♂ per	0; 1,500 ppm (2,700 mg.m^{-3}), 7 hours/day, 5 days/week,	1,500 ppm (2,700 mg.m^{-3}): after 39 weeks increased mortality, slight anaemia, increased protein level in urine, increased activity of	LOAEC = 1,500 ppm (2,700 mg.m^{-3}) (only one concentration tested)	Feron 1979 in 1982 supplement

group)	for 52 weeks, whole-body exposure	glutamate oxaloacetate transaminase in urine, no histological changes in the kidneys, histological changes in the nasal mucosa and trachea (hyperplasia, squamous metaplasia, inflammation)	Degeneration of the olfactive epithelium	
Syrian golden hamster (n = 30 ♂/30 ♀ per group)	0; 2,500/1,650 ppm, (4,500/2,970 mg.m^{-3}) 7 hours/day, 5 days/week, whole-body exposure, 52 weeks, after 9 weeks concentration reduced in steps to 1,650 ppm (from week 45) because of delayed growth, early mortality	2,500/1,650 ppm (4,500/2,970 mg.m^{-3}): increased mortality, decreased body weights; ♀: increased relative lung and kidney weights, increased activity of phosphatase alkaline in serum; nose: rhinitis, thinning and degeneration of the olfactive epithelium, hyperplasia and metaplasia of the respiratory epithelium and thickening of the submucosa, almost exclusively in the dorsomedial section of the nasal cavity, metaplastic layered squamous epithelium; larynx: slight to moderate focal metaplasia in the epithelium, atrophic, inflammatory, hyperplastic and metaplastic changes; trachea: slight to moderate focal epithelial metaplasia	LOAEC = 2,500/1,650 ppm (4,500/2,970 mg.m^{-3}) (the only dose tested) Degeneration of the olfactive epithelium	Feron, Kruysse and Woutersen 1982
Rat Wistar (n = 10 ♂/10 ♀ per group)	0; 400; 1000; 2,200; 5,000 ppm (720; 1,800; 3,960 or 9,000 mg.m^{-3}) 6 hours/day, 5 days/week for 4 weeks whole-body exposure	From 400 ppm (720 mg.m^{-3}): ♂ and ♀: Alteration of olfactive epithelium of the nose ≥ 2,200 ppm (≥ 3,960 mg.m^{-3}): hyperplasia and metaplasia of the nose olfactive epithelium, degeneration of the respiratory epithelium of the nose, degeneration of the epithelium of the larynx and the trachea, hyperplasia and metaplasia of larynx and trachea; 5,000 ppm (9,000 mg.m^{-3}):	LOAEC = 400 ppm (720 mg.m^{-3}) Degeneration of the olfactive epithelium without hyperplasia and metaplasia	Appelman, Woutersen and Feron 1982

		♂ and ♀: increased number of neutrophils, decreased lymphocytes, ♂: increased monocytes; ♂ and ♀: degeneration of the respiratory epithelium of the nose, hyperplasia and metaplasia; ♂: focal irritation of the interstitium of the lungs, focal haemorrhages in the lungs, small foci of compacted, alveolar septa with a high cell density in the lungs, with an increased number of macrophages		
Rat Wistar (n = 20 ♂/20 ♀ per group)	13–52 weeks, 0; 735; 1,412; 3,000 ppm (highest concentration (0; 1,322; 2,541; 5,400 mg.m^{-3}): reduced to 1977 ppm because of delayed growth, transient body weight loss and early mortality), 6 hours/day, 5 days/week, whole-body exposure, interim examinations after weeks 13, 26 and 52	<u>General effects:</u> ≥1,412 ppm (≥2,541 mg.m^{-3}): after 52 weeks: delayed growth; 3,000/977 ppm (5,400/1,758 mg.m^{-3}): after 4 months: delayed growth, after 6 months: laboured breathing, salivation, breathing through the mouth, after 52 weeks: increased mortality <u>Effects on olfactive epithelium:</u> ≥ 735 ppm (≥1,322 mg.m^{-3}): after 13/26 weeks: concentration-dependent degeneration (flattening, cell necrosis, giant cells); after 52 weeks: degeneration, focal basal cell hyperplasia, 3,000 ppm (5,400 mg.m^{-3}): loss of Bowman's glands and bundled nerve fibres <u>Effects on respiratory epithelium:</u> <u>≥</u> 735 ppm (≥1,322 mg.m^{-3}): after 13 weeks: degeneration ≥ 1,412 ppm (≥2,541 mg.m^{-3}): after 26 weeks: degeneration, hyperplasia and metaplasia, hyperkeratosis,	LOAEC = 750 ppm (1,350 mg.m^{-3}) Degeneration of olfactive epithelium	Woutersen et al. 1984

		3,000 ppm (5,400 mg.m^{-3}): rhinitis; <u>Trachea degeneration:</u> ≥ 1,412 ppm (≥ 2,541 mg.m^{-3}): degeneration after 52 weeks: 3,000 ppm (5,400 mg.m^{-3}): hyperplastic and metaplastic changes with keratinisation, papillomatous hyperplasia, proliferation of atypical basal cells, rhinitis, sinusitis <u>Larynx</u>: 3,000 ppm (5,400 mg.m^{-3}): after 13/26 weeks: hyperplasia, squamous cell metaplasia, time-dependent lesions; ≥ 1,412 ppm (≥2,541 mg.m^{-3}): after 52 weeks: hyperplasia, metaplasia, keratinisation		
Rat Wistar (n = 12 ♂ per group)	0, 243 ppm (437 mg.m^{-3}), 8 hours/day, 5 days/week for 5 weeks whole-body exposure	243 ppm (437 mg.m^{-3}): increased functional residual capacity, increased residual volume, increased total lung capacity, increased respiration rate, pronounced subacute inflammatory reactions in the nasal cavities, hyperplasia in the nasal olfactive epithelium, polymorph nuclear and mononuclear infiltrations in the submucosa; lower respiratory tract and parenchyma of the lungs without noticeable findings	LOAEC = 243 ppm (437 mg.m^{-3}) Hyperplasia of olfactive epithelium	Saldiva et al. 1985
Rat Wistar (n = 10 ♂ per group)	0, 150, 500 ppm (270 and 900 mg.m^{-3}), 6 hours/day, 5 days/week for 4 weeks whole-body exposure	500 ppm (900 mg.m^{-3}): olfactive epithelium degeneration, loss of microvilli, thinning and disarrangement of the epithelium irritation of the eyes and nose 150 ppm (270 mg.m^{-3}): no histological changes; interruptions in the daily exposure of 1 ½ hours had no influence on the histological changes;	LOAEC = 500 ppm (900 mg.m^{-3}) degeneration of olfactive epithelium NOAEC = 150 ppm (270 mg.m^{-3})	Appelman et al. 1986

		Biochemical parameters of lung lavage did not give unusual findings		
Rats F-344 (n=60 ♂ per group)	fed ad libitum, 0, 50, 150, 500 and 1,500 ppm acetaldehyde (0, 90, 270, 900, 2,700 $mg.m^{-3}$) (60 rats per dose group) For 13 weeks	Nose: Effects on respiratory and olfactive epithelia 500 ppm (900 $mg.m^{-3}$) and 1,500 ppm (2,700 $mg.m^{-3}$): Minimal to mild metaplasia from day 4 in both groups; minimal to mild hyperplasia respectively from day 14 and day 4 in both dose groups. Larynx: 1,500 ppm (2,700 $mg.m^{-3}$) and at the end of the experiment in rats exposed to 500 ppm (i900 $mg.m^{-3}$) for 65 days: minimal to mild squamous cell metaplasia at the base of the epiglottis Lungs and trachea: No apparent treatment-related effects observed in the lungs or trachea. 1,500 ppm (2,700 $mg.m^{-3}$): evidence of a minimal inflammatory response in the respiratory epithelium after 14 days of exposure and, after 65 days of exposure, inflammation, hyperplasia and metaplasia (from the nostrils to the dorsal olfactive tissue). No mortality, no loss of body weight gain, and no significant difference in rat weights at the end of the experiment.	LOAEC = 150 ppm (270 $mg.m^{-3}$) NOAEC = 50 ppm (90 $mg.m^{-3}$) Degeneration of olfactive epithelium	Dorman et al. 2008

3.5 Reproductive toxicity and developmental toxicity

No human data are available in the literature on the reproductive or developmental effects of acetaldehyde after inhalation. It should be noted that the involvement of acetaldehyde, the main metabolite of ethanol, in the aetiology of human foetal alcohol syndrome is not known (Anses 2014).

No animal data have been identified on reproductive effects following oral, inhalation or dermal exposure. In mice, no significant effect was observed in testis, seminal vesicles or sperm after intraperitoneal injection of 62.5, 125 or 250 $mg.kg^{-1}$acetaldehyde daily for 5 days (Lahdetie, 1988). Intra-amniotic exposure resulted in chromosomal aberrations in rat embryos and in mouse bone marrow cells (Bariliak and Kozachuk 1983 ; Kunugita et al. 2008 ; Health Council of the Netherlands 2012).

Several animal studies on the developmental effects of acetaldehyde have been performed, primarily to investigate its role in ethanol-induced teratogenicity. In these studies, acetaldehyde was administered by amniotic or intraperitoneal injection. Dose-related embryotoxic, fetotoxic and teratogenic effects were observed in most of these studies, particularly in rats, but maternal toxicity was often not assessed adequately, or reported. Dose-related embryotoxic effects were observed in *in vitro* studies on rat embryos exposed to acetaldehyde. Effects on the placenta have also been observed following intraperitoneal injections of acetaldehyde in pregnant rats (IARC 2010; Anses 2014).

3.6 Genotoxicity

Human studies are relatively limited, but show that acetaldehyde forms DNA adducts, DNA breakage, DNA crosslinks and DNA-protein crosslinks, as presented below.

3.6.1 DNA adduct formation

- **In humans**

Average levels of acetaldehyde-DNA adducts were measured in human lymphocytes and were of an order of magnitude higher in alcoholic subjects compared to controls (Fang and Vaca 1997). In alcoholic patients, a statistically significant increase in DNA adducts in granulocytes and lymphocytes was observed compared to healthy controls (Matsuda et al. 2006). According to the authors, this increase is attributable to acetaldehyde, the main metabolite of ethanol (Health Council of the Netherlands 2012). The most abundant DNA adduct resulting from the reaction of acetaldehyde with DNA is N^2-ethylidenedeoxyguanosine which is too unstable to be purified and isolated, but can be converted into the stable adduct N^2-ethyldeoxyguanosine (N^2-EtdG) (IARC, 2010 and 2012). Levels of acetaldehyde-derived DNA adducts were also elevated in peripheral blood cells of Japanese alcoholic beverage abusers with the ALDH2 1*2 genotype compared with those with the ALDH2*1*1 genotype (Matsuda et al. 2006; Health Council of the Netherlands 2012).

Acetaldehyde also forms methy-hydroxy-lpropanodeoxyguanosine (M-OH-PdG) adducts, which have been found in humans. Several studies have shown that these adducts are associated with increased mutation frequency in *in vitro* experiments (IARC 2010 and 2012).

Taken together, the data show that acetaldehyde can form DNA adducts in humans.

- ***In vitro*: Human/mammalian cells/cell lines**

Following *in vitro* exposure to acetaldehyde, DNA adducts have been observed in isolated DNA (Fang and Vaca 1995; Fraenkel-Conrat and Singer 1988; Inagaki et al. 2003; Vaca, Fang, and Schweda 1995; Wang et al. 2000) and in mammalian cells. Incubation of human buccal epithelial cells with acetaldehyde at concentrations of 0, 10, 30 or 100 mM led to a concentration-dependent increase in N^2-ethyldeoxyguanosine adducts (Vaca et al. 1998). No other DNA adducts were investigated.

Taken together, the data show that acetaldehyde can form DNA adducts in vitro.

- ***In vivo***

In animal models, DNA adducts have also been detected in the liver and brain of rats following exposure to concentrations equivalent to those measured in city atmospheres (14 $\mu g.m^{-3}$ continuously for 50 days) (Sanchez et al. 2018).

3.6.2 DNA breakage, DNA crosslinks (in vitro)

In the comet assay using isolated human cells, DNA strand breaks were induced in lymphocytes and different types of human cells (mucosa cells of stomach and colon) (Blasiak et al. 2000). DNA breaks occurred in lymphocytes in a concentration-dependent manner after exposure to acetaldehyde (Singh and Khan 1995). In alkaline elution assays with lymphocytes, leucocytes and human bronchial epithelial cells, no single strand breaks were found, although DNA crosslinks did occur (Lambert et al. 1985 ; (Saladino 1985 ; Grafstrom et al. 1994). However, some of these studies only tested one concentration, and two studies reported positive results for DNA cross-links, together with negative results for DNA single strand-breaks. The presence of DNA crosslinks may affect the outcomes of an alkaline elution test.

The alkaline elution technique in rodent cells (CHO cells) showed DNA crosslinks following exposure to acetaldehyde (Marinari et al. 1984).

3.6.3 DNA-protein crosslinks

- **In humans**

In humans, the methy-hydroxy-lpropanodeoxyguanosine (M-OH-PdG) adduct described above (section 3.6.1) can undergo ring-opening in double-stranded DNA, and then react with proteins to generate DNA-protein cross-links. With a deoxyguanosine residue at the opposite strand, a DNA inter/intrastrand crosslink can be formed. These inter/intrastrand crosslinks are also mutagenic (IARC 2010).

- **In animal models: *in vivo* studies (see Table 9)**

A dose-dependent increase in DNA-protein crosslinks in the respiratory and olfactive mucosa was observed in rats following acetaldehyde exposure by inhalation. Lam et al. reported the formation of DNA-protein crosslinks in the nasal tissue of male Fischer-344 rats following exposure to acetaldehyde by inhalation. The animals were exposed to *a*cetaldehyde at concentrations of 0, 180, 540, 1 800 and 5 400 $mg.m^{-3}$ (0, 100, 300, 1000, 3000 ppm) for a single 6 hours, or to 5 400 $mg.m^{-3}$ (3000 ppm) 6 hours a day for 5 consecutive days (Lam, Casanova, and Heck 1986). Immediately after the final exposure, the animals were killed and nasal respiratory mucosa was obtained for

further examination. After a single inhalation, a dose-dependent increase in DNA-protein crosslinks was observed in the respiratory mucosa, but not in the olfactive mucosa. In the short-term repeated inhalation exposure study, acetaldehyde induced DNA-protein crosslinks were found both in the respiratory and in the olfactive mucosa.

However, in the study by Dorman et al., no increases in DNA-protein crosslinks (DPX) were observed in rats exposed to acetaldehyde by inhalation up to 1500 ppm (Dorman et al. 2008). Likewise, Stanek and Morris did not observe an increase in DPX formation in the respiratory epithelium of F344 rats following exposure to 1500 ppm acetaldehyde for 6 hours (Stanek and Morris 1999).

Taken together, the data show that acetaldehyde can damage DNA directly and induce mutations *in vivo*.

Table 9 : Summary of *in vivo* studies

Endpoints	Species/ tissues	Exposure route/ Concentrations	Results	Reference
DNA-protein crosslinks	Male Fisher-344 rats; DNA protein cross-links studies in nasal respiratory mucosa and olfactive cells	1) Inhalation; 0, 180, 540, 1 800 and 5 400 $mg.m^{-3}$ (0, 100, 300, 1000 and 3000 ppm for a single 6-hour exposure 2) inhalation: 5,400 $mg.m^{-3}$ (1000 ppm), 6 hours/day, 5-days (samples of three rats were combined)	1) positive in the respiratory mucosa (dose-dependent increase), but negative in the olfactive mucosa 2) positive in the respiratory mucosa and in the olfactive mucosa	Lam et al. 1986
DNA-protein crosslinks	Male Fisher-344 rats	fed ad libitum; 0, 50, 150, 500 and 1 500 ppm acetaldehyde (0, 90, 270, 900, 2 700 $mg.m^{-3}$) (60 rats per dose group) for 13 weeks	no increase in DNA-protein crosslinks	Dorman et al. 2008
DNA-protein crosslinks	Male Fisher-344 rats	1500 ppm acetaldehyde for 6 hours	no increase in DNA-protein crosslink formation in the respiratory epithelium	Stanek and Morris 1999

3.6.4 Mutagenicity, clastogenicity and aneuploidicity (*in vivo*)

- **In humans**

No human studies are available.

- **In animal models**

The results of animal studies are presented below. A summary of *in vivo* mutagenicity results in somatic and germ cells are presented in annex 2, based on the evaluations of Anses in 2014, ECHA in 2016, DGF in 2013 and ACGIH® in 2014.

- Somatic cells

In vivo, studies of acetaldehyde exposure *via* the intraperitoneal or intra-amniotic route showed chromosomal aberrations and sister chromatid exchanges in bone marrow cells in mice, hamsters, and rats.

Acetaldehyde induced micronuclei in bone marrow and peripheral blood cells in mice and rats (Ma 1985 ; Hynes et al. 2002; Wakata et al. 1998; Morita et al. 1997) and sister chromatid exchange in the bone marrow of mice (Obe and Beek 1979) and hamsters (Korte et al. 1981) after intraperitoneal injection. These tests showed limitations (methodological shortcomings, insufficient data regarding toxicity or the inadequate purity of the test substance).

In addition, Kunugita et al. studied the induction of gene mutations and micronuclei in knock-out mice having an inactive acetaldehyde dehydrogenase 2 gene. Both wild type and knockout mice inhaled acetaldehyde at concentrations of 0, 225 or 900 $mg.m^{-3}$, continuously for two weeks. In addition, groups of mice (5-10 animals per group) were given acetaldehyde orally at doses of 0 or 100 $mg.kg\ bw^{-1}$, once a day for two weeks. Two weeks after the last exposure, all animals were sacrificed, and the number of reticulocytes with micronuclei was determined. Mutations in the *TCR* gene in T-lymphocytes were also measured. The study reported increased mutation frequencies in T-lymphocytes and increased micronuclei frequencies in reticulocytes in ALDH2 knock-out mice but not in wild-type mice, suggesting metabolism is an important factor in the ability of acetaldehyde to to generate clastogen or mutagen effects (Kunugita et al. 2008).

- Germ cells

Intraperitoneal exposure of mice to acetaldehyde induced an increase in sister chromatid exchanges in spermatogonial cells. No micronuclei formation in early spermatids and no sperm abnormalities were observed after intraperitoneal injection of 62.5-250 $mg.kg^{-1}$ acetaldehyde in mice (Lahdetie 1988).

Intra-amniotic exposure resulted in chromosomal aberrations in rat embryos and in mouse bone marrow cells (Bariliak and Kozachuk 1983; Kunugita et al. 2008; Health Council of the Netherlands 2012).

3.6.5 Mutagenicity, clastogenicity and aneuploidicity (*in vitro*)

Numerous studies in prokaryotic and eukaryotic organisms and in mammalian cells are available (Dellarco 1988). Acetaldehyde is mutagenic, clastogenic and aneugenic *in vitro*. It also induced gene mutations, sister chromatid exchanges in mammalian cells in the absence of metabolic activation.

- ***In vitro* mutagenicity of acetaldehyde**

  - Bacteria

In bacterial tests, acetaldehyde did not induce mutations in *Salmonella typhimurium* (strains TA98, TA97a, TA100, TA102, TA104, TA1535, TA1537 and TA1538) and *Escherichia Coli* (WP2 uvrA), in the presence or absence of a metabolic activation system (doses of 0.1 to 1 mL). Overall, negative outcomes were found in bacteria using the reverse mutation assay**.**

  - Yeast

In one study, acetaldehyde (> 300 µg.mL^{-1}) induced chromosomal aberrations in *Aspergillus nidulans* in the absence of a metabolic activation system (S9-mix) (Crebelli et al. 1989).

- Plants

In a forward mutation test, acetaldehyde gave positive equivocal results. There was a dose-related increase in chromosomal aberrations in Vicia faba root tips exposed to acetaldehyde (220-2205 µg.mL^{-1}) (Rieger & Michaelis, 1960 cited in WHO 1995). Acetaldehyde also induced chromosome aberrations, micronuclei, and sister chromatid exchanges (SCEs) in the root meristem cells of Allium cepa (7.5-7500 µg.mL^{-1}) (Cortes et al., 1986 cited in WHO 1995).

Acetaldehyde induced mutations in single strand DNA (ssDNA), but not double strand DNA, with a strong preference strand-biased mutations on guanines (G→T) in a sensitive yeast Saccharomyces cerevisiae reporter system at a concentration of 0.2%. This study identified a mutational guanine-centred signature (GG to TT mutations) in this yeast reporter system, which was also found in a whole exon sequenced samples of an oesophageal cancer dataset from patients with a known history of alcohol or tobacco usage (Vijayraghavan et al. 2022). However, in this study, clinical data for most of the data set did not allow correlation between exposure and enrichment of the mutational signature. Another recent study using the same yeast reporter system demonstrated that formaldehyde and acetaldehyde generate common mutation patterns in ssDNA, with concentration-dependent increases in C/G to A/T transversions. Interestingly, in this study, acetaldehyde, but not formaldehyde induced deletions of 5 or more bases (Thapa et al. 2022).

- Mammalian cells

Most *in vitro* assays with mammalian cells gave positive results. Acetaldehyde induced gene mutations. A summary of *in vitro* studies in rodent and human cell models included in the evaluation by Anses in 2014, ECHA in 2016, DFG in 2013 and ACGIH® in 2014 is available in annex 2. Acetaldehyde, without metabolic activation, induced gene mutations at the *tk* locus in mouse lymphoma L5178Y cells (Wangenheim et al. 1988). In contrast, no significant increases in gene mutations at the *hprt* locus were observed in a study by Budinsky *et al.* (Budinsky et al. 2013). No explanation was provided by the authors to explain this difference.
Overall, acetaldehyde is considered to induce mutagenicity in mammalian cells *in vitro*.

- ***In vitro* clastogenicity of acetaldehyde**

Acetaldehyde without metabolic activation induced chromosomal aberrations and micronuclei in Sprague Dawley (SD) rat primary skin fibroblasts (Bird et al., 1982). The induction of these chromosomal aberrations was concentration-dependent. Acetaldehyde also induced chromosome aberrations in embryonic diploid fibroblasts of Chinese hamsters and micronuclei in V79 Chinese hamster lung fibroblasts (Kayani and Parry 2010; Speit et al. 2008). In human lymphocytes, concentration-dependent increases in micronuclei were observed (Migliore and Nieri 1991; Migliore, Cocchi and Scarpato 1996). Aldehyde was also shown to induce chromosomal aberrations in human lymphocytes (Veghelyi and Osztovics 1978; Bohlke, Singh and Goedde 1983). Other more recent studies are in agreement with these results. Indeed, acetaldehyde induced chromosomal aberrations in HepG2, Hep3B and in Chinese hamster ovary (CHO) cells (Majer et al. 2004; Mechilli et al. 2008). Acetaldehyde produced dose-dependent increased in micronulei induction in a study using the MCL-5 genetically engineered human lymphoblastoid cell line. The MCL-5 cell line was established from human lymphoblastoid TK+/− cells transfected with cDNAs of human cytochrome P450s (CYP1A2, CYP2A6, CYP2E1, and CYP3A4) and microsomal epoxide hydrolase (Kayani and Parry 2010). Overall, these results suggest a clastogenic genotoxicity of acetaldehyde.

- ***In vitro* aneuploidicity of acetaldehyde**

Acetaldehyde has been shown to induce aneuploidy *in vitro*. It was tested in a chromosomal aberration testing in Chinese hamster embryonic diploid fibroblast at 0.002–0.006% (0.35–1.1 mM; 16–48 µg.mL^{-1} without metabolic activation S9). Acetaldehyde induced an increase in aneuploidy and chromosome breaks and exchanges. However, following treatment with acetaldehyde, the most notable effect was an increase in hypodiploid cells and a parallel increase in multinucleated interphase cells, the frequency of hyperdiploid cells was considerably lower. A strong correlation ($r = 0.93$, $P < 0.01$) between the frequency of hypodiploid cells to the frequency of hyperdiploid plus multinucleated interphase cells has been observed (Dulout and Furnus 1988).

3.6.6 Conclusion

In humans, genotoxicity studies are relatively limited and aimed to assess the effects of ethanol in alcoholic individuals. These studies show that acetaldehyde has a genotoxic potential.

In vitro, acetaldehyde is genotoxic. It is mutagenic, clastogenic and aneugenic. It induces gene mutations, DNA-strand breaks, DNA-adducts, DNA-protein crosslinks, in both rodent and human cells in the absence of metabolic activation. Acetaldehyde also induces DNA damage as shown by comet assay, micronuclei (MN), chromosomal aberrations (CA), etc.

In vivo, there are very few studies on the genotoxicity of acetaldehyde and even fewer by inhalation.

Intraperitoneal exposure to acetaldehyde resulted in increases in micronuclei and sister chromatid exchanges (SCE) at very high doses. The study by Kunugita *et al.* shows that acetaldehyde has genotoxic potential *via* inhalation (increase in micronuclei, increase in mutations) in *Aldh2* Knock Out mice, but not in Wild Type mice treated with acetaldehyde (500 ppm, 2 weeks by inhalation and 100 mg.kg^{-1}, once a day for 2 weeks orally) (Kunugita et al. 2008).

The acetaldehyde-induced formation of DNA adducts and DNA-protein crosslinks are likely to appear at concentrations above a certain threshold at which the mechanisms of detoxification will be saturated. The capacity of these detoxification mechanisms depends on enzymatic activity of detoxification enzymes, including ALDH2, as well as the intracellular concentrations of reactive thiols, which inhibit the interaction of acetaldehyde with proteins, peptides and DNA.

As with formaldehyde (and other aldehydes), irritation is associated with hyperplasia, metaplasia, degeneration of the olfactive epithelium. Cytotoxicity is responsible for regenerative cell proliferation and a contribution of (local) genotoxicity at high concentrations, above saturation concentrations of metabolism and defense mechanisms.

According to Budinsky *et al.*, the key event for acetaldehyde genotoxicity involves an intermediary Schiff's base in the formation of DNA-protein crosslinks to elicit genotoxicity and/or cytotoxicity (Budinsky et al. 2013). DNA repair, apoptosis and other stress-related adaptive responses, and replacement of proteins or redundancy in protein function all act in opposition of these adducts.

3.7 Carcinogenicity

3.7.1 Human data

No studies on the carcinogenicity of acetaldehyde exposure in humans are available.

3.7.2 Animal data

Results from carcinogenicity studies indicate that the nasal and respiratory tract epithelia are the primary targets of acetaldehyde in rodents after repeated respiratory exposure. It should be noted that available data are relatively limited, and the studies are rather old. No recent data on the carcinogenicity of acetaldehyde have been identified.

- **Inhalation exposure**

Inhalation exposure to acetaldehyde has been shown to produce nasal tumours in rats and laryngeal tumours in hamsters. The US EPA and IARC classifications are based on 4 main studies: (Feron 1979; Feron, Kruysse and Woutersen 1982; Woutersen et al. 1984, 1986).They are described below.

In the studies by Woutersen et al. (Woutersen et al. 1984, 1986; Woutersen and Feron 1987), male and female Wistar rats (105 of each sex per group) were exposed to 0, 735, 1,412 or initially 3,000 ppm (0, 1,322, 2,541 or 5,400 $mg.m^{-3}$) acetaldehyde, 6 hours per day and 5 days per week, for 28 months. The highest concentration was gradually decreased to 977 ppm (1,758 $mg.m^{-3}$), because of severe growth retardation, loss of body weight and early mortality. Surviving rats were killed and autopsied at the termination of the study (weeks 120-122). All rats found dead during the course of the study were also autopsied. Incidence of nasal adenocarcinoma of respiratory epithelium was significantly increased in all treated groups, both in male and female rats[15]. Incidence of squamous cell carcinoma of nasal respiratory epithelium was significantly elevated in male rats of each of the two groups with the highest exposures. In female, a statistically significant increase was observed only for the top-exposure group[16]. No increase of any tumour incidence was observed neither in the larynx nor in the lungs (Woutersen et al. 1984, 1986; Woutersen and Feron 1987). New cases of both nasal adenocarcinoma and nasal squamous cell carcinoma were observed during a 26-52 week "recovery period", after discontinuation of exposure (Woutersen and Feron 1987). A summary of respiratory tract tumor incidences in rats is presented in Table 10 : Respiratory tract tumour incidences in rats, which were exposed by inhalation to acetaldehyde for 28 months (Woutersen et al. 1986; Woutersen and Feron 1987)

Some limitations of this study should be noted:

- a high mortality rate
    - animals in the highest concentration group from both sexes died before termination of exposure;
    - only a few animals survived in the mid-exposure group when the study was terminated;
- the concentrations selected for this study were very high.

[15] After 28 months of exposure, the incidence of adenocarcinomas in male control rats and those from the low, middle and high exposure groups was 0/49, 16/52 ($p<0.001$), 31/53 ($p<0.001$), 21/49 ($p<0.001$); it was 0/50, 6/48, 26/53 ($p<0.001$), 21/53 ($p<0.001$), in females.

[16] After 28 months of exposure, the incidence of nasal squamous cell carcinomas in control male rats and in male rats from the low, middle and high exposure groups was ;1/49, 1/52, 10/53 ($p<0.05$), 15/49 ($p<0.001$) respectively; in females, it was 0/50, 0/48, 5/53, 17/53 ($p<0.001$).

Table 10 : Respiratory tract tumour incidences in rats, which were exposed by inhalation to acetaldehyde for 28 months (Woutersen et al. 1986; Woutersen and Feron 1987)

Exposure level (ppm)	**0**	**735 (1,322, mg.m^{-3})**	**1,412 (2,541 mg.m^{-3})**	**3,000/977 (5,400/1,758 mg.m^{-3})**
Male animals				
Nose:				
Papilloma	0/49	**0/52**	**0/53**	**0/49**
Squamous cell carcinoma	1/49	1/52	10/53*	15/49**
Carcinoma in situ	0/49	0/52	0/53	1/49
Adenocarcinoma	0/49	16/52**	31/53**	21/49**
Larynx: carcinoma *in situ*	0/50	0/50	0/51	0/47
Lungs: poorly differentiated adenocarcinoma	0/55	0/54	0/55	0/52
Female animals				
Nose:				
Papilloma	0/50	1/48	0/53	0/53
Squamous cell carcinoma	0/50	0/48	5/53	17/53**
Carcinoma in situ	0/50	0/48	3/53	5/53
Adenocarcinoma	0/50	6/48*	26/53**	21/53**
Larynx: carcinoma *in situ*	0/51	0/46	1/47	0/49
Lungs: poorly differentiated adenocarcinoma	0/53	1/52	0/54	0/54

Statistical significance Fisher exact test: *p<0.05, ** p<0.001

Two older studies carried out in hamsters (Feron 1979; Feron, Kruysse and Woutersen 1982) showed that repeated inhalation of acetaldehyde increased the incidence of nasal and laryngeal tumours.

In the first study, 35 male Syrian golden hamsters were exposed to acetaldehyde concentrations of 0 and 1,500 ppm (0 and 2,700 mg.m^{-3}) for 52 weeks, 7 hours per day, 5 days a week. No neoplastic effects associated with exposure to acetaldehyde alone were observed (Feron 1979). In contrast, exposure of animals to acetaldehyde vapour associated with intratracheal instillation of benzo[a]pyrene increased by 2-fold the incidence of squamous cell carcinomas when compared to exposure to benzo[a]pyrene alone (potentiating effect of acetaldehyde).

In the second study, Syrian golden hamsters (36 animals of each sex per group) were exposed to acetaldehyde vapour 7 hours per day and 5 days per week for 52 weeks (Feron, Kruysse, and Woutersen 1982). The acetaldehyde concentration was 2,500 ppm (4,500 mg.m^{-3}) during the first 9 weeks, 2250 ppm (4,050 mg.m^{-3}) during weeks 10-20, 2000 ppm (3,600 mg.m^{-3}) during weeks 21-29, 1800 ppm (3,240 mg.m^{-3}) during weeks 30-44, and 1,650 ppm (2,970 mg.m^{-3}), during weeks 45-52. This progressive reduction of exposure level was necessary to avoid mortality and severe growth retardation. Control animals were exposed only to air. A proportion of the animals was given simultaneously either intratracheal instillations of benzo[a]pyrene or subcutaneous injections of diethylnitrosamine in addition to the acetaldehyde exposure. Acetaldehyde exposure induced rhinitis, hyperplasia and metaplasia of the nasal, laryngeal and tracheal epithelia. The exposed animals also developed laryngeal carcinomas with a few laryngeal polyps, nasal polyps and carcinomas. A non-statistically significant increase in nasal tumours and a statistically significant increase in the incidence of laryngeal tumours were observed. The incidence of respiratory tract tumours was 0/30 (males, control), 8/29 (males, exposed to only a single concentration that was reduced throughout the treatment schedule), 0/28 (females, control) and 5/29 (females, exposed) (see Table 11 : Respiratory tract tumour incidence in hamsters exposed by inhalation to gradually reduced concentrations of acetaldehyde from 2,500 to 1,650 ppm (500 to 2,970 mg.m^{-3}) acetaldehyde for 52 weeks (Feron, Kruysse and Woutersen 1982). No tumours were observed in the bronchi and bronchioles.

Table 11 : Respiratory tract tumour incidence in hamsters exposed by inhalation to gradually reduced concentrations of acetaldehyde from 2,500 to 1,650 ppm (500 to 2,970 mg.m^{-3}) acetaldehyde for 52 weeks (Feron, Kruysse and Woutersen 1982)

	Incidence of tumours			
	males		females	
	Control	Acetaldehyde	Control	Acetaldehyde
Nose				
Adenoma	0/24	1/27	**0/23**	**0/26**
Adenocarcinoma	0/24	0/27	0/23	1/26
Anaplastic carcinoma	0/24	1/27	-	-
Larynx				
Polyp/Papilloma	0/20	1/23	0/22	1/20
Carcinoma in situ	0/20	3/23	0/22	0/20
Squamous cell carcinoma	0/20	2/23	0/22	1/20
Adeno-squamous cell carcinoma	-	-	0/22	2/20
Total	**0/30**	**08/29***	**0/28**	**5/29**

Statistical significance (Fisher's exact test) *p<0.05,

Data on animal carcinogenicity studies which were evaluated by ECHA in 2016 are summarised in Table 12.

Table 12 : Summary of data on carcinogenicity in animals following inhalation exposure (ECHA 2016)

Species (number)	Design	Exposure	Observations	references
Inhalation				
Rats Wistar (n = 105 animals/sex/group)	6 hours/day, 5 days/week for 28 months, gross necropsy and histopathological examination	0, 1,322, 2,541, 5,400/1758 mg.m^{-3} (0, 735, 1,412 or 3,000/ 977 ppm) Due to toxicity, the highest exposure level was gradually reduced to 1758 mg.m^{-3} over a period of 11 months	Lower survival and body-weight observed in exposed animals compared to controls Lesions: exposure induced irritant effects and malignant tumours in the respiratory tract at all doses tested. Note: only the respiratory tract was examined for the presence of abnormalities.	Woutersen et al. 1986
Rats Wistar (number of animals not given; Male/female)	exposure for 52 weeks followed by 26 weeks (n=20) and 52 weeks (n=10) recovery	0, 1,322, 2,541, 5,400/1758 mg.m^{-3} (0, 735, 1,412 or 3,000/ 977 ppm)	Increased incidence of nasal tumours after discontinuation of exposure	Woutersen and Feron 1987
Hamster, Syrian golden (n = 36 animals/sex/group)	7 hours/day, 5 days/week for 52 weeks, week 53-81, post-exposure period; gross necropsy and histopathological examination; 6 animals/sex were	4,500 mg.m^{-3} (week 1-9), 4,050 mg.m^{-3} (week 10-20), 3,600 mg.m^{-3} (week 21-29), 3,240 mg.m^{-3} (week 30-44) and 2,970 mg.m^{-3} (week 45-52); due to considerable growth retardation	General: from week 4, significant reduced body-weight in exposed animals compared to controls; reduction diminished partly in the post-exposure period. Lesions:	Feron, Kruysse and Woutersen 1982

	killed for interim examination	and to avoid early death, exposures were reduced gradually during experiment	rhinitis, hyperplasia and metaplasia in the nasal, laryngeal and tracheal epithelia. laryngeal and nasal carcinomas and polyps observed; respiratory tract tumours: 0/30 - 8/29 (male, control-exposed) 0/28 - 5/29 (female, control-exposed)	
Hamster, Syrian golden (n = 35 animals/group; males only)	7 hours/day; 5 days/week for 52 weeks, animals killed after 78 weeks; at week 52, 5 animals were killed for interim examination, gross necropsy and histopathological examination	0 or 2,700 mg.m^{-3} (0 or 1500 ppm)	Only one sex used, only one dose applied General: body-weight slightly lower in exposed animals than in controls. mortality increased more rapidly in exposed animals than in controls in the last part of the exposure period. Lesions: no substance-related tumours found. Acetaldehyde induced hyperplastic, metaplastic and inflammatory changes.	Feron 1979
Subcutaneous exposure				
Rats (n = 14 to 20)	subcutaneous injection	(Total) dose not specified; repeated injections	Data from secondary source, original study in Japanese, no abstract available General: no data Lesions: spindle-cell sarcomas at site of injections (4 animals survived in the period up to 554 day)	Watanabe and Sugimoto 1956

- **Subcutaneous exposure**

Watanabe and Sugimoto reported the induction of sarcoma *in situ* in rats after repeated subcutaneous injections of acetaldehyde (Watanabe and Sugimoto 1956). This study had limitations (dose injected not specified, original study in Japanese, no abstract was available).

Mechanism of action

Due to the physicochemical and toxicokinetic characteristics of acetaldehyde (high solubility in water, high reactivity with proteins and nucleic acids in the cells of the body, very rapid metabolism, …), its acute or chronic inhalation leads to local irritation in the respiratory tract. Irritant effects at the site of contact (upper respiratory route) have been observed following both acute and chronic exposures. These irritant effects appear at lower doses than carcinogenic effects, specifically in the upper respiratory tract, leading to lesions of the epithelium after repeated exposure.

Acetaldehyde is a highly reactive substance which can directly react at the site of contact by addition, condensation or polymerisation, with the primary amine (NH_2), thiol (SH) and hydroxyl groups of many substances (nucleophilic sites of DNA, proteins, peptides and amino acids (cysteine, glutathione).

Acetaldehyde appears to be a direct genotoxic agent, and is genotoxic *in vitro* and *in vivo*. In vitro, it induced gene mutations, clastogenic effects, sister chromatid exchanges in mammalian cells, in the absence of metabolic activation, as specified in chapter 5.7.2. In vitro studies demonstrate that acetaldehyde reacts with DNA isolated from the nasal epithelium to form DNA-protein and DNA-DNA crosslinks. In this light, acetaldehyde presents significant similarities with formaldehyde (formation of DNA-protein crosslinks[17]) in terms of the mechanism of action. By analogy with formaldehyde, the reactivity of acetaldehyde is preferential for single-stranded DNA rather than double-stranded DNA. A comparative in vitro study of the kinetics of the histone-DNA cross-link reaction by aldehydes also showed that acetaldehyde reacts 1000 times slower than formaldehyde (Lam, Casanova and Heck 1986).

As with formaldehyde, analysis of this mechanism of action indicates that the carcinogenesis process would occur at exposure levels inducing cytotoxicity associated with regenerative cell proliferation. In the rat study by Woutersen et al., acetaldehyde exposure at a concentration of 1412 ppm induced a cytotoxic response (hyperplasia and metaplasia of the respiratory mucosa) associated with an increase in tumor incidence. At 735 ppm, fewer non-neoplastic lesions were observed and there was no significant increase in the incidence of cancerous tumours. This phenomenon was also observed for formaldehyde (Woutersen et al. 1984).Therefore, in the current state of knowledge, acetaldehyde and formaldehyde toxicities have many similarities[18]. Although these mechanisms of action remain relatively better documented for formaldehyde than for acetaldehyde, it seems reasonable to consider acetaldehyde as a genotoxic carcinogen, and that a dose threshold may exist for acetaldehyde-induced nasopharyngeal cancers following exposure by the respiratory route. This is supported by the presence of a saturating local defense mechanism at high concentrations.

These elements suggest that upper airway irritation (eyes, nose, and throat) could be retained as a critical effect as it is highly probable that irritation lesions must be associated to genotoxic effects to produce cancer locally and as respiratory irritation lesions are observed at much lower concentrations than those for which the possible occurrence of cancer is associated. In other words, protecting against prolonged irritation would also protect against nasal cavity cancer. An analogous reasoning was already used by Anses with formaldehyde for nasopharyngeal cancer (considering a threshold effect for cancer) (Anses 2018).

In conclusion, while the exact mechanism of carcinogenesis of acetaldehyde remains to be better understood, the hypothesis of the mode of action retained for cancer of the nasal cavity induced by acetaldehyde is an increase in regenerative proliferation of epithelial cells of the nasal mucosa

[17] Incomplete repair of these bypasses can then lead to mutations

[18] High water solubility, electrophilic compounds, high retention in the upper respiratory system, high chemical reactivity with biological macromolecules located at the point of contact

(cytotoxicity) inducing an increase in the number of replications of DNA and in the probability of formation of DNA and DNA-protein adducts. This chain reaction leads to more frequent errors in replication, and subsequent mutations. This hypothesis was confirmed by the demonstration of local genotoxicity *in vitro* and *in vivo* only at high doses resulting in cytotoxicity.

3.8 Sensitive populations

Studies conducted in Caucasian and East-Asian volunteers showed that individuals with previous bronchial hyperreactivity (people with rhinitis or asthma in these studies) are more sensitive to the respiratory irritant effects of inhaled acetaldehyde than healthy subjects (Myou et al. 1993; Myou et al. 1994; Fujimura et al. 1999; Prieto et al. 2000; Prieto et al. 2002a; Prieto et al. 2002b).

All elements suggest that populations presenting respiratory diseases (asthma, allergic rhinitis) have an exacerbated sensitivity to acetaldehyde respiratory irritant effects.

4 Establishment of OELs

In accordance to the Anses methodological guidance document (Anses, upcoming), the HRV Committee considers that it is justified to recommend an 8 hour-OEL and a 15 min-STEL for acetaldehyde. The recommended 8 hour-OEL aims to protect workers from long-term effects and a 15-min-STEL from short-term effects.

4.1 Establishment of an 8-hour occupational exposure level (8h-OEL)

4.1.1 Choice of the critical effect

No systemic effects are reported from acetaldehyde inhalation or skin exposure, in humans or animals (see above).

The documented effects after exposure to airborne acetaldehyde are irritative and carcinogenic (see chapter 3)

Acetaldehyde is classified as possibly carcinogenic to humans by several organisations (IARC: category 2B; European Union: category 1B; US EPA: category B2, ACGIH: category A2; etc.) (cf. chapter 1.3). No study investigating the carcinogenicity of acetaldehyde exposure in humans was found in the literature. In animal models, nasal tumours in rats and laryngeal tumours in hamsters have been reported following exposure to acetaldehyde vapour at concentrations of 735 ppm (1,322 $mg.m^{-3}$) and higher (Woutersen et al. 1986), and in hamsters exposed at 1,650 ppm (2,970 $mg.m^{-3}$) (Feron, Kruysse and Woutersen 1982). However, it should be noted that the lowest concentration tested (750 ppm) is very high, and the high mortality observed in both studies make it difficult to interpret the data.

Analysis of the mechanism of action of carcinogenic effects indicates that they would occur at exposure levels inducing cytotoxicity associated with regenerative cell proliferation (see chapter 3.8). This was also observed for formaldehyde, for which exposure to lower concentration levels (5.6 ppm) than those inducing tumours induces local cytotoxicity but does not increase the incidence of tumours (Anses 2018). Based on the available data, and as was suggested for nasopharyngeal cancers induced by formaldehyde in the Anses report of 2018, a threshold dose may exist for carcinogenic effects of acetaldehyde, the existence of which is supported by the presence of a local defence mechanism saturating at high concentrations. Moreover, the local reactivity and rapid metabolism of acetaldehyde, is such that toxicity is only observed above a certain threshold.

The HRV Committee retained the existence of a threshold for the carcinogenic effects of acetaldehyde in the nasal cavity (cytotoxicity associated with regenerative proliferation, genotoxicity at cytotoxic doses).

Chronic inhalation of acetaldehyde leads to local irritation in the respiratory tract at the site of contact due to its physicochemical and toxicokinetic characteristics. These irritant effects appear at lower doses than carcinogenic effects, in particular in the upper respiratory tract leading to lesions of the epithelium after repeated exposure. These effects are therefore retained as critical effects, both for non-carcinogenic and carcinogenic effects. In other words, protecting against prolonged irritation would also protect against cancer.

Based on all evidence, alteration of the olfactory epithelium was retained by the HRV Committee as the critical endpoint to derive an 8h-OEL which will protect against both non-carcinogenic and carcinogenic effects of acetaldehyde inhalation exposure.

4.1.2 Choice of the key study

According to the methodology used by Anses for the derivation of OELs (Anses, upcoming), at equal quality, human data are considered more relevant than animal data for the establishment of an 8h-OEL. However, no relevant chronic human study is available.

Most animal studies were performed over exposure periods too short to be considered relevant for the establishment of an 8h-OEL. Among the available sub chronic studies (Kruysse, Feron and Til 1975; Feron 1979; Feron, Kruysse and Woutersen 1982 and 1984 ; Dorman et al. 2008), the study of Dorman et al. was selected as the most relevant and robust study (Klimisch score 1; see Klimisch file in annex 3) which observed the critical effect retained at the lowest doses (Dorman et al. 2008).

This study investigated the effects of acetaldehyde on the upper respiratory tracts (histopathology, cell proliferation) in rats. Fisher F344 rats (n = 60/concentration) were exposed to acetaldehyde at 0, 50, 150, 500 or 1,500 ppm (0, 90, 270, 900, 2,700 $mg.m^{-3}$), 6 hours per day, 5 days per week for 13 weeks. At 1500 ppm, effects were observed on respiratory epithelium (hyperplasia and metaplasia from the nostrils to the dorsal olfactory tissue). At concentrations equal and up to 500 ppm, observed effects on respiratory and olfactive epithelium (metaplasia and hyperplasia minimal to moderate) and effects on larynx (metaplasia of squamous cells minimal to moderate) were reported. The histopathological modifications occurring at the lowest doses correspond to neuronal losses in the olfactive epithelium, which were observed from 150 ppm. The severity and distribution of these lesions increased with exposure duration and concentration.

The 2008 study of Dorman *et al*. is selected by the HRV Committee as key study for the establishment of the 8h-OEL. This is indeed the most robust study available by inhalation, with the longest duration of exposure (13 weeks).

4.1.3 Identification of the point of departure (PoD)

In the study by Dorman *et al*., a degeneration of olfactive epithelium was observed in rats from 150 ppm. This concentration is therefore considered as a LOAEC and the concentration of 50 ppm (90 $mg.m^{-3}$) as a NOAEC (Dorman et al. 2008). In the absence of quantified results at a concentration of 50 ppm, it was not possible to perform benchmark dose (BMD) modelling.

The NOAEC of 50 ppm (90 $mg.m^{-3}$) is retained as the PoD.

4.1.4 Temporal adjustment

In the study by Dorman et al., F344 rats were exposed for 13 weeks (6h/d, 5d/week) by inhalation. Considering that acetaldehyde is an irritant that induces tissue lesions in the olfactive epithelium through repeated exposure, and in order to take into account the difference in exposure time between animals (6h/day) and workers (8h/day), a time adjustment was applied to the NOAEC as following:

$$NOAEC_{ADJ} = NOAEC \times 6h/8h = 67.5\ mg.m^{-3}\ (37.5\ ppm)$$

4.1.5 Dosimetric adjustment

A human equivalent NOAEC ($NOAEC_{HEC}$) was calculated based on the $NOAEC_{ADJ}$ derived from the key study to take into account the dosimetry differences between the animal species (rats) and humans. Acetaldehyde is considered as a Category 1 gas, which according to the US EPA causes a local extra thoracic damage to the upper respiratory tract (US EPA 1994b; US EPA 2009).

The HRV Committee applied the following formula:

$$NOAEC_{ADJ\ HEC} = NOAEC_{ADJ} \times \text{Regional Gas Dose Ratio} = NOAEC \times (V_A/SA_A)/(V_H/SA_H)$$

$$NOAEC_{ADJ\ HEC} = 67.5 \times [(0.2/15) / (10/200)] = 17.6\ mg.m^{-3}\ (10\ ppm)$$

Considering: $NOAEC_{ADJ\ HEC}$ = NOAEC in humans

$NOAEC_{ADJ}$ = NOAEC in animals with a time adjustment

V_A = ventilation rate in rat = 0.20 m^3/day

SA_A = surface area of extra thoracic region in rat = 15 cm^2

V_H = ventilation rate in worker = 10 m^3/day

SA_H = surface area of extra thoracic region in human = 200 cm^2

4.1.6 Application of uncertainty factors

An uncertainty factor of 10 was applied to $NOAEC_{ADJ\ HEC}$ which is set as follows:

- Inter-species variability: **$UF_{A\text{-}TD}$ =** 1

To address inter-species variability, the dosimetry adjustment previously performed made it possible to calculate an equivalent concentration in humans. Acetaldehyde is an irritant. As rats and humans breathe differently (through the nose and mouth for humans and the nose only for rats), higher exposure of the respiratory and olfactory mucous membranes is expected in rats. Consequently, no additional uncertainty factor was applied according to Anses methodological guidance document (Anses, upcoming).

- Intra-species variability: **$UF_H = \sqrt{10}$**

An UF_H of $\sqrt{10}$ was applied because the key study was carried out in rodents, few parameters could be evaluated with regard to the effects that could be observed in humans (e.g. induction of bronchial hyperresponsiveness, ciliary motility in the upper respiratory tract or bronchi), the variability is considered to be less for an irritant effect. Therefore the HRV Committee decided to apply an UF_H of $\sqrt{10}$.

- PoD is a NOAEC : **$UF_{L/B} = 1$**

- Extrapolation from mid to long term exposure: **$UF_S = \sqrt{10}$**

Due to the lack of data on effects related to long term exposure, extrapolation from sub chronic effects was conducted. The duration of the selected key study, considered in toxicology to be "sub chronic" (the animals were exposed 5 days a week for 13 weeks), corresponds to approximately 10% of the animals' lives, which, in humans, would correspond to about 7 years of exposure according to conventions. As shown in the 2008 key study of Dorman et al., irritant effects can be cumulative. At the start of exposure, only minimal damage to the olfactive epithelium is observed, then on a longer period, it is expected to observe cumulative irritant effects (such as necrosis, metaplasia, hyperplasia and signs of inflammation). Similarly, there is insufficient data to assess whether similar effects could occur following chronic exposure to concentrations lower than those tested in sub chronic studies. In addition, other effects, not observed in sub chronic exposure studies, could occur following repeated long-term exposure (chronic respiratory diseases, repeated DNA mutations).
Therefore, the HRV Committee applied a value of $\sqrt{10}$ for this uncertainty factor.

- Due to completeness of data : **$FI_D = 1$**

Therefore, the HRV Committee recommends an 8h-OEL of 1,76 mg.m^{-3} rounded to 1.8 mg.m^{-3} (1 ppm). Preventing respiratory irritation, this 8h-OEL is expected to protect also against nasal cavity cancer.

4.2 Establishment of a 15 minutes short term exposure level (15 min-STEL)

4.2.1 Choice of the critical effect

Acetaldehyde is a respiratory and eye irritant which, due to its high reactivity, mainly exerts its toxicity at the point of entry into the body. Symptoms observed following aerial exposure include irritation of upper and lower respiratory tract. Although the nasal and ocular tissues seem to be the most sensitive targets, available studies on these endpoints were judged to be of insufficient quality. Acetaldehyde was shown to be a sensory irritant in rats, with a 50% reduction in respiratory rate (RD50 at 3,046 ppm (5,500 mg.m-3)) (Cassee et al. 1996). Steinhagen and barrow (1984) also show RD50 values of 2845 and 2932 ppm (5121 and 5277 mg.m-3) in mice. Such sensory irritation is expected to appear at lower doses than 'lesional' irritation, the last inducing irreversible damage. The human studies of Prieto et al. (2000) and Myou et al. (Myou et al. 1993, 1994), all estimated to be of good quality, focused on bronchoconstriction induced via the mouth (inhalation by mouth, clipped nose) in asthmatics. The bronchial hyper-reactivity corresponding to a sensory irritative effect is chosen as critical effect, since it appears earlier than respiratory tract lesion.
The most sensitive effect in humans, that is to say the bronchoconstriction induced in asthmatics, is retained as critical effect.

4.2.2 Choice of the key study

According to the Anses methodological guidance document (Anses, upcoming), at equal quality, human data is considered more relevant than animal data.

Three studies following the same experimental protocol showed bronchoconstriction in asthmatics: (Prieto et al. 2000); (Myou et al. 1993); (Myou et al. 1994).

Groups of healthy and asthmatic volunteers were exposed by inhalation to an aerosol of acetaldehyde (140, 280, 560, 1120 mg.m^{-3}) for 2 minutes. Results showed a significant dose-dependent reduction in FEV1 in asthmatic volunteers (compared with healthy controls) (Myou et al. 1993). In another study, asthmatic volunteers of Japanese origin were exposed to a sub-clinical concentrations - i.e. not causing bronchoconstriction - of a 0.8 mg.mL^{-1}saline solution of acetaldehyde. The aim of this study was to find out whether acetaldehyde exposure decreased the dose methacholine inducing à 20 % decrease of FEV1 (Myou et al. 1994). Myou *et al.*'s studies were not selected for the construction of a 15min-STEL because of the small number of subjects (n=9) and because they did not aim to study the dose-response relationship on acetaldehyde-induced bronchoconstriction.

The study of Prieto et al. described the exposure of asthmatic volunteers to acetaldehyde for a few minutes (Prieto et al. 2000). In this study, sixty-one (61) asthmatic adult subjects (and 20 control non-asthmatic subjects) were exposed under experimental conditions identical to those of the 1994 study of Myou *et al.* (Prieto et al. 2000). However, the studied health effects were different compared with those taken into account by Myou *et al.*; the effect of acetaldehyde exposure on the bronchoconstriction was documented by measuring the decrease in FEV1. The results presented in the article by Prieto *et al.* indicate that the atmospheric concentration of acetaldehyde inducing a 20% (PC20) decrease in FEV1 was: 142 mg.m^{-3} (79 ppm). This study was judged to have a good

sensitivity, as bronchoconstriction was observed in almost all asthmatic subjects, while no healthy volunteers presented this effect. Although the study was considered sufficiently robust to be retained as a key study, it has certain limitations: short exposure duration (2 min), absence of atmospheric measurement.

The 2000 study of Prieto *et al*. is selected as the key study for the proposal of a 15 min-STEL.

4.2.3 Identification of the point of departure (PoD)

In the 2000 study of Prieto et al., the LOAEC corresponds to an air acetaldehyde concentration of 142 $mg.m^{-3}$ (79 ppm) (cf. chapter 3.2.1) (Prieto et al. 2000).

The LOAEC of 79 ppm (142 $mg.m^{-3}$) is retained as point of departure.

4.2.4 Temporal adjustment

The toxicity of sensory irritants such as acetaldehyde is more dependent on the concentration than on the duration of exposure (Belkebir et al. 2011). Indeed, the mode of action responsible for sensory irritation effects is similar among chemical agents giving rise to irritation effects. It derives mainly from the activation of the body's alert and protective reflex to chemicals that come into contact with the mucous membranes of the eyes and respiratory tract. This reflex is mediated by the trigeminal nerve. The sensory effects appear at lower doses than 'lesional' irritation, the latter inducing irreversible damage.

Therefore, no time adjustment was applied to the LOAEC.

4.2.5 Application of uncertainty factors

An uncertainty factor of 10 was applied to the $LOAEC_{ADJ}$ which is established as follows:

- Inter-species variability: **UF_A = 1**

The key study is used to elaborate a 15min-STEL based on a human population and on a subgroup particularly sensitive (asthmatics), so an uncertainty factor of 1 is applied.

- Intra-species variability: **UF_H: 1**

The key study was carried out on individuals particularly sensitive to respiratory irritants (asthmatics). Protecting this population also protects the entire population of workers. Individuals with asthma are known to present bronchial hyperresponsiveness. Using the methacholine challenge test for measuring bronchial reactivity, PD20 and PC20 (dose and concentration of methacholine inducing a 20 % decrease in FEV1) of asthmatic patients are lower than those of non-asthmatic subjects. Asthmatics are therefore a sensitive population.

- Use of LOAEC/severity of effects: **$UF_{L/B}$ = √10**

A value of √10 is retained to consider the use of a LOAEC rather than a NOAEC. The HRV Committee applied a value of √10 and not 10 for this uncertainty factor because of the nature of the critical effect observed in a sensitive population (bronchoconstriction in asthmatics).

- Quality of the database: **UF_D = √10**

A value of √10 is selected to take into account the use of aerosol exposure via a saline solution and the experimental modalities of exposure to acetaldehyde using a nebuliser (saline aerosol) which remains an usual technique in asthmatic testing.

The HRV Committee recommends a 15 min-STEL of 14.2 mg.m^{-3} (7.9 ppm).

4.3 “Skin” notation

Due to its high reactivity at contact site, acetaldehyde is expected to binds to compounds in epidermis and not reaching derma, leading to weak passage in the systemic circulation. Only one study provided quantitative data on dermal absorption (Thredgold et al. 2020) (cf. chapter 3.1.1.) However, this study presents some limitations and does not allow to determine the contribution of the dermal route to overall exposure of workers.

In the absence of sufficient data on dermal absorption, **no "skin” notation is recommended for acetaldehyde.**

4.4 “Noise” notation

As there are no data on possible interactions during co-exposure to noise and acetaldehyde, **the "noise" notation is not recommended.**

5 Conclusions of the collective expert appraisal on health effects

Table 13 : Summary table of recommended occupational threshold OELs

Type of OEL		**8h-OEL**	**15 min-STEL**
RV	Organisation	Anses	Anses
	Year	2024	2024
	Value	**1.8 mg.m^{-3} (1 ppm at 25°C and 0.9 ppmat 20°C*)**	**14.2 mg.m^{-3} (7.9 ppm at 25°C and 7.1 ppm at 20°C*)**
Target population		Workers	Workers
Critical Effect		Olfactive epithelium alteration	Bronchoconstriction in asthmatic adults
Key study	Reference	Dorman et al. 2008	Prieto et al. 2000
	Population of the study or species	Adult F344 rats	Asthmatic adults
	Exposure (time, route)	13 weeks (6h/d, 5d/week), inhalation	2 min (nebuliser)
Point of departure (PoD)		NOAEC = 90 mg.m^{-3} (ie 50 ppm)	LOAEC = 4.72 mg.mL^{-1} = 142.3 mg.m^{-3} (ie 79 ppm**)
Time Adjustment		NOAEC$_{ADJ}$= 90 x 6/8 = 67.5 mg.m^{-3} =.37.5 ppm	/
Dosimetry Adjustment		NOAEC$_{ADJ\ HEC}$ = NOAEC$_{ADJ}$ x RGDR = 17.6 mg.m^{-3} (10 ppm)	/
Uncertainty Factors (UF)		10 (UF$_{A\text{-}TD}$:1; UF$_H$: √10 ; UF$_{L/B}$: 1; UF$_S$: √10; UF$_D$:1)	10 (UF$_A$: 1 ; UF$_H$: 1; UF$_{L/B}$: √10 ; UF$_D$: √10)
« skin » notation		None	
« noise » notation		None	

** considering a Hudson 1720 nebuliser: air flow per minute = 6 L; nebulisation time = 2 to 4 minutes; acetaldehyde solution output = 0.18 mL.min^{-1}:
4.72 mg.mL^{-1} * [(0.18 mL.min^{-1} / 0.006 m^{-3}.min^{-1})] = 4.72 mg.mL^{-3}*30 mL.m^{-3} =142 mg.m^{-3}

***** Additionally, as the European directives setting OELs take into account a conversion factor at 20°C, the recommended values also are expressed using this conversion factor. 1 ppm (mL.m^{-3}) equivalent to 2 mg.m^{-3} at 20°C.

Part B: Report on the assessment of methods for measurement of exposure levels in workplace atmospheres

1 Presentation and discussion of methods for measuring acetaldehyde in workplace air

The methods for measuring the concentration of a substance in workplace air are evaluated to recommend one or more methods for carrying out concentration measurements of the substance for comparison with the OELs recommended by the Anses committee or with the OELs established in the European directives.

The objective is not to classify all the methods according to a numerical scoring system but rather to present in a structured and systematic way the criteria enabling the final choice based on a scientific judgement (Figure 2).

The general principle is as follows:

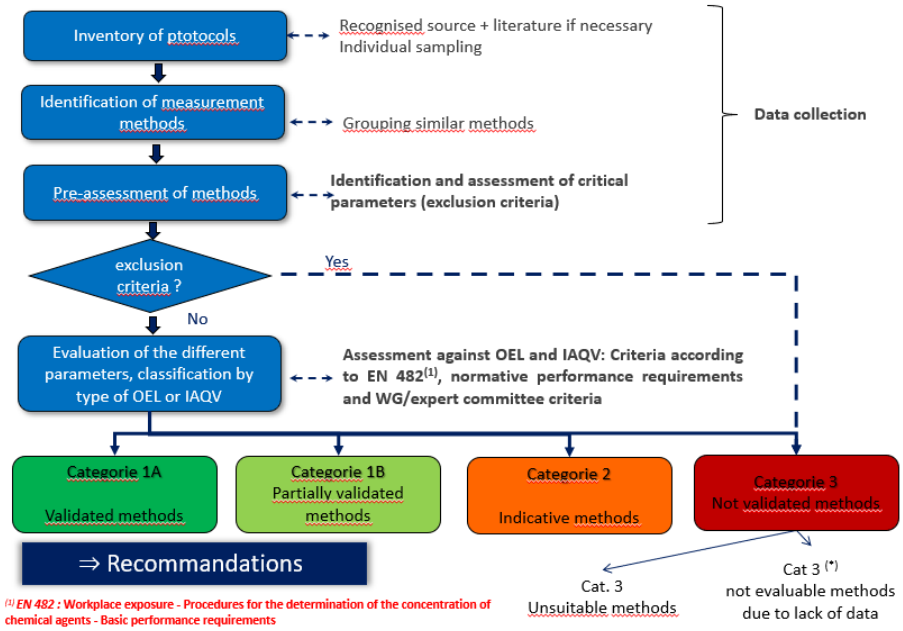


Figure 3 : General principle for the classification of analytical methods (Anses 2020)[19]

[19] The term "complementary standards" refers to the available standards setting additional requirements to those of standard NF EN 482 to be met for certain particular types of measuring procedures and devices. The WG acronym in this figure refers specifically to the working group Metrology in charge of the work of evaluating measurement methods.

1.1 Mapping measurement methods

Acetaldehyde is a very volatile carbonyl compound with a boiling point of 20.1 °C. Respiratory occupational exposure to acetaldehyde mainly occurs in gaseous form.

As acetaldehyde falls within the scope of the OEL work program, only workplace air measurement methods were evaluated in detail. The working group did not select protocols developed exclusively for measuring ambient exposure levels in a work area or to control environmental or indoor air quality and not suitable to personal exposure sampling like the use of canisters as described in the US-EPA TO-15 method. In addition, it rejected the use of portable glass bubblers containing a reagent in a solvent and placed close to the breathing zone as described in the NIOSH 3507 method taking into account their main drawbacks, namely solvent evaporation over long sampling periods, discomfort for workers in their movements and postures, and risk of glass bubblers breaking. Given the disadvantages mentioned and the fact that other methods have also been referenced, these methods have not been evaluated.

Table 14 presents the measurement methods that were identified and evaluated in the present report.

Table 14 : Summary table of methods for measuring acetaldehyde in workplace air

Methods						
	Similar protocols		Sampling	Supports	Desorption/ extraction	Analysis
	Occupational exposure	Indoor/ambient exposure				
A	NF X 43 264 (2011) ; INRS M-66 (2016) ; US EPA TO-11A (1999) ; NIOSH 2018 (2013) ; HSE MDHS 102 (2010) ; DFG method 1 (1990) DFG Method 2 (1995)	NF ISO 16000-3 (2011)	Active	Silica gel, polymeric adsorbent or glass fiber filter coated with acidified 2,4 dinitrophenylhydrazine (DNPH)	Acetonitrile	HPLC-UV
B	OSHA 68 (1988), NIOSH 2538 (1994), IRSST 385		Active	Polymeric adsorbent coated with hydroxymethylpyperidine (hmp)	Toluene	GC-NPD or GC-FID
C		LCSQA 2007, LCSQA 2013, Radiello®	Passive	Silica and magnesium oxides coated with acidified 2,4 DNPH	Acetonitrile	HPLC-UV

The main performance criteria for sampling and analytical methods used in the workplace atmosphere are summarised in chapter 1.2.

Details in terms of sampling media, sample processing, analysis and validation data are given in Annex 4.

1.2 Detailed assessment of the methods

Requirements: Considering the 8h-OEL and the 15 min-STEL recommended by the Committee, methods should be validated in the following concentration range:

- 0.1 to 2 *8h-OEL: 0.18 – 3.6 mg·m^{-3} (for the technical regulatory control)
- 0.1 to 2 *15min-STEL: 1.4 – 28 mg·m^{-3} (for the technical regulatory control)
- 0.5 to 2 *15-min-STEL: 7 – 28 mg·m^{-3} (for the monitoring of short exposure)

The following table presents the rating of identified methods relevant to measure worker's acetaldehyde exposure (Table 15). The evaluation is described in the following paragraphs.

Table 15 : Rating of monitoring methods for workplace acetaldehyde assessment

Acetaldehyde Monitoring				
	Methods	Protocols	8h-OEL	15min-STEL
			Technical regulatory control	Technical regulatory control
A	Active sampling on a DNPH-coated silica gel in a sampling tube or on glass filters – desorption with acetonitrile – Determination by liquid chromatography using a UV/visible detector	(NF ISO 16000-3 (2011) ; NF X 43 264 (2011) ; INRS M-66 (2016) ; US EPA TO-11A (1999) ; NIOSH 2018 (2013) ; HSE MDHS 102 (2010) ; DFG method 1 (1990) DFG Method 2 (1995)	**1B** **DNPH coated silica gel, (2x4 h)** **2** **DNPH coated filter cassette**	**1B** **DNPH coated silica gel** **2** **DNPH coated filter cassette**
B	Active sampling on XAD-2® adsorbent resin coated with 2-HMP – desorption with toluene - Determination by gas chromatography – FID/NDP/mass detector	OSHA 68 (1988), NIOSH 2538 (1994), IRSST 385	**2**	**3** **(2 for monitoring of short exposure)**
C	Passive sampling on a DNPH/H_3PO_4-coated badge (3 types of badge) – Determination by liquid chromatography using a UV/visible detector	HSE MDHS 102 (2010) LCSQA, 2007; LCSQA 2013	**3**	**3**

Figures 4 and 5 present the ranges for which the various methods were tested and their limits of quantification for the 8h-OEL and 15 min-STEL values recommended by the Committee.

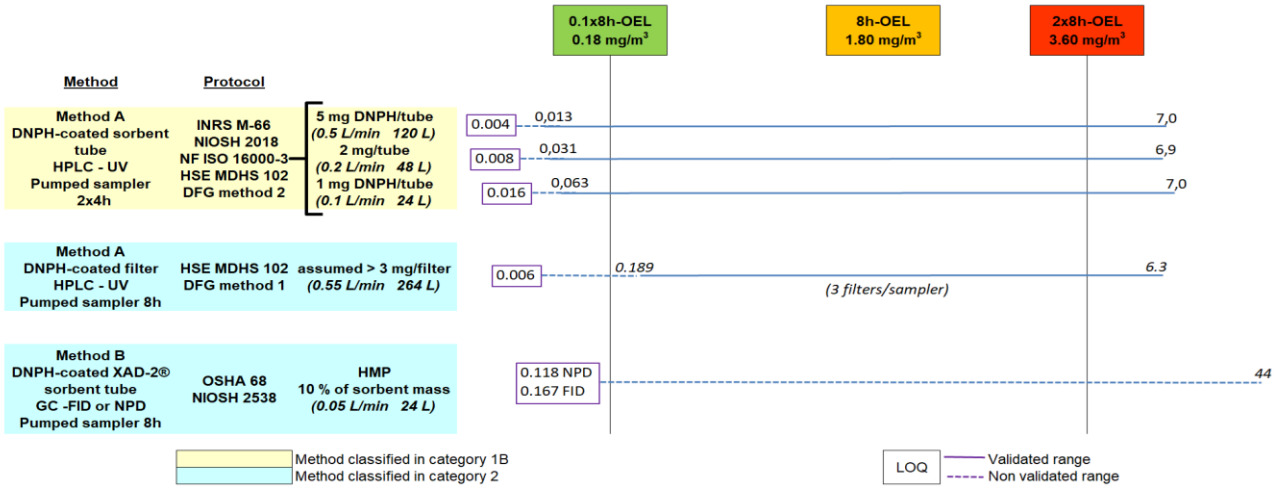


Figure 4: Working range and quantification limits of the different methods classified as 1B and 2 compared to the 8h-OEL (NF ISO 16000-3, 75% safety level)

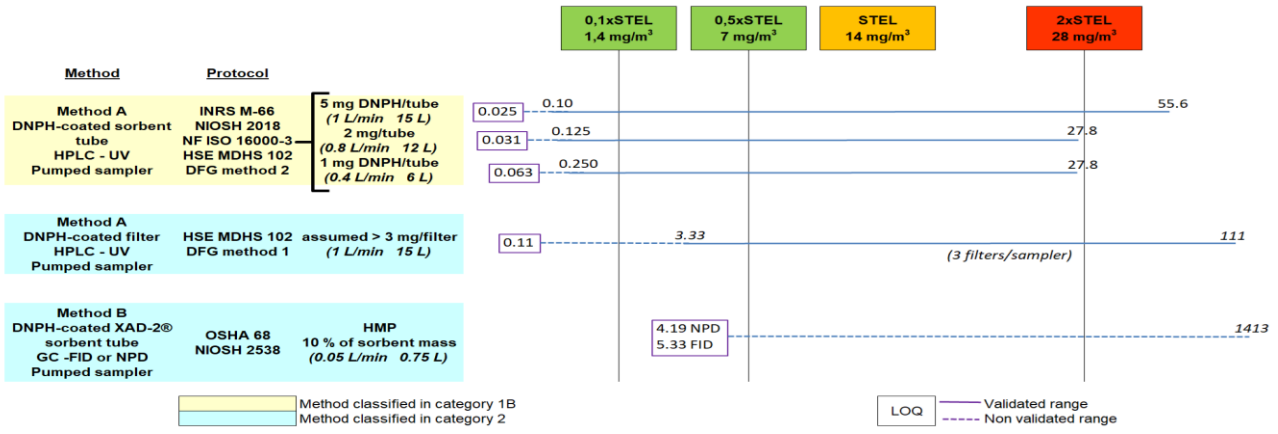


Figure 5 : Working range and quantification limits of the different methods classified as 1B or 2 compared to the 15min-STEL (NF ISO 16000-3, 75% safety level)

1.3 Detailed assessment of method A: Active sampling on a DNPH-coated silica gel in a sampling tube – desorption with acetonitrile – Determination by liquid chromatography using a UV/visible detector

This method is described by eight protocols: NIOSH 2018, HSE MDHS 102, INRS M-66, EPA TO-11A, and DFG methods 1 and 2, NF ISO 16000-3 and NF X43-264. Although standard 16000-3 concerns indoor exposure, it describes this method in detail and forms the basis of the MDHS 102 protocol for occupational exposure. Method A involves trapping acetaldehyde through chemisorption by derivatizing it into hydrazone using 2,4-DNPH (2,4-dinitrophenylhydrazine) impregnated on a surface, typically a silica gel or a glass fibre filter. The acetaldehyde reacts with the hydrazine to form a non-volatile derivative (melting point 164°C) that remains fixed on the surface of the support (see Figure 4). The sampling is active, with air being pumped at a known flow rate through a tube containing the impregnated silica gel. The HSE MDHS 102 and DFG method 1 protocols offer an alternative where the silica gel can be replaced by one or more DNPH-impregnated glass fibre filters arranged in a filter cassette.

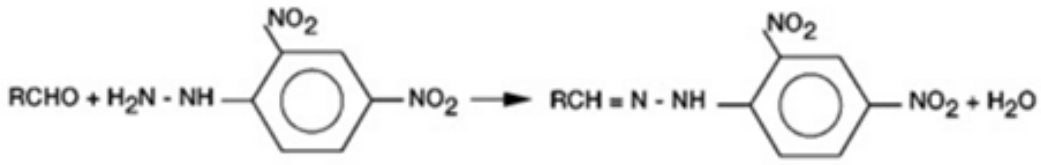


Figure 6: Reaction principle of the chemisorption of acetaldehyde into hydrazone in the presence of 2,4-DNPH

For analysis, the impregnated silica gel, or the glass fibre filter, is desorbed directly into a vial or by percolation with a known volume of acetonitrile. The acetaldehyde hydrazone, solubilised in the solvent, is then injected into a high-performance liquid chromatography (HPLC) system coupled with a UV spectrophotometric detector at 360-365 nm. The separation column is a reverse-phase C18, and the eluent is a mixture of acetonitrile and water, with proportions varying according to the protocol. The acetaldehyde hydrazone is identified and quantified by comparison to a range of reference standards analysed using the same protocol.

The NF X43-264 protocol only describes the various steps of the method and sets performance specifications without providing validation data. The EPA TO-11A protocol is specific to formaldehyde but dedicates Chapter 14 to the adaptation of chromatographic conditions for the measurement of other aldehydes.

Characteristics of the sampling support

The studied standards and protocols describe the method for preparing the DNPH-impregnated support. Between 350 and 500 mg of support with a particle size of 55 to 105 mesh is used, with a DNPH concentration on the support ranging from 0.29% to 2% by mass. The use of commercially available media is also considered in NF ISO 16000-3, NF X 43 264, US EPA TO-11A, NIOSH 2018, HSE MDHS 102, and DFG method 2 protocols. The main commercially available sampling media mentioned are the SKC™ silica gel-DNPH tube, the Supelco™ LpDNPH cartridge, and the Waters™ Sep-Pak DNPH cartridge. The DFG method 1 and HSE MDHS 102 protocols use DNPH-impregnated glass fibre filters, and DFG method 1 combines 2 to 3 filters, 37 mm in diameter, in the same filter holder cassette.

Support capacity

When using these different impregnated media, the molar concentration of carbonyl compounds, including acetaldehyde, in the sampled air volume should not exceed the molar capacity of DNPH. Each milligram of DNPH corresponds to 0.005 millimoles (molar mass of DNPH: 198.14 $g.mol^{-1}$). The NF ISO 16000-3 standard states that, generally, a sample size less than 75% of the DNPH mass load contained in the sampling support is considered acceptable. The NIOSH 2018 protocol, which is more conservative, applies for the Supelco™ LpDNPH cartridge a safety factor of 2/3 of the consumed DNPH.

Cartridges prepared according to the NF ISO 16000-3 and the EPA TO-11A protocols contain about 2 mg of DNPH, corresponding to an estimated capacity of 334 µg of acetaldehyde, at the 75% safety level. The cartridge prepared according to the MétroPol M-66 protocol from INRS contains 5 mg of DNPH, corresponding to an estimated capacity of 834 µg of acetaldehyde. The commercial cartridges mentioned in the NF ISO 16000-3, EPA TO-11A, NIOSH 2018, HSE MDHS 102, and DFG method 2 protocols contain approximately 1 mg of DNPH, with an estimated trapping capacity of 167 µg of acetaldehyde.

The amount of DNPH impregnated on the filter in the HSE MDHS 102 protocol is not specified. The DFG method 1 protocol provides details on the impregnation method: the 37 mm diameter glass

fibre filter is immersed for 10 seconds in a solution of 30 mg.mL^{-1} DNPH in acetonitrile acidified with phosphoric acid, then placed on a grid for 90 seconds. This operation is repeated 3 times before drying in a desiccator. The final DNPH load available for sampling is not specified, but it is likely to exceed 10 mg, as the protocol recommends using 2 or even 3 filters in the filter holder cassette.

For these last two protocols, sampling is performed with a filter holder cassette that can contain 1 to 3 filters. Since this involves a gas, sampling can be done with the cassette either open or closed. This parameter, which determines the air velocity through the filter(s), is not specified and may affect trapping efficiency if the aldehyde derivatization reaction kinetics are not adhered to.

Based on the NF ISO 16000-3 protocol, the trapping capacities of the different media are presented in Table 16, considering a 75% safety level.

Table 16 : Trappable acetaldehyde mass based on the mass of DNPH impregnated on the support, with a 75% safety factor (cf. NF ISO 16000-3)

Support	Mass DNPH/support (mg)	Conc. mol. DNPH (millimole)	Trappable acetaldehyde mass (mg)
Silica gel cartridge prepared according to INRS	5	0.0252	0.834
Silica gel cartridge prepared according to NF ISO 16000-3	2	0.0101	0.333
Tube Supelco™ LpDNPH, & SKC™silica gel DNPH & Waters™Sep-Pak	1	0.005	0.167
Coated filter cassette prepared according DFG method 1	≈ 10	≈ 0.0505	≈ 1.667

Sampling characteristics

Sampling flow rate:

- NF ISO 16000-3 and NIOSH 2018: Sampling flow rate between 0.1 and 1.5 L.min^{-1}. This standard specifies that the pressure drop caused by the tube at 1.5 L.min^{-1} may affect the pump, and ultimately recommends a maximum flow rate of 1.2 L.min^{-1}.
- EPA TO-11A: Flow rate of 1 L.min^{-1} (but 0.1 to 2 L.min^{-1} possible) for cartridges with a single bed, and 0.8 L.min^{-1} for those with a second bed to detect potential breakthrough (SKC™ silica gel DNPH).
- INRS M-66: Flow rate between 0.2 and 1 L.min^{-1}.
- DFG method 2: Flow rate on the Waters™ Sep-Pak cartridge is 0.1 L.min^{-1}.
- HSE MDHS 102: The impregnated filter supports a flow rate between 0.1 and 1 L.min^{-1}.
- DFG method 1: The three filters combined in a filter holder cassette support a flow rate of 0.33 L.min^{-1}.

Sampling duration and sampled volume:

- NIOSH 2018: No specific duration given, apart from the possibility of conducting both short- and long-term measurements, with a maximum sampled volume of 15 litres for an atmosphere containing 7 to 15 $mg.m^{-3}$ of acetaldehyde.
- NF ISO 16000-3: Sampling duration ranges from 5 minutes to 24 hours, i.e., 18 litres in 15 minutes at 1.2 $L.min^{-1}$ and 240 litres at a flow rate of 0.5 $L.min^{-1}$.
- EPA TO-11A: The sampling volume should be adapted based on the suspected concentration and the DNPH load of the sampling support. The sampling duration should be determined based on this volume and the selected flow rate between 0.1 and 2 $L.min^{-1}$.
- INRS M-66: 15 minutes and 15 litres at 1 $L.min^{-1}$, and 8 hours and 96 litres sampled at 0.2 $L.min^{-1}$.
- DFG method 2: 6 litres for one hour.
- HSE MDHS 102: For aldehyde concentrations below 2 $mg.m^{-3}$, 15 minutes and 1.5 litres sampled over 8 hours and 480 litres.
- DFG method 1: 20 litres sampled over one hour.

Validation domain:

The method has been validated across different domains for two protocols, as presented in Table 17.

Table 17 : Validation domains identified in the protocols

Protocols	Sampling support	Sampled volume (L)	Concentration range ($mg.m^{-3}$)	Fraction of the 8h-OEL	Fraction of the 15min-STEL
NIOSH 2018	Supelco™ LpDNPH & Waters™ Sep-Pak	15	0.045 to 10.5 at 85% RH to 11.9 at 10% RH	0.025 to 3.88	0.003 to 0.5
DFG method 1	Coated filter cassette	20	0.167 to 25	0.093 to 13.9	0.011 to 1.8

The concentration ranges validated by the two protocols cover the range targeted for the 8h-OEL ; however, they do not cover the range required to encompass 0.1 to 2 times the 15min-STEL. The flow rates needed for 8 hours sampling to respect these ranges are 0.03 $L.min^{-1}$ for tubes and 0.04 $L.min^{-1}$ for filters, which are much lower than the flow rates prescribed in the protocols, namely 0.1 and 0.33 $L.min^{-1}$, respectively however such flow rates can be easily used in the field.

The EPA TO-11A, INRS M-66, DFG method 2, HSE MDHS 102 protocols and the NF ISO 16000-3 and NF X 43-264 standards do not specify the studied concentration range.

Breakthrough volume:

Based on the potentially trappable acetaldehyde masses on the different media, with the 75% safety level prescribed by the NF ISO 16000-3 protocol, it is possible to determine the maximum sampling volumes to reach the 8h-OEL or the 15min-STEL without exceeding the support's capacity.

$$V_{max}\ (L) = (\text{Potentially trappable aldehyde mass} \times 1000) / (2 \times \text{Limit Value}).$$

From these volumes, presented in table 18, the corresponding flow rates to be implemented can be derived.

Table 18 : Maximum sampling volumes without exceeding the trapping capacity of the different media

Sampling support	DNPH /support (mg)	Trappable acetaldehyde mass (mg)	Maximum volume for 2*8h-OEL (L)	Maximum volume for 2*15 min-STEL (L)
Silica gel cartridge prepared according to tube INRS	5	0.834	232	29.8
Silica gel cartridge prepared according to tube NF ISO 16000-3	2	0.333	92.5	11.9
Tube Supelco™ LpDNPH, & SKC™silica gel DNPH & Waters™Sep-Pak	1	0.167	46.4	6.0
Coated filter cassette prepared according DFG method 1	≈10	1.667	463	59.5

Table 19 : Maximum applicable flow rates for 8 hours and 15 minutes sampling

Sampling media	DNPH /media (mg)	2*8h-OEL		2*15 min-STEL	
		Maximum volume (L)	Maximum flow rate ($L.min^{-1}$)	Maximum volume. (L)	Maximum flow rate ($L.min^{-1}$)
Silica gel cartridge prepared according to tube INRS	5	232	0.483 rounded to 0.5	29.8	1.99 rounded to 2.0
Silica gel cartridge prepared according to tube NF ISO 16000-3	2	92.5	0.193 rounded to 0.2	11.9	0.79 rounded to 0.8
Tube Supelco™ LpDNPH, & SKC™silica gel DNPH & Waters™Sep-Pak	1	46.4	0.097 rounded to 0.1	6.0	0.4
Coated filter cassette prepared according to DFG method 1	≈10	463	0.965 rounded to 1	59.5	3.97 rounded to 4

The NF ISO 16000-3 standard specifies that beyond a flow rate of 1.5 $L.min^{-1}$, the pressure drop introduced by the sampling tube may affect the pump's operation and recommends a maximum flow rate of 1.2 $L.min^{-1}$. The INRS protocol sets a maximum flow rate of 1.0 $L.min^{-1}$ for a tube containing 500 mg of silica gel.

The HSE MDHS 102 protocol specifies that 480 L can be sampled for exposures not exceeding 2 $mg.m^{-3}$. For 3.6 $mg \cdot m^{-3}$ over 8 hours, the volume will be limited to 265 L with a flow rate of 0.55 $L.min^{-1}$.
Standard NF X43-264 recommends validating the absence of breakthrough by checking on two-section tubes that the concentration measured in the back section does not exceed 5% of that in the front section. For tubes, cartridges, or filters without a second section of adsorbent, the standard recommends verifying that the chromatographic peak of DNPH in the sampled extract represents at least 10% of that found in a blank analysis.

Limit of detection:

NIOSH 2018: 0.2 µg on a Supelco™ LpDNPH cartridge, 1 mg DNPH, 13.3 $\mu g.m^{-3}$ for 15 litres sampled and desorbed in 10 mL of acetonitrile.

DFG method 2: 4 $\mu g.m^{-3}$ for 6 litres sampled and desorbed in 5 mL of acetonitrile.

DFG method 1: 50 $\mu g.m^{-3}$ for 20 litres sampled and desorbed in 10 mL of acetonitrile.

HSE MDHS 102: 0.5 to 3 $\mu g.m^{-3}$ for 15 litres depending on the type of sampling media, DNPH-impregnated filter desorbed in 2 mL of acetonitrile or commercial cartridges desorbed in the volume of acetonitrile specified by the manufacturer, generally 5 mL.

Limit of quantification:

NIOSH 2018: 0.68 µg on a Supelco™ LpDNPH cartridge (1 mg DNPH), corresponding to 45.3 $\mu g/m^3$ for 15 litres sampled and desorbed in 10 mL of acetonitrile, or 0.34 µg for 5 mL.

DFG method 2: Estimated from the LOD, 0,0132 $mg.m^{-3}$ for 6 litres sampled, with a quantification limit of 0.08 µg for desorption in 5 mL of acetonitrile.

DFG method 1: Estimated from the LOD, 0,165 $mg.m^{-3}$ for 20 litres sampled and desorption in 10 mL of acetonitrile, with a quantification limit of 1.65 µg for 5 mL.

HSE MDHS 102: Ranges from 0,0016 to 0,01 $mg.m^{-3}$ for 15 litres, depending on the type of sampling support. Impregnated filters are desorbed in 2 mL of acetonitrile, or commercial cartridges in 5 mL, giving a limit between 0.024 to 0.375 µg regardless of the support.

The highest quantification limit is 0.375 µg for a sampler packed with DNPH-coated silica gel, and 1.65 µg for an impregnated filter, both desorbed in 5 mL.

Table 20 provides quantification limits in $mg.m^{-3}$ based on these values and the sampled volumes for each support. These limits are below one-tenth of the 8h-OEL and 15min-STEL thresholds.

Table 20 : Determination of the Limit of quantification of various sampling supports for 8 hours and 15 minutes samples for comparison with the 8h-OEL and the 15 min-STEL

Sampling support	DNPH /sampling support (mg)	LOQ/sampling support (µg)	8h-OEL		15 min-STEL	
			Flow rate ($L.min^{-1}$)	LOQ ($mg.m^{-3}$)	Flow rate ($L.min^{-1}$)	LOQ ($mg.m^{-3}$)
Silica gel cartridge prepared according to tube INRS	5	0.375	0.5	0.0016	1	0.025
Silica gel cartridge prepared according to tube NF ISO 16000-3	2		0.2	0.0039	0.8	0.0313
Tube Supelco™ LpDNPH, & SKC™silica gel DNPH & Waters™Sep-Pak	1		0.1	0.0078	0.4	0.625
Coated filter cassette prepared according to DFG method 1	≈10	1.65	0.55	0.0063	1	0.11

Measurement Range

The accessible measurement range depends on the quantification limit and the maximum acetaldehyde mass that can be trapped. The maximum volume is calculated to avoid exceeding 75% consumption of the derivatization reagent present in the sampling tube. The quantification limit is influenced by the volume of acetonitrile used to desorb the sample, with the values in Table 21 and 22 provided for desorption using 5 mL.

Table 21 : Method applicability domain for monitoring the 8h-OEL

Sampling support	DNPH /sampling support (mg)	8h-OEL (8 hours sampling)		
		Flow rate ($L.min^{-1}$)	LOQ at maximal capacity ($mg.m^{-3}$)	Fraction of 8h-OEL
Silica gel cartridge prepared according to tube INRS	5	0.5	0.002 to 3.48	0.001 to 1.93
Silica gel cartridge prepared according to tube NF ISO 16000-3	2	0.2	0.004 to 3.47	0.002 to 1.93
Tube Supelco™ LpDNPH, & SKC™silica gel DNPH & Waters™Sep-Pak	1	0.1	0.008 to 3.48	0.004 to 1.93

Coated filter cassette prepared according to DFG method 1	≈10	0.55	0.006 to 6.31	0.002 to 3.51

Table 22 : Method applicability domain for monitoring the 15min-STEL

Sampling support	DNPH /sampling support (mg)	15min-STEL		
		Flow rate ($L.min^{-1}$)	LOQ at maximal capacity ($mg.m^{-3}$)	Fraction of 15min-STEL
Silica gel cartridge prepared according to tube INRS	5	1	0.025 to 55.6	0.002 to 3.97
Silica gel cartridge prepared according to tube NF ISO 16000-3	2	0.8	0.031 to 27.75	0.002 to 1.98
Tube Supelco™ LpDNPH, & SKC™silica gel DNPH & Waters™Sep-Pak	1	0.4	0.063 to 27.8	0.005 to 1.99
Coated filter cassette prepared according to DFG method 1	≈10	1.0	0.11 to 111.1	0.008 to 7.94

Trapping and desorption efficiency

The HSE MDHS 102 protocol reports a trapping efficiency for aldehydes greater than 99%.

In the DFG Method 2, trapping and desorption efficiency was tested only for formaldehyde.

The NIOSH 2018 protocol provides trapping and desorption efficiency values for acetaldehyde by spiking tubes with liquid solutions of acetaldehyde. Between 20 and 90 µL of solution at concentrations ranging from 50 to 300 $µg.mL^{-1}$ were added to each tube, with six tubes tested per concentration level. Table 23 outlines these values.

Table 23: Average percentage of acetaldehyde mass recovered after liquid spiking and analysis (n=6, desorption volume = 10 mL)

Spiked mass	1.5 µg	3.00 µg	6.00 µg	12.0 µg	20.0 µg	Standard deviation
Recovery %	106	99	108	106	100	3.1
$Mg.m^{-3}$ for 15 L sampled	0.1	0.2	0.4	0.8	1.33	

The DFG method 1 protocol also tested the efficiency of trapping and desorption by doping a cassette containing three triply impregnated filters before pumping 20 L of air through the cassette. The acetaldehyde spiking was carried out at two concentration levels, 50 and 500 µg. m^{-3}, or 2.5 and 25 $mg.m^{-3}$ for 20 L sampled, with apparently one test per level. The recovery rates were 88.8% and 93.3%, respectively.

Storage and efficiency

NIOSH 2018: That protocol is the only one to determine a recovery rate after storage. Storage of sampled tubes and solutions after desorption at 5°C in the dark. Recovery rate: 102% for tubes after 30 days (n=6). Solutions remain stable beyond 36 days ([acetaldehyde] = 1.25 µg.mL^{-1}).

NF ISO 16000-3: Minimize the period of non-refrigeration to less than two days. Samplers are refrigerated and stored for up to 30 days before analysis.

INRS M-66: Preservation for at least 8 days at 5°C.

HSE MDHS 102: Impregnated filters can be stored after sampling for at least two weeks at room temperature.

Linearity of detection

Calibration is external, performed using solutions with known concentrations of aldehyde hydrazone in acetonitrile.

According to NIOSH 2018, the response is linear between 0.68 and at least 150 µg per sampling support, with 20 µL injected into the analyser. For a sample of 15 L, this corresponds to a concentration range of 0.045 to over 10 mg.m^{-3}.

For DFG method 1, calibration is linear between 1 and 25 µg.mL^{-1} for a 20 µL injection, which corresponds to a range of 0.5 to 12.5 mg.m^{-3} for a sample of 20 liters desorbed in 10 mL of acetonitrile.

Interferences and environmental conditions during sampling

The standards and most protocols emphasize the importance of identifying the presence of aldehydes other than acetaldehyde, acetone, nitrogen oxides, and ozone in the sampled atmosphere. The first ones will react with DNPH, competing with acetaldehyde; nitrogen oxides and ozone will degrade both the DNPH and the formed hydrazones through photo-oxidation, with the latter mechanism being influenced by solar radiation.

The 16000-3 standard indicates a decrease in trapping efficiency at temperatures below 10°C, without observing a decrease at high temperatures. Conversely, the INRS protocol notes faster cartridge saturation at higher temperatures and under high humidity. The NIOSH 2018 protocol reports a 12.6% reduction in the capacity of the Supelco™ LpDNPH tube for samples taken at ambient temperature and 85% humidity compared to those taken at 10% humidity. As silica gel is hydrophilic, in very humid atmospheres, the formation of an aqueous condensate on the support is possible.

The HSE MDHS 102 and DFG method 1 protocols do not provide information on the influence of interferences, with HSE MDHS 102 simply citing reduced trapping capacity in the presence of acetone and other oxidants such as nitrogen dioxide and ozone.

Specificity

The specificity of the method in all protocols depends on the applied chromatographic conditions, including the relative concentrations of acetonitrile and water in the eluent, the elution gradient, the flow rate, the separator column specifications, and the absorption wavelength (360 or 365 nm). With the exception of the NF X43-264 standard, all mentioned protocols specify analysis conditions that allow for a good resolution of the acetaldehyde hydrazone peak.

Uncertainties

The NIOSH 2018 protocol studied the analytical repeatability for spiking 5 batches of six tubes with acetaldehyde in solution at different concentration levels ranging from 1.5 to 20 µg per tube, or 0.1 to 1.33 mg.m^{-3} for 15 L of air sampled. The average recovery per level varied between 99% and

108%, and the pooled relative standard deviation across all measurements was homogeneous according to Bartlett's test at 3.1%.

The DFG method 1 protocol tested analysis reliability with 10 filters spiked at 50 µg (2.5 $mg.m^{-3}$), with a relative standard deviation of 4.3% and a spread of 9.6%.

Annex A of the ISO 16000-3 standard presents results from a U.S. study conducted in 14 cities, with analyses performed in 16 laboratories across the U.S., Canada, and Europe. The absolute percentage difference between sets of duplicate samples was 14.5% for acetaldehyde (n=346).

In the same annex, during the program, analyses performed by one laboratory on 13 DNPH cartridges spiked at approximately 0.5, 5, and 10 µg showed an average uncertainty of 13.8% for acetaldehyde. Another laboratory, under similar conditions with 30 spiked DNPH cartridges, reported an average uncertainty of 5.1%. Applying an expansion factor of 2 to the average uncertainty of 13.8% (U factor in EN 482), the expanded uncertainty becomes 27.8%.

The key factor in this method is the amount of DNPH available on the sampling support to react with acetaldehyde. Table 8 shows that for monitoring the 8h-OEL, the tubes and cartridges, at specified flow rates and with a 75% safety margin, cannot measure 2 times the 8h-OEL over an 8 hours sample, but cover 96% of that value. The flow rates given in Table 8 respect the range recommended by the protocols for the different samplers, 0.1 to 1 $L.min^{-1}$. Conducting two 4 hours samples, at these flow rates, stabilizes the method and allows near fourfold coverage of the 8h-OEL, with a quantification limit still below one-tenth of the value and enhanced protection against interferences.

The method validation data show that the NF EN 482 standard's requirements for measurement range and uncertainty are met for the 8h-OEL monitoring with two consecutive samples using tubes and cartridges containing silica gel impregnated with at least 1 mg of DNPH.

For monitoring 15 minutes exposures, the method is validated with tubes and cartridges containing at least 1 mg of DNPH impregnated on silica gel and at the maximum flow rates specified in Table 9.

These findings categorise the method using a DNPH-impregnated silica gel tube in category 1B for regulatory technical control of the 8h-OEL. The method is also classified as category 1B for regulatory technical control of the 15 min-STEL and short-term exposure monitoring.

The method using one or more DNPH-impregnated fibre glass filters, as described in the DFG method 1 and HSE MDHS 102 protocols, can be performed over 8 hours and 15 minutes. It is considered indicative due to uncertainties about the actual amount of DNPH available to react with acetaldehyde, the trapping efficiency, which seems lower and more variable than with tubes and cartridges (requiring further testing), and the lack of information on environmental conditions' influence. The potential for leaks at the cassette rings or along the filter edges if the cassette is not tightly pressed is another factor that has not been studied, especially for a cassette that can hold up to three filters of significant thickness.

These factors place the method using DNPH-impregnated filter trapping of acetaldehyde, as described by the DFG method 1 and HSE MDHS 102 protocols, in category 2 for the technical control of the 8h-OEL, the regulatory technical control of the 15 min-STEL, and short-term exposure monitoring.

For tubes, cartridges, and filters without a second range, the group recommends ensuring no breakthrough by following the NF X43-264 standard's recommendation, i.e., verifying that the chromatographic peak of DNPH in the collected sample represents at least 10% of that found in the analysis of a blank sample.

1.4 Detailed assessment of the method B: Active sampling on XAD-2® adsorbent resin coated with 2-HMP – desorption with toluene - Determination by gas chromatography – FID/NDP/mass detector

This method is described in three protocols: OSHA 68, NIOSH 2538 (an adaptation of the former), and IRSST 385. The NIOSH protocol builds on OSHA's evaluation by adding 21 days preservation tests. It's worth noting that the IRSST 385 protocol is quite brief, only mentioning the sampling process, specifically the tube reference, flow rate, volume, and the need for freezing samples before and after collection.

Method B involves trapping acetaldehyde by chemisorption on XAD-2® resin impregnated with 2-HMP. XAD-2® is a porous, hydrophobic organic polymer. Acetaldehyde reacts with 2-HMP according to the reaction scheme shown in Figure 7, forming a non-volatile derivative. After desorption in toluene, gas chromatography analysis reveals two derivatives of acetaldehyde with identical molecular masses (enantiomers). The ratio between the two remains relatively constant, regardless of how they are formed—whether through liquid spiking or gas generation in a chamber. The two 2-HMP derivatives of acetaldehyde differ in the position of the methyl group and hydrogen atom attached to the asymmetric carbon (marked with an asterisk in the scheme), forming syn and anti derivatives (OSHA 68).

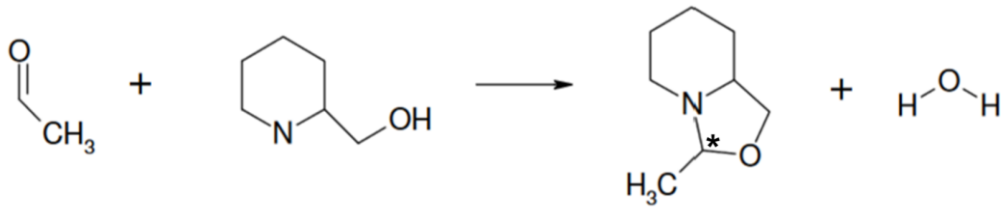


Figure 7 : Reaction mechanism of chemisorption of acetaldehyde in the presence of 2-HMP

After sampling, the impregnated resin is desorbed in toluene. The desorbate is then injected into a gas chromatography system coupled with a flame ionization detector (FID) or a nitrogen-phosphorus detector (NPD). The separation column can be a wide-bore silica capillary column (15 m x 0.32 mm, 1 µm DB 1301) or any other column capable of separating the 2-HMP acetaldehyde derivative from other compounds. The acetaldehyde derivative is identified and quantified by comparison with a calibration curve, analysed under the same protocol.

Characteristics of the sampling media:

The IRSST 385 protocol prescribes the use of the commercially available Orbo™ 23 tube (ref. 2186). This tube consists of two sections, 120 mg and 60 mg, of XAD-2® resin impregnated with 2-HMP (2-hydroxymethylpiperidine), with a particle size of 20/40 mesh. The NIOSH 2539 protocol, used for aldehyde screening without precise quantification, employs the Orbo™ 23 tube and also indicates a 10% impregnation by weight of 2-HMP.

The OSHA 68 and NIOSH 2538 protocols recommend using a tube (11 cm x 6 mm internal diameter) with two sections, 450 mg and 225 mg, of XAD-2® impregnated with 10% by weight of 2-HMP. This tube, previously marketed as "Orbo™ 25," is no longer available but can be prepared following the instructions in the OSHA and NIOSH protocols. Another tube, Orbo™ 24, is currently available, which consists of two sections: 150 mg and 75 mg of XAD-2® impregnated with 2-HMP, likely also at 10% by weight, given that the same adsorbent phase is used across these three Orbo tube formats.

Capacity of the sampling media:

When using these impregnated media, it is essential to ensure that the molar concentration of acetaldehyde in the sampled air volume does not exceed the molar capacity of the 2-HMP impregnated on the resin. 1 mg of 2-HMP corresponds to 8.7 µmoles, so the theoretical capacity of the front section of the Orbo™ 25 tube is 391 µmoles of acetaldehyde, equivalent to 17.21 mg; for the Orbo™ 24 and Orbo™ 23 tubes, the capacities are 5.74 mg and 4.58 mg, respectively.

Sampling characteristics

Sampling flow rate:

With the 450/225 mg tube: OSHA 68 recommends a flow rate of 0.05 $L \cdot min^{-1}$, while NIOSH 2538 suggests between 0.01 and 0.05 $L \cdot min^{-1}$.

With the 120/60 mg tube: IRSST 385 specifies a flow rate of 0.1 L·min-1, while NIOSH 2539 recommends between 0.01 and 0.05 $L \cdot min^{-1}$.

Sampling duration and collected volume:

With the 450/225 mg tube: OSHA 68 recommends a collected volume of 3 L at 0.05 $L.min^{-1}$, which corresponds to a maximum sampling time of 60 minutes. NIOSH 2538 allows a maximum volume of 12 L, making an 8hours sampling duration possible at 0.025 $L.min^{-1}$, and a 15 minutes sampling of 0.75 L at 0.05 $L.min^{-1}$.

With the 120/60 mg tube: IRSST 385 specifies a maximum volume of 10 L at 0.1 $L.min^{-1}$, for a sampling duration of 100 minutes.

Validation domain:

The most comprehensive validation data are provided in OSHA 68. It validates the tube's capacity for 3 L sampled at an acetaldehyde concentration of 840 $mg.m^{-3}$ by atmospheric generation and analytical precision between 108 and 720 $mg.m^{-3}$, which is well above the domains studied.

The NIOSH protocol extends the OSHA validation range, defining an applicable range from 1.3 to 730 $mg.m^{-3}$ for 3 L sampled, while also indicating that up to 12 L can be collected.

No validation domain is described in IRSST 385.

Breakthrough volume:

The OSHA protocol studied the capacity of the 450/225 mg tube by generating controlled acetaldehyde atmospheres.

In the first test, an atmosphere of 840 $mg.m^{-3}$ in air at 78% RH and 28°C was sampled. After 304 minutes and 15.2 L sampled at 0.05 $L.min^{-1}$, with 12.8 mg of acetaldehyde collected, 5% of the total concentration was found in the back section. At this breakthrough level, 74% of the theoretical 2-HMP concentration on the first section was consumed.

In the second test, to determine whether the amount of water in the sampled air affected the sampler's capacity, an atmosphere containing 823 $mg.m^{-3}$ of acetaldehyde in air at 19% RH and 27°C was sampled. A 14% breakthrough was observed after sampling 3 L. Although breakthrough occurred, the amount of acetaldehyde recovered corresponded to 99.7% of the generated amount, or 2.46 mg across the entire tube, with 2.12 mg on the front section. This breakthrough confirms the influence of water on the derivatization reaction and justifies the 3 L maximum sampling volume in dry air.

Detection limit:

OSHA 68: The analytical detection limit is based on the largest peak, 595 pg, using an NPD detector, with 0.9 µL injected, and desorption in 5 mL of toluene.

NIOSH 2538: Detection limit of 2 µg per sample, using FID detection, with 1 µL injected, and desorption in 5 mL of toluene.

Quantification limit:

OSHA 68: Quantification limit of 3.14 µg for 3 L sampled, or 1.05 $mg.m^{-3}$ (NPD detection, 0.9 µL injected, desorption in 5 mL).

NIOSH 2538: 4 µg for 3 L sampled, or 1.33 $mg.m^{-3}$ (FID detection, 1 µL injected, desorption in 5 mL).

Accessible measurement range:

Based on the amount recovered on the front section during OSHA's breakthrough test in dry air (2.12 mg @ 3L) and applying a high safety factor, e.g. 50 %, sampling for 8 hours at 0.05 $L.min^{-1}$ (24 L) in dry air, the most unfavourable condition for trapping capacity, allows for sampling with no risk of breakthrough an acetaldehyde concentration of 44 $mg.m^{-3}$.

For short-term exposure assessments, sampling for 15 minutes at 0.05 $L.min^{-1}$ (flow rate cannot be increased to respect the derivatization reaction kinetics), an acetaldehyde concentration of 1 413 $mg.m^{-3}$ can be measured without risk of breakthrough.

With 24 L sampled, desorption in 5 mL of solvent, and 1 µL injected, the quantification limit for OSHA with NPD detection is 0.118 $mg.m^{-3}$, and for NIOSH with FID detection, it is 0.167 $mg.m^{-3}$. For the 15min-STEL and 0.75 L sampled, the quantification limits under OSHA and NIOSH analysis conditions are 4.19 $mg.m^{-3}$ and 5.33 $mg.m^{-3}$, respectively. These limits of quantification can be lowered by doubling the injection volume.

For the 8h-OEL, the accessible measurement range covers the desired range of 0.18 to 3.6 $mg.m^{-3}$, with 8 hours of sampling at 0.05 $L.min^{-1}$.

For the 15min-STEL, the accessible measurement range does not allow for reaching one-tenth of the 15min-STEL for regulatory technical control but covers the desired range of 7 to 28 $mg.m^{-3}$ for short-term exposure assessment.

Trapping and desorption efficiency

The OSHA protocol tested the trapping efficiency by generating acetaldehyde-polluted atmospheres at levels near 830 $mg.m^{-3}$, with high and low humidity, 78% and 19% RH. Trapping efficiency was over 95% for up to 16.7 L sampled in a humid atmosphere and over 99% for up to 3 L, and 91% for up to 6.5 L in a dry atmosphere.

OSHA specifies that desorption efficiency is not a limiting factor as long as standards are prepared by doping the XAD-2® phase impregnated with 2-HMP and treated analytically the same way as the samples. Desorption efficiency, evaluated by comparison with doping done directly in toluene containing 9 mg/mL of 2-HMP, showed a variation of 2 to 6% for standards ranging from 62 to 434 $µg.mL^{-1}$.
NIOSH tested desorption efficiency during storage tests, showing 100% efficiency for supports doped with 26.8 and 107 µg of acetaldehyde, corresponding to concentrations of 1.1 and 4.5 $mg.m^{-3}$ for an 8 hours sampling period at 0.05 $L.min^{-1}$, 0.6 and 2.5 times the 8h-OEL, 35.7 and 142.7 $mg.m^{-3}$ for an 15 minutes sampling period at 0.05 $L.min^{-1}$, 2.6 and 10.2 times the 15min-STEL.

Storage and recovery rate

OSHA 68: Storage at 23°C of tubes sampled in an atmosphere at 28°C and 80% RH containing 415 mg/m^3 of acetaldehyde showed a recovery rate of 96.6 to 99.3% at 23 days. Storage at -20°C of tubes sampled in an atmosphere at 27°C and 81% RH containing 380 $mg.m^{-3}$ of acetaldehyde resulted in a recovery rate of 92.4 to 98.4% at 16 days.

NIOSH 2358: Storage by doping at 26.8 and 107 µg of commercial tubes (0.6 and 2.5 times the 8h-OEL,2.6 and 10.2 times the 15min-STEL) after 21 days in a refrigerated chamber, gave a recovery rate of 100%.

Detection linearity

OSHA 68: Calibration is external, but an internal standard is also added to the toluene used as the solvent for the standards and desorption of the tubes. The internal standard is dimethylformamide at a rate of 20 µL for 100 mL of toluene. The NPD detector's response is linear between 97.6 and 390.6 µg injected in 0.9 µL, corresponding to concentrations of 22.6 and 90.4 $mg.m^{-3}$ for an 8 hours sampling period at 0.05 $L.min^{-1}$, 12.6 and 50.2 times the 8h-OEL, 723 and 2893 $mg.m^{-3}$ for a 15 minutes sampling period at 0.05 $L.min^{-1}$, 51.7 and 207 times the 15min-STEL. The instrument response is not linear over this range.

NIOSH 2538: The FID detector's response is linear between 0.8 and 440 ng injected in 1 µL corresponding to concentrations of 0.17 and 91.7 $mg.m^{-3}$ for an 8 hours sampling period at 0.05 $L.min^{-1}$, 0.93 and 50.9 times the 8h-OEL, 5.3 and 2933 $mg.m^{-3}$ for a 15 minutes sampling period at 0.05 $L.min^{-1}$, 0.39 and 210 times the 15min-STEL.

Interferences and environmental conditions during sampling

OSHA highlights the role of humidity in reducing trapping capacity, especially in low-humidity atmospheres. OSHA also warns about the presence of mineral acids, which can neutralize 2-HMP, and carbonyl-containing compounds, which may compete with acetaldehyde in reacting with the support.

Specificity

The method's specificity depends on the applied chromatographic conditions. The cited protocols outline the analytical conditions necessary to resolve the acetaldehyde derivative peaks.

Uncertainty

OSHA 68 derives the overall uncertainty from 23-day storage tests at room temperature, including a systematic error of ±5% for sampling. Based on these data, the overall uncertainty is ±25% or better, at a 95% confidence level. It is important to note that this uncertainty is calculated from data at high atmospheric acetaldehyde concentrations, 416 mgm^{-3} for 3 L sampled, with 1.25 mg on the support. The NIOSH 2538 protocol provides the following information: for concentrations between 180 and 720 $mg.m^{-3}$ for 3 L sampled (0.54 to 2.16 mg on the support), the bias is 0.2%, the overall precision is 12%, and the expanded uncertainty (accuracy) is ±23.7%.

For this method, most validation data are available in the OSHA 68 and NIOSH 2538 protocols but were determined at concentration ranges far above twice the 8h-OEL and twice the 15min-STEL. The OSHA 68 and NIOSH 2018 protocols are based on a target concentration of 360 $mg.m^{-3}$, 200 times higher than the 8h-OEL and the 15min-STEL recommended by the Committee. **However, it is possible to adapt the method by maintaining the sampling flow rate at 0.05 L/min. For 8 hours exposure measurement, sampling 24 litres covers the full duration without exceeding the tube's trapping capacity. For short-term exposure measurements, doubling the injection volume in the analyser (2 µL instead of 1 µL) allows for sufficient sensitivity to evaluate 0.5 times the 15min-STEL, though not reaching one-tenth of it.**

These elements lead to the classification of the method using the XAD-2® tube impregnated with 10% by mass of 2-HMP as a category 2 method for regulatory technical control of the 8h-

OEL. The method is also classified as category 2 for short-term exposure monitoring, but as category 3 for regulatory technical control of the 15min-STEL.

1.5 Main features of the method C: Passive sampling on a DNPH/H_3PO_4-coated badge (3 types of badge) – Determination by liquid chromatography using a UV/visible detector

This method is illustrated by two LCSQA reports, from 2007 and 2013/2014. The 2007 report focuses on formaldehyde evaluation with some data on acetaldehyde, while the 2013/2014 report is specific to acetaldehyde. The method consists of sampling acetaldehyde by diffusion onto a badge tube containing a granular support impregnated with DNPH and phosphoric acid. Desorption is carried out with a few millilitres of acetonitrile before analysis by liquid chromatography with UV spectrometry detection.

Only the Radiello® 165 badge, combined with the R 120 diffusion body, was tested in the LCSQA 2007 report. The tests were conducted in a polluted atmosphere generation bench as well as in the aisle of a shopping mall. For the bench tests, conducted over 8 hours, the concentration was monitored using active sampling on Waters™ Sep-Pak cartridges. One test at 11.7 µg.m^{-3} of acetaldehyde showed a recovered average concentration of 4.8 µg.m^{-3} on six Radiello® tubes, which is a 41% recovery rate with a dispersion of 49.8%. Another test at 36.7 µg.m^{-3} showed a better recovery rate of 111% with a dispersion of 9.3%.

Field measurements, also compared to active sampling, showed discrepancies ranging from -27.2 to -36.4% over three 24 hours sampling series with concentrations close to 100 µg.m^{-3} (99.3 to 133.5).

The LCSQA 2013/2014 report summarises the results of tests conducted in 2013 and 2014 for acetaldehyde measurement, both in real-world settings and in a generation bench. Sampling was done using the Radiello® 165 badge and the Supelco™ DSD-DNPH. Real-world tests were conducted in 2013 in a closed office and an urban area, both for 4.5 days, with repeat tests in 2014. In 2013, concentrations were 7.7 to 9.2 µg.m^{-3} in the office and 2.4 to 3 µg.m^{-3} in the urban area. In 2014, they were 3.0 to 4.5 and 0.9 to 1.2 µg.m^{-3}, respectively. The relative differences between the two sampling media were 16% for the office and 20% for the urban site in 2013, and 42% and 30% in 2014.

In 2013 and 2014, bench generation tests were conducted over 4.5 days, with concentrations ranging from 27.3 to 82.0 µg.m^{-3}, alongside formaldehyde pollution in the generated atmosphere. The relative difference between active and passive sampling ranged from 10 to 15% at low formaldehyde concentrations (formaldehyde/acetaldehyde molar ratio ≤ 1) but increased rapidly to 60 and 74% at higher concentrations (molar ratio ≥ 1.5).

In this latter report, the uncertainty due to inter-sample variability and analytical uncertainty is given for Radiello® and DSD-DNPH exposed in an exposure chamber during acetaldehyde generation alone, being 34% for Radiello® and 47% for DSD-DNPH, with an expansion factor of 2.

To supplement this information, regarding the Radiello® 165, the Maugeri Foundation, the designer of the Radiello, notes in a recent document, *Radiello sampler for aldehydes - Rev 1.0 2023*, that the applicable range for acetaldehyde extends from 41 to 1458 µg.m^{-3} for an 8 hours sampling period.

The LCSQA reports' data do not validate the passive sampling method using a badge containing a DNPH-impregnated support. The ranges studied in these reports are far from the target and extend from 4.8 to 36.7 µg.m^{-3} over 8 hours. Furthermore, these are not validation domains, as validation criteria such as storage and sampling media capacity are not provided.

The technical document from the Maugeri Foundation also informs about the applicable range of the Radiello® 165, 0.04 to 1.46 mg·m^{-3} for an 8 hours sampling period. To achieve a measurement at twice the 8h-OEL limit (2*8h-OEL), three consecutive 160 minutes samplings could be used, but no data are available on uncertainty related to diffusion rate variation, which is generally high during the first hours of sampling.

For a short sampling period of 15 minutes, the variation in the diffusion rate is generally considered to be very high, while the diffusion flux stabilises. In the absence of available data on the acetaldehyde diffusion rate on the Radiello®165 over this short period of time, it should be considered that this measurement method does not meet the quality criteria defined in standard EN 482.

For all these reasons, the passive sampling method using a DNPH-impregnated support is classified as category 3 for the regulatory control of the 8h-OEL and the 15min-STEL, as well as for short-term exposure monitoring.

2 Conclusions and recommendations

Three methods have been identified for determining acetaldehyde in workplace atmospheres.

Method A involves trapping acetaldehyde through chemisorption by derivatizing it into hydrazone using 2,4-DNPH (2,4-dinitrophenylhydrazine) impregnated on a surface, typically a silica gel or a glass fibre filter. The acetaldehyde reacts with the hydrazine to form a non-volatile derivative. The sampling is active, with air being pumped at a known flow rate through a tube containing the impregnated silica gel. For analysis, the impregnated support is desorbed into a vial or by percolation with a known volume of acetonitrile then injected into a high-performance liquid chromatography (HPLC) system coupled with a UV spectrophotometric detector at 360-365 nm.

Method B involves trapping acetaldehyde by chemisorption. The sampling is active, with air being pumped at a known flow rate through a tube containing XAD-2® resin impregnated with 2-HMP. Acetaldehyde reacts with 2-HMP, forming a non-volatile derivative. After desorption in toluene, gas chromatography, NPD or FID detection, analysis reveals two derivatives of acetaldehyde with identical molecular masses (enantiomers). The ratio between the two remains relatively constant.

Method C consists of sampling acetaldehyde by diffusion onto a badge tube containing a granular support impregnated with DNPH and phosphoric acid. Desorption is carried out with a few millilitres of acetonitrile before analysis by liquid chromatography with UV spectrometry detection.

Among these three methods, method A has been selected and is considered partially validated. This method, across various protocols, has most of the validation data but needs to be adapted to cover an 8 hours exposure assessment. The limiting factor is the amount of DNPH in the sampling tube available for trapping acetaldehyde. For the 8h-OEL monitoring, two consecutive 4 hours samplings are recommended, as the capacity of the different tubes studied is slightly insufficient to evaluate twice the 8h-OEL with a 75% safety margin in a single 8 hours sampling. Two 4 hours samplings allow the evaluation of exposure levels up to four times the 8h-OEL limit while maintaining a limit of quantification below one-tenth of the limit value. However, the maximum flow rates recommended in Table 24, which account for the available DNPH quantity for trapping, must be respected.

Table 24 : Maximum flow rates recommended for monitoring the 8h-OEL (2x 4 hours sampling)

Sampling support	DNPH /sampling support (mg)	Flow rate ($L.min^{-1}$)
DNPH-coated silica gel in a tube or cartridge	5	0.5
	2	0.2
	1	0.1

For short-term exposure monitoring over a 15-minutes period, the method is validated by using tubes and cartridges containing at least 1 mg of DNPH impregnated on silica gel. The maximum flow rates specified in Table 25 must be adhered to for effective sampling and reliable results.

Table 25 : Maximum flow rates recommended for monitoring the 15 min-STEL

Sampling support	DNPH /sampling support (mg)	Flow rate ($L.min^{-1}$)
DNPH-coated silica gel in a tube or cartridge	5	1
	2	0,8
	1	0,4

These elements lead to the classification of the method involving a silica gel tube impregnated with at least 1 mg of DNPH as category 1B for regulatory technical monitoring of the 8-hour OEL. The method is also classified as category 1B for the regulatory technical control of the 15min-STEL and short-term exposure monitoring.

Certain protocols of method A implement sampling over 8 hours and 15 minutes using one or more DNPH-impregnated fibreglass filters. This method, as an alternative to the impregnated silica gel, is considered indicative due to uncertainties regarding the actual amount of DNPH available to react with acetaldehyde and its lower and variable trapping efficiency compared to tubes and cartridges. The performance of this collection method would benefit from further testing with one, two, or even three filters placed in the sampling cassette.

This approach using DNPH-impregnated filters is classified as category 2 for the technical control of the 8h-OEL, the 15min-STEL, and short-term exposure monitoring.

A second method is considered adaptable and therefore indicative (method B), which uses active sampling on XAD-2® resin impregnated with 10% by mass of 2-HMP. After desorption in toluene, analysis is conducted via gas chromatography with FID or NPD detection. For this method, most validation data are available though determined at concentrations much higher than 2 x 8h-OEL and *2 x* 15min STEL. It is possible to adapt this method by sampling 24 L at a flow rate of 0.05 L/min. This volume covers an 8 hours period without exceeding the tube's trapping capacity. For 15 minutes exposure measurements, adjusting the injected volume to 2 µL instead of 1 µL provides sufficient sensitivity to assess 0.5*15min-STEL, though not reaching one-tenth of the limit.

This method involving XAD-2® resin impregnated with 2-HMP is classified as category 2 for the regulatory technical control of the 8h-OEL when sampling 24 L. The method is also classified as category 2 for short-term exposure monitoring but category 3 for the regulatory technical control of the 15min-STEL.

The recommended methods for personal acetaldehyde exposure in a workplace atmosphere are summarised in Table 26**.**

Table 26 : Recommended methods for acetaldehyde assessment in workplace atmosphere in light of the 8h-OEL and 15min-STEL

Method	Active sampling on a DNPH-coated silica gel in a sampling tube – desorption with acetonitrile – Determination by liquid chromatography using a UV/visible detector	
Protocols	(NF ISO 16000-3 (2011) ; NF X 43 264 (2011) ; INRS M-66 (2016) ; US EPA TO-11A (1999) ; NIOSH 2018 (2013) ; HSE MDHS 102 (2010) ; DFG method 1 (1990) DFG method 2 (1995)	
For regulatory technical control of the 8h-OEL	**Category**	**1B**
	Conditions of use	Silica gel coated with minimum 1 mg DNPH/sampler, Maximum flow rate vs mg DNPH/sampler : 0.5 $L.min^{-1}$ for 5 mg 0.2 $L.min^{-1}$ for 2 mg 0.1 $L.min^{-1}$ for 1 mg Sampling duration : **2x4 h**
For regulatory technical control of the 15min-STEL and short-term exposure monitoring	**Category**	**1B**
	Conditions of use	Silica gel coated with minimum 1 mg DNPH/sampler Maximum flow rate vs mg DNPH/sampler : 1 $L.min^{-1}$ for 5 mg 0.8 $L.min^{-1}$ for 2 mg 0.4 $L.min^{-1}$ for 1 mg

Date de validation du rapport d'expertise collective par le CES « Valeurs sanitaires de référence » : 12 décembre 2024

Signature :

Maisons-Alfort,

Au nom des experts du CES
« Valeurs sanitaires de référence »,
J Thireau
Président du CES

3 References

Scientific publications

ACGIH® 2014. "Acetaldehyde." In *Documentation of TLVs and BEIs, ACGIH*. Cincinnati, OH, USA.

ACGIH® 2018. *TLVS® and BEIS®: based on the documentation of the threshold limit values for chemical substances and physical agents & biolological exposure indices*. Cincinnati, OH: ACGIH S®.

AFSSET 2008. "Agence française de sécurité sanitaire de l'environnement et du travail). 2008. Evaluation des risques sanitaires liés à la présence de formaldéhyde dans les environnements intérieurs et extérieurs. Toxicité du formaldéhyde. Etat des connaissances sur la caractérisation des dangers et choix des valeurs toxicologiques de référence. ." *Afsset, Maisons-Alfort, 79p.*

Abernethy DJ, Frazelle JH, and Boreiko CJ. 1982. "Effects of ethanol, acetaldehyde and acetic acid in the C3H/10T1/2 C18 cell transformation system." *Environ Mutagen* 4: 331.

Anses 2018. "AVIS et RAPPORT de l'Anses relatif à la Mise à jour des valeurs guides de qualité d'air intérieur – Formaldéhyde."

Anses 2014. *Propositions de Valeurs Guides de qualité de l'Air intérieur - Acétaldéhyde.* Anses (Maisons-Alfort). https://www.anses.fr/fr/system/files/AIR2013sa0076Ra.pdf. 133-p.

Anses. (upcoming, à paraitre). Guide d'élaboration et de choix des valeurs de référence. Saisine n°2020-SA-0019. Maisons-Alfort : Anses.

Appelman L.M, Woutersen RA, and Feron VJ. 1982. "Inhalation toxicity of acetaldehyde in rats. I. Acute and subacute studies." *Toxicology* 23 (4): 293-307. https://doi.org/10.1016/0300-483x(82)90068-3

Appelman LM, Woutersen RA, Feron VJ, R. Hooftman N, and Notten WR. 1986. "Effect of variable versus fixed exposure levels on the toxicity of acetaldehyde in rats." *J Appl Toxicol* 6 (5): 331-6. https://doi.org/10.1002/jat.2550060506

Babiuk C, Steinhagen WH, and Barrow CS. 1985. "Sensory irritation response to inhaled aldehydes after formaldehyde pretreatment." *Toxicol Appl Pharmacol* 79 (1): 143-9. https://doi.org/10.1016/0041-008x(85)90376-x

Belkebir E, Rousselle C, Duboudin C, Bodin L, Bonvallot N (2011). Haber's rule duration adjustments should not be used systematically for risk assessment in public health decision making. Toxicol. Lett. 204, 148-155.

Bariliak IR and SIu Kozachuk. 1983. "[Embryotoxic and mutagenic activity of ethanol and acetaldehyde in intra-amniotic exposure]." *Tsitol Genet* 17 (5): 57-60.

Bergh M, and Karlberg AT. 1999. "Sensitizing potential of acetaldehyde and formaldehyde using a modified cumulative contact enhancement test (CCET)." *Contact Dermatitis* 40 (3): 139-45. https://doi.org/10.1111/j.1600-0536.1999.tb06011.x

Billionnet C, Gay E, Kirchner S, Leynaert B, and Annesi-Maesano I. 2011. "Quantitative assessments of indoor air pollution and respiratory health in a population-based sample of French dwellings." *Environ Res* 111 (3): 425-34. https://doi.org/10.1016/j.envres.2011.02.008

Bird RP, Draper HH and Basrur PK. 1982. "Effect of malonaldehyde and acetaldehyde on cultured mammalian cells. Production of micronuclei and chromosomal aberrations." *Mutat Res* 101 (3): 237-46. https://doi.org/10.1016/0165-1218(82)90155-0

Blakley PM and Scott WJ Jr. 1984. "Determination of the proximate teratogen of the mouse fetal alcohol syndrome. 2. Pharmacokinetics of the placental transfer of ethanol and

acetaldehyde." *Toxicol Appl Pharmacol* 72 (2): 364-71. https://doi.org/10.1016/0041-008x(84)90321-1

Blasiak J, Trzeciak A, Malecka-Panas E, Drzewoski J, and Wojewódzka M. 2000. "In vitro genotoxicity of ethanol and acetaldehyde in human lymphocytes and the gastrointestinal tract mucosa cells." *Toxicol In Vitro* 14 (4): 287-95. https://doi.org/10.1016/s0887-2333(00)00022-9.

Bohlke JU, Singh S and Goedde HW. 1983. "Cytogenetic effects of acetaldehyde in lymphocytes of Germans and Japanese: SCE, clastogenic activity, and cell cycle delay." *Hum Genet* 63 (3): 285-9. https://doi.org/10.1007/BF00284666

Booze TF and Oehme FW. 1986. "An investigation of metaldehyde and acetaldehyde toxicities in dogs." *Fundam Appl Toxicol* 6 (3): 440-6. https://doi.org/10.1016/0272-0590(86)90217-4.

Budinsky R, Gollapudi B, Albertini RJ, Valentine R, Stavanja M, Teeguarden J, Fensterheim R, Rick D, Lardie T, McFadden L, Green A, and Recio L. 2013. "Nonlinear responses for chromosome and gene level effects induced by vinyl acetate monomer and its metabolite, acetaldehyde in TK6 cells." *Environ Mol Mutagen* 54 (9): 755-68. https://doi.org/10.1002/em.21809. Epub 2013 Aug 28.

CEPA 1993. "California Environmental Protection Agency (CEPA). Air ressources board.Acetaldehyde as a toxic air contaminant." *Part B. Health assessement. Stationery source division*. https://doi.org/https://ww2.arb.ca.gov/sites/default/files/classic/toxics/id/summary/acetaldehyde_b.pdf.

CERI 2007. "HAZARD ASSESSMENT REPORT ACETALDEHYDE CAS No. 75-07-0 Chemicals Evaluation and Research Institute (CERI), Japan. in collaboration with National Institute of Technology and Evaluation (NITE) under the sponsorship of New Energy and Industrial Technology Development Organization (NEDO).". https://doi.org/https://www.cerij.or.jp/ceri_en/hazard_assessment_report/pdf/en_75_07_0.pdf.

Cassee FR, Groten JP, and Feron VJ. 1996. "Changes in the nasal epithelium of rats exposed by inhalation to mixtures of formaldehyde, acetaldehyde, and acrolein." *Fundam Appl Toxicol* 29 (2): 208-18. https://doi.org/10.1006/faat.1996.0024

Cederbaum AI, and Rubin E. 1976. "Protective effect of cysteine on the inhibition of mitochondrial functions by acetaldehyde." *Biochem Pharmacol* 25: 963-973.

Corley RA, Kabilan S, Kuprat A, Carson JP, Jacob RE, Minard KR, Teeguarden JG, Timchalk C, Pipavath S, Glenny R, and Einstein DR. 2015. "Comparative Risks of Aldehyde Constituents in Cigarette Smoke Using Transient Computational Fluid Dynamics/Physiologically Based Pharmacokinetic Models of the Rat and Human Respiratory Tracts." *Toxicol Sci* 146 (1): 65-88. https://doi.org/10.1093/toxsci/kfv071. Epub 2015 Apr 8.

Crebelli R, Conti G, Conti L, and Carere A. 1989. "A comparative study on ethanol and acetaldehyde as inducers of chromosome malsegregation in Aspergillus nidulans." *Mutat Res* 215 (2): 187-95. https://doi.org/10.1016/0027-5107(89)90183-8

DFG 1982. The MAK-Collection for occupational Health and Safety Acétaldehyde MAK value Documentation.

DFG 2013a. "The MAK-Collection for occupational Health and Safety. Documentations and methods." *MAK Value Documentations*: 320. https://doi.org/10.1002/3527600418.mb7507e3013.

DFG 2013b. "Acetaldehyde [MAK Value Documentation, 2013b]." In *The MAK-Collection for Occupational Health and Safety*, edited by Forschungsgemeinschaft Deutsche and Area Commission for the Investigation of Health Hazards of Chemical Compounds in the Work, 320 pages. Wiley.

Dellarco VL. 1988. "A mutagenicity assessment of acetaldehyde." *Mutat Res* 195 (1): 1-20. https://doi.org/10.1016/0165-1110(88)90013-9

Deng XS, and Deitrich RA. 2009. "Putative role of brain acetaldehyde in ethanol addiction." *Curr Drug Abuse Rev.Curr Drug Abuse Rev. Author manuscript; available in PMC* 1 (1): 3-8.

Dey A, Dhawan A, Kishore Seth P, and Parmar D. 2005. "Evidence for cytochrome P450 2E1 catalytic activity and expression in rat blood lymphocytes." *Life Sci* 77 (10): 1082-93. https://doi.org/10.1016/j.lfs.2005.01.021

Dorman DC, Struve M, Wong BA, Gross EA, Parkinson C, Willson GA, Tan YM, Campbell JL, Teeguarden JG, Clewell HJ, 3rd, and Andersen ME. 2008. "Derivation of an inhalation reference concentration based upon olfactory neuronal loss in male rats following subchronic acetaldehyde inhalation." *Inhal Toxicol* 20 (3): 245-56. https://doi.org/10.1080/08958370701864250

Dulout FN, and Furnus CC. 1988. "Acetaldehyde-induced aneuploidy in cultured Chinese hamster cells." *Mutagenesis* 3 (3): 207-11. https://doi.org/10.1093/mutage/3.3.207

ECHA 2016. "RAC. Opinion proposing harmonised classification and labelling at EU level of acetaldehyde; ethanal. ." In *European Chemicals Agency*.

Egle JL Jr. 1970. "Retention of inhaled acetaldehyde in man." *J Pharmacol Exp Ther* 174 (1): 14-9.

Eker P, and Sanner T. 1986. "Initiation of in vitro cell transformation by formaldehyde and acetaldehyde as measured by attachment-independent survival of cells in aggregates." *Eur J Cancer Clin Oncol* 22 (6): 671-6. https://doi.org/10.1016/0277-5379(86)90164-1

Fang JL, and Vaca CE. 1995. "Development of a 32P-postlabelling method for the analysis of adducts arising through the reaction of acetaldehyde with 2'-deoxyguanosine-3'-monophosphate and DNA." *Carcinogenesis* 16 (9): 2177-85. https://doi.org/10.1093/carcin/16.9.2177

Fan, JL, and Vaca CE. 1997. "Detection of DNA adducts of acetaldehyde in peripheral white blood cells of alcohol abusers." *Carcinogenesis* 18 (4): 627-32. https://doi.org/10.1093/carcin/18.4.627

Feron VJ. 1979. "Effects of exposure to acetaldehyde in syrian hamsters simultaneously treated with benzo(a)pyrene or diethylnitrosamine." *Prog Exp Tumor Res* 24: 162-76. https://doi.org/10.1159/000402094

Feron V J, Kruysse A, and Woutersen RA. 1982. "Respiratory tract tumours in hamsters exposed to acetaldehyde vapour alone or simultaneously to benzo(a)pyrene or diethylnitrosamine." *Eur J Cancer Clin Oncol* 18 (1): 13-31. https://doi.org/10.1016/0277-5379(82)90020-7

Fraenkel-Conrat H, and Singer B. 1988. "Nucleoside adducts are formed by cooperative reaction of acetaldehyde and alcohols: possible mechanism for the role of ethanol in carcinogenesis." *Proc Natl Acad Sci U S A* 85 (11): 3758-61. https://doi.org/10.1073/pnas.85.11.3758.

Fujimura M, Myou S, Kamio Y, Ishiura Y, Iwasa K, Hashimoto T, and Matsuda T. 1999. "Increased airway responsiveness to acetaldehyde in asthmatic subjects with alcohol-induced bronchoconstriction." *Eur Respir J* 14 (1): 19-22. https://doi.org/10.1034/j.1399-3003.1999.14a05.x

Garberg P, Åkerblom E-L, and Bolcsfoldi G. 1988. "Evaluation of a genotoxicity test measuring DNA strand breaks in mouse lymphoma cells by alkaline unwinding and hydroxyapatite elution." *Mutat Res* 203: 155-176.

Goedde HW, and Agarwal DP. 1987. "Polymorphism of aldehyde dehydrogenase and alcohol sensitivity." *Enzyme* 37 (1-2): 29-44. https://doi.org/10.1159/000469239

Grafstrom RC, Dypbukt JM, Sundqvist K, Atzori L, Nielsen I, Curren RD, and Harris CC. 1994. "Pathobiological effects of acetaldehyde in cultured human epithelial cells and fibroblasts." *Carcinogenesis* 15 (5): 985-90. https://doi.org/10.1093/carcin/15.5.985

He SM, and Lambert B. 1990. "Acetaldehyde-induced mutation at the hprt locus in human lymphocytes in vitro." *Environ Mol Mutagen* 16 (2): 57-63. https://doi.org/10.1002/em.2850160202

Health Council of, the Netherlands. 2014. *Acetaldéhyde, Re-evaluation of the carcinogenicity and genotoxicity. Subcommittee on the classification of carcinogenic substances of the Dutch expert Committee on occupational safety, a committee of the health council of the netherlands. DGV/BMO/U-932542. U-8234/JR/cn/246-W19. To the minister of social affairs abd employment.* https://doi.org/advisory-report-acetaldehyde.

Health Council of the Netherlands. 2012. *Evaluation of the carcinogenicity and genotoxicity.*

Heap L, Ward RJ, Abiaka C, Dexter D, Lawlor M, Pratt O, Thomson A, Shaw K, and Peters TJ. 1995. "The influence of brain acetaldehyde on oxidative status, dopamine metabolism and visual discrimination task." *Biochem Pharmacol* 50 (2): 263-70. https://doi.org/10.1016/0006-2952(94)00539-x

Heit C, Dong H, Chen Y, Thompson DC, Deitrich R., and Vasiliou V. 2013. "The Role of CYP2E1 in Alcohol Metabolism and Sensitivity in the Central Nervous System." *Subcell Biochem* 67: 235-247. https://doi.org/10.1007/978-94-007-5881-0_8.

Hobara N, Watanabe A, Kobayashi M, Nakatsukasa H, Nagashima H, Fukuda T, and Araki Y. 1985. "Tissue distribution of acetaldehyde in rats following acetaldehyde inhalation and intragastric ethanol administration." *Bull Environ Contam Toxicol* 35 (3): 393-6. https://doi.org/10.1007/BF01636528

Hynes GM, Torous DK, Tometsko CR, Burlinson B, and Gatehouse DG. 2002. "The single laser flow cytometric micronucleus test: a time course study using colchicine and urethane in rat and mouse peripheral blood and acetaldehyde in rat peripheral blood." *Mutagenesis* 17 (1): 15-23. https://doi.org/10.1093/mutage/17.1.15

IARC 1999. Acetaldehyde. IARC Summary & Evaluation, Volume 71, 1999.

IARC 2010. "Alcohol consumption and ethyl carbamate." *IARC Monogr Eval Carcinog Risks Hum* 96: 3-1383.

IARC 2012. *Personal Habits and Indoor Combustions.* Lyon: Centre international de recherche sur le cancer.

INERIS 2018. *Acétaldéhyde - Fiche de données toxicologiques et environnementales des subs tances chimiques.* https ://substances.ineris.fr/fr/. 39-91.

INRS 2022. *Acetaldehyde Fiche toxicologique n°120.*

Inagaki S, Esaka Y, Deyashiki Y, Sako M, and Goto M. 2003. "Analysis of DNA adducts of acetaldehyde by liquid chromatography-mass spectrometry." *J Chromatogr A* 987 (1-2): 341-7. https://doi.org/10.1016/s0021-9673(02)01948-9

JSOH May 2021. *Recommendation of occupational exposure limits (2021–2022) The Japan Society for Occupational Health.* https://www.sanei.or.jp/english/files/topics/oels/OEL.pdf.

Kayani MA., and Parry JM. 2010. "The in vitro genotoxicity of ethanol and acetaldehyde." In *Toxicology in Vitro*, 56-60.

Kharchenko NK. 1998. "[Effect of acetaldehyde on the formation of alcohol dependence in animal experiments]." *Ukr Biokhim Zh (1978)* 70 (5): 122-8.

Klyosov AA, Rashkovetsky LG, Tahir MK, and Keung WM. 1996. "Possible role of liver cytosolic and mitochondrial aldehyde dehydrogenases in acetaldehyde metabolism." *Biochemistry* 35 (14): 4445-56. https://doi.org/10.1021/bi9521093

Korte A, Obe G, Ingwersen I, and Ruckert G. 1981. "Influence of chronic ethanol uptake and acute acetaldehyde treatment on the chromosomes of bone-marrow cells and peripheral lymphocytes of Chinese hamsters." *Mutat Res* 88 (4): 389-95. https://doi.org/10.1016/0165-1218(81)90030-6

Kruysse A. 1970. *Acute Inhalation Toxicity of Acetaldehyde in Hamsters.* Central Institute for Nutrition and Food Research (The Netherlands).

Kruysse A, Feron VJ, and Til HP. 1975. "Repeated exposure to acetaldehyde vapor. Studies in Syrian golden hamsters." *Arch Environ Health* 30 (9): 449-52. https://doi.org/10.1080/00039896.1975.10666748

Kunugita N, Isse T, Oyama T, Kitagawa K, Ogawa M, Yamaguchi T, Kinaga T, and Kawamoto T. 2008. "Increased frequencies of micronucleated reticulocytes and T-cell receptor mutation in Aldh2 knockout mice exposed to acetaldehyde." *J Toxicol Sci* 33 (1): 31-6. https://doi.org/10.2131/jts.33.31

Lahdetie J. 1988. "Effects of vinyl acetate and acetaldehyde on sperm morphology and meiotic micronuclei in mice." *Mutat Res* 202 (1): 171-8. https://doi.org/10.1016/0027-5107(88)90179-0.

Lam CW, Casanova M, and Heck HD. 1986. "Decreased extractability of DNA from proteins in the rat nasal mucosa after acetaldehyde exposure." *Fundam Appl Toxicol* 6 (3): 541-50. https://doi.org/10.1016/0272-0590(86)90228-9.

Lambert B, Chen Y, He SM, and Sten M. 1985. "DNA cross-links in human leucocytes treated with vinyl acetate and acetaldehyde in vitro." *Mutat Res* 146 (3): 301-3. https://doi.org/10.1016/0167-8817(85)90072-0.

Latge C, Lamboeuf Y, Roumec C, and de Saint Blanquat G. 1987. "Effect of chronic acetaldehyde intoxication on ethanol tolerance and membrane fatty acids." *Drug Alcohol Depend* 20 (1): 47-55. https://doi.org/10.1016/0376-8716(87)90075-5.

Ma T, Miller TL, & Xu Z 1985. "Mouse peripheral erythrocyte micronucleus assay on the genotoxicity of acetaldehyde." *Environ Mutagen* 7(Suppl.3) (28).

Madrigal-Bujaidar E, Velazquez-Guadarrama N, Morales-Ramirez P, and Mendiola MT. 2002. "Effect of disulfiram on the genotoxic potential of acetaldehyde in mouse spermatogonial cells." *Teratog Carcinog Mutagen* 22 (2): 83-91. https://doi.org/10.1002/tcm.10003.

Majer, BJ, Mersch-Sundermann V, Darroudi F, Laky B, de Wit K, and Knasmuller S. 2004. "Genotoxic effects of dietary and lifestyle related carcinogens in human derived hepatoma (HepG2, Hep3B) cells." *Mutat Res* 551 (1-2): 153-66. https://doi.org/10.1016/j.mrfmmm.2004.02.022.

Marinari UM, Ferro M, Sciaba L, Finollo R, Bassi AM, and Brambilla G. 1984. "DNA-damaging activity of biotic and xenobiotic aldehydes in Chinese hamster ovary cells." *Cell Biochem Funct* 2 (4): 243-8. https://doi.org/10.1002/cbf.290020411.

Matsuda T, Yabushita H, Kanaly RA, Shibutani S, and Yokoyama A. 2006. "Increased DNA damage in ALDH2-deficient alcoholics." *Chem Res Toxicol* 19 (10): 1374-8. https://doi.org/10.1021/tx060113h.

Matura M, Bodin A, Skare L, Nyren M, Hovmark A, Lindberg M, Lundeberg L, Wrangsjo K, and Karlberg AT. 2004. "Multicentre patch test study of air-oxidized ethoxylated surfactants." *Contact Dermatitis* 51 (4): 180-8. https://doi.org/10.1111/j.0105-1873.2004.00436.x.

Mechilli M, Schinoppi A, Kobos K, Natarajan AT, and Palitti F. 2008. "DNA repair deficiency and acetaldehyde-induced chromosomal alterations in CHO cells." *Mutagenesis* 23 (1): 51-6. https://doi.org/10.1093/mutage/gem042. Epub 2007 Nov 7.

Migliore L, Cocchi L, and Scarpato R. 1996. "Detection of the centromere in micronuclei by fluorescence in situ hybridization: its application to the human lymphocyte micronucleus assay after treatment with four suspected aneugens." *Mutagenesis* 11 (3): 285-90. https://doi.org/10.1093/mutage/11.3.285.

Migliore L, and Nieri M. 1991. "Evaluation of twelve potential aneuploidogenic chemicals by the in vitro human lymphocyte micronucleus assay." In *Toxicol In vitro*, 325-336.

MoritaT, Asano N, Awogi T, Sasaki Y, Sato S, Shimada H, Sutou S, Suzuki T, Wakata A, Sofuni T, and Hayashi M. 1997. "Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (groups 1, 2A and 2B) the summary report of the 6th collaborative study by CSGMT/JEMS MMS. Collaborative Study of the Micronucleus Group Test. Mammalian Mutagenicity Study Group." *Mutat Res* 389 (1): 3-122. https://doi.org/10.1016/s1383-5718(96)00070-8.

Morris J. 1997. "Dosimetry, toxicity and carcinogenicityof inspired acetaldehyde in the rat." *Mutat Res* 380: 113-124.

Morris JB, and Blanchard KT. 1992. "Upper respiratory tract deposition of inspired acetaldehyde." *Toxicol Appl Pharmacol* 114 (1): 140-6. https://doi.org/10.1016/0041-008x(92)90106-3.

Muttray A, Gosepath J, Brieger J, Faldum A, Pribisz A, Mayer-Popken O, Jung D, Rossbach B, Mann W, and Letzel S. 2009. "No acute effects of an exposure to 50 ppm acetaldehyde on the upper airways." *Int Arch Occup Environ Health* 82 (4): 481-8. https://doi.org/10.1007/s00420-008-0354-9. Epub 2008 Aug 21.

Myou S, Fujimura M, Nishi K, Matsuda M, Ohka T, and Matsuda T. 1994. "Potentiating effect of inhaled acetaldehyde on bronchial responsiveness to methacholine in asthmatic subjects." *Thorax* 49 (7): 644-8. https://doi.org/10.1136/thx.49.7.644.

Myou S, Fujimura M, Nishi K, Ohka T, and Matsuda T. 1993. "Aerosolized acetaldehyde induces histamine-mediated bronchoconstriction in asthmatics." *Am Rev Respir Dis* 148 (4 Pt 1): 940-3. https://doi.org/10.1164/ajrccm/148.4_Pt_1.940.

NTP 2014. "Acetaldehyde. CAS No. 75-07-0." *Report on Carcinogens, Fifteenth Edition*.

Nomiyama, T. 2021. "Occupational exposure limits for acetaldehyde, 2-bromopropane, glyphosate, manganese and inorganic manganese compounds, and zinc oxide nanoparticle, and the biological exposure indices for cadmium and cadmium compounds and ethylbenzene, and carcinogenicity, occupational sensitizer, and reproductive toxicant classifications." *J Occup Health* 63 (1): e12294. https://doi.org/10.1002/1348-9585.12294.

OEHHA 2008. "OEHHA (Office of Environmental Health Hazard." In *Air oxics Hot Spots Program Technical Support Document for the Derivation of Noncancer Reference Exposure Levels p4-41 (OEHHA*, 131. Oakalnd, California.

Obe G, Ristow H, and Herha J. 1978. "Mutagenic activity of alcohol in man. In: Mutations: their origin, nature and potential relevance to genetic risk in man. ." Proceedings Yearly Conference 1977 of German Acetaldehyde (EHC 167, 1995) Page 40 of 51. https://inchem.org/documents/ehc/ehc/ehc167.htm 08/09/2022.Research Society. Boppard, Harald Boldt, pp 151-161.

Obe G, and Beek B. 1979. "Mutagenic activity of aldehydes." *Drug Alcohol Depend* 4 (1-2): 91-4. https://doi.org/10.1016/0376-8716(79)90044-9.

Otsuka M, Mine T, Ohuchi K, and Ohmori S. 1996. "A detoxication route for acetaldehyde: metabolism of diacetyl, acetoin, and 2,3-butanediol in liver homogenate and perfused liver of rats." *J Biochem* 119 (2): 246-51. https://doi.org/10.1093/oxfordjournals.jbchem.a021230.

Pettersson H, and Kiessling K. 1977. "Acetaldehyde occurrence in cerebrospinal fluid during ethanol oxidation in rats and its dependence on the blood level and on dietary factors." *Biochem Pharmacol,* 26: 237-240.

Prasanna C.V., and Ramakrishnan S. 1984b. "Effect of acetaldehyde on arbohydrate metabolism in rat brain." *ndian J Biochem Biophys* 21 (2): 121-123.

Prasanna CV, and Ramakrishnan S. 1987. "Effect of acetaldehyde on hepatic and blood lipids." *Indian J Med Res* 85: 206-211.

Prieto L, Gutierrez V, Cervera A, and Linana J. 2002a. "Airway obstruction induced by inhaled acetaldehyde in asthma: Repeatability and relationship to adenosine 5'-monophosphate responsiveness." *J Invest Allerg Clin Immuno* 12 (2): 91-98.

Prieto L, Sanchez-Toril F, Brotons B, Soriano S, Casan R, and Belenguer JL. 2000. "Airway responsiveness to acetaldehyde in patients with asthma: relationship to methacholine responsiveness and peak expiratory flow variation." *Clin Exp Allergy* 30 (1): 71-8. https://doi.org/10.1046/j.1365-2222.2000.00672.x.

Prieto L, Sanchez-Toril F, Gutierrez V, and Marin MJ. 2002b. "Airway responsiveness to inhaled acetaldehyde in subjects with allergic rhinitis: relationship to methacholine responsiveness." *Respiration* 69 (2): 129-35. https://doi.org/10.1159/000056315.

Proctor NH, Hughes JP, Willey AJ, Lechner JF, Grafstrom RC, LaVeck M, and Harris CC. 1978. "Acetaldehyde." *Chemical Hazards of the Workplace* 45 (6): 79-80.

RIFM 1976. *Report on Human Maximization Studies*. Vol. RIFM Report No. 1796, : Research Institute for Fragrance Materials (RIFM).

SCCS 2012. "Scientific Committee on Consumer Safety SCCS OPINION on Acetaldehyde. 16th Plenary meeting of 18 September 2012." *SCCS/1468/12 Revision of 11 december 2012*. https://doi.org/10.2772/8968. .

Saladino AJ, Willey JC, Lechner JF, Grafstrom RC, LaVeck M, and Harris CC. 1985. "Effects of formaldehyde, acetaldehyde, benzoyl peroxide, and hydrogen peroxide on cultured normal human bronchial epithelial cells." *Cancer Res* 45 (6): 2522-2526.

Saldiva PH, do Rio Caldeira MP, Massad E, Calheiros D, Cardoso LM, Bohm GM, and Saldiva CD. 1985. "Effects of formaldehyde and acetaldehyde inhalation on rat pulmonary mechanics." *J Appl Toxicol* 5 (5): 288-92. https://doi.org/10.1002/jat.2550050505.

Sanchez AB, Garcia CCM, Freitas FP, Batista GL, Lopes FS, Carvalho VH, Ronsein GE, Gutz I R., Di Mascio P, and Medeiros MHG. 2018. "DNA Adduct Formation in the Lungs and Brain of Rats Exposed to Low Concentrations of [(13)C(2)]-Acetaldehyde." *Chem Res Toxicol* 31 (5): 332-339. https://doi.org/10.1021/acs.chemrestox.8b00016. Epub 2018 May 9.

Santé Canada. 2000. "Loi canadienne sur la protection de l'environnement. Liste des substances d'intérêt prioritaire. Rapport d'évaluation pour l'acétaldehyde. ." In *Ministere des Travaux publics et des Services gouvernementaux*. Ottawa, Ontario.

Serio NR, and Gudas LJ. 2020. "Modification of stem cell states by alcohol and acetaldehyde." In *Chem Biol Interact*, 316-108919.

Shiohara E., Tsukada M, and Chiba S. 2007. "Effect of chronic administration of acealdehyde by inhalation on (Na+ and K+)-activated adenosine triphosphatase activity of rat brain membranes." *Toxicology* 34: 277-84.

Shmunes E, and Kempton RJ. 1980. "Allergic contact dermatitis to dimethoxane in a spin finish." *Contact Dermatitis* 6 (6): 421-4. https://doi.org/10.1111/j.1600-0536.1980.tb04986.x.

Silverman ., Schuttle H, and First MW. 1946. "Further studies on sensory response to certain industrial solvent vaports." *J Ind Hyg Toxicol* 28: 262-266.

Sim VM, and Pattle RE. 1957. "Effect of possible smog irritants on human subjects." *J Am Med Assoc* 165 (15): 1908-13. https://doi.org/10.1001/jama.1957.02980330010003.

Sina JF, Bean CL, Dysart GR, Taylor VI, and Bradley MO. 1983. "Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential." *Mutat Res* 113 (5): 357-91. https://doi.org/10.1016/0165-1161(83)90228-5.

Singh NP, and Khan A.. 1995. "Acetaldehyde: genotoxicity and cytotoxicity in human lymphocytes." *Mutat Res* 337 1): 9-17. https://doi.org/10.1016/0921-8777(95)00006-6.

Speit G, Frohler-Keller M, Schutz P, and Neuss S. 2008. "Low sensitivity of the comet assay to detect acetaldehyde-induced genotoxicity." *Mutat Res* 657 (2): 93-7. https://doi.org/10.1016/j.mrgentox.2008.07.010. Epub 2008 Aug 5.

Stanek JJ, and Morris JB. 1999. "The effect of inhibition of aldehyde dehydrogenase on nasal uptake of inspired acetaldehyde." *Toxicol Sci* 49 (2): 225-31. https://doi.org/10.1093/toxsci/49.2.225.

Stanek J, Symanowicz PT, Olsen JE, Gianutsos G, and Morris JB. 2001. "Sensory-nerve-mediated nasal vasodilatory response to inspired acetaldehyde and acetic acid vapors." *Inhal Toxicol* 13 (9): 807-22. https://doi.org/10.1080/08958370120057.

Steinhagen WH, and Barrow CS. 1984. "Sensory irritation structure-activity study of inhaled aldehydes in B6C3F1 and Swiss-Webster mice." *Toxicol Appl Pharmacol* 72 (3): 495-503. https://doi.org/10.1016/0041-008x(84)90126-1.

Stotts J, and Ely WJ. 1977. "Induction of human skin sensitization to ethanol." *J Invest Dermatol* 69 (2): 219-22. https://doi.org/10.1111/1523-1747.ep12506333.

Teeguarden JG, Bogdanffy MS, Covington TR, Tan C, and Jarabek AM. 2008. "A PBPK model for evaluating the impact of aldehyde dehydrogenase polymorphisms on comparative rat and human nasal tissue acetaldehyde dosimetry." *Inhal Toxicol* 20 (4): 375-90. https://doi.org/10.1080/08958370801903750.

Terelius Y, Norsten-Höög C, Cronholm T, and Ingelman-Sundber M. 1991. "Acetaldehyde as a substrate for ethanol-inducible cytochrome P450 (CYP2E1)." *Biochemical and Biophysical Research Communications* 179 (1): 689-694.

Thapa MJ, Fabros RM, Alasmar S, and Chan K. 2022. "Analyses of mutational patterns induced by formaldehyde and acetaldehyde reveal similarity to a common mutational signature." *G3 (Bethesda)* 12 (11): jkac238. https://doi.org/10.1093/g3journal/jkac238.

Thredgold L, Gaskin S, Heath L, Pisaniello D, Logan M, and Baxter C. 2020. "Understanding skin absorption of common aldehyde vapours from exposure during hazardous material incidents." *Journal of Exposure Science & Environmental. Epidemiology* 30: 537-546.

US EPA 1991. "Acetaldehyde - Reference Concentration for Chronic Inhalation Exposure (RfC)." *U.S*. https://doi.org/https://cfpub.epa.gov/ncea/iris2/chemicalLanding.cfm?substance_nmbr=290.

US EPA 1994b. "US. EPA. Methods For Derivation Of Inhalation Reference Concentrations (RfCs) And Application Of Inhalation Dosimetry. U.S. Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment, Washington, DC, EPA/600/8-90/066F.".

US EPA 2009. "Status Report: Advances In Inhalation Dosimetry of Gases and Vapors With Portal of Entry Effects In the Upper Respiratory Tract. U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-09/072, 2009.".

Umulis, DM, Gurmen NM, Singh P, and Fogler HS. 2005. "A physiologically based model for ethanol and acetaldehyde metabolism in human beings." *Alcohol* 35 (1): 3-12. https://doi.org/10.1016/j.alcohol.2004.11.004.

Vaca CE, Fang JL, and Schweda EK. 1995. "Studies of the reaction of acetaldehyde with deoxynucleosides." *Chem Biol Interact* 98 (1): 51-67. https://doi.org/10.1016/0009-2797(95)03632-v.

Vaca CE, Nilsson JA, Fang JL, and Grafstrom RC. 1998. "Formation of DNA adducts in human buccal epithelial cells exposed to acetaldehyde and methylglyoxal in vitro." *Chem Biol Interact* 108 (3): 197-208. https://doi.org/10.1016/s0009-2797(97)00107-5.

Veghelyi PV, and M. Osztovics. 1978. "The alcohol syndromes: the intrarecombigenic effect of acetaldehyde." *Experientia* 34 (2): 195-6. https://doi.org/10.1007/BF01944673.

Vijayraghavan SL, Porcher PA, Mieczkowski and Saini N.. 2022. "Acetaldehyde makes a distinct mutation signature in single-stranded DNA." *Nucleic Acids Res* 50 (13): 7451-7464. https://doi.org/10.1093/nar/gkac570.

Von Burg R and Stout T. 1991. "Acetaldehyde." *J Appl Toxicol* 11 (5): 373-6. https://doi.org/10.1002/jat.2550110513.

Von Wartburg P. 1987. "Acute aldehyde syndrome and chronic aldehydism." *Muta Reseach/Reviews in Genetic Toxicology* 186 (3): 249-259.

WHO 1995. *Acetaldehyde. IPCS. Environmental health criteria*. Geneva: WHO.

Wakata A, Miyamae Y, Sato S, Suzuki T, Morita T, Asano N, Awogi T, Kondo K, and Hayashi M. 1998. "Evaluation of the rat micronucleus test with bone marrow and peripheral blood: summary of the 9th collaborative study by CSGMT/JEMS. MMS. Collaborative Study Group for the Micronucleus Test. Environmental Mutagen Society of Japan. Mammalian Mutagenicity Study Group." *Environ Mol Mutagen* 32 (1): 84-100.

Wang M, McIntee EJ, Cheng G, Shi Y, Villalta PW, and Hecht SS. 2000. "Identification of DNA adducts of acetaldehyde." *Chem Res Toxicol* 13 (11): 1149-57. https://doi.org/10.1021/tx000118t.

Wangenheim J, and Bolcsfoldi G. 1988. "Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds." *Mutagenesis,* 3 (3): 193-206.

Watanabe F, and Sugimoto S. 1956. "[Study on the carcinogenicity of aldehyde. 3. Four cases of sarcomas of rats appearing in the areas of repeated subcutaneous injections of acetaldehyde]." *Gan* 47 (3-4): 599-601.

Westcott JY, Weiner ., Schultz J, and Myers RD. 1980. "In vivo acetaldehyde in the brain of the rat treated with ethanol." *Biochem. Pharmacol* 29 (411-417).

Wilkin JK, and Fortner G. 1985. "Cutaneous vascular sensitivity to lower aliphatic alcohols and aldehydes in Orientals." *Alcohol Clin Exp Res* 9 (6): 522-5. https://doi.org/10.1111/j.1530-0277.1985.tb05596.x.

Woodruff RC, Mason JM, Valencia R, and Zimmering S. 1985. "Chemical mutagenesis testing in Drosophila. V. Results of 53 coded compounds tested for the National Toxicology Program." *Environ Mutagen* 7 (5): 677-702. https://doi.org/10.1002/em.2860070507.

Woutersen RA, Appelman LM, Vander Heijden CA, and Feron VJ. 1984. "Inhalation toxicity of acetaldehyde in rats. II. Carcinogenicity study : interim results after 15 months." *Toxicology* 31 (2): 123-133. https://doi.org/10.1016/0300-483X(84)90004-0.

Woutersen RA, Appelman LM, Van Garderen-Hoetmer A, and Feron VJ. 1986. "Inhalation toxicity of acetaldehyde in rats. III. Carcinogenicity study." *Toxicology* 41 (2): 213-31. https://doi.org/10.1016/0300-483x(86)90201-5.

Woutersen RA, and Feron VJ. 1987. "Inhalation toxicity of acetaldehyde in rats. IV. Progression and regression of nasal lesions after discontinuation of exposure." *Toxicology* 47 (3): 295-305. https://doi.org/10.1016/0300-483x(87)90059-x.

Zhu L, Pei W, Thiele I, and Mahadevan R. 2021. "Integration of a physiologically-based pharmacokinetic model with a whole-body, organ-resolved genome-scale model for characterization of ethanol and acetaldehyde metabolism." *PLoS Comput Biol* 17 (8): e1009110. https://doi.org/10.1371/journal.pcbi.1009110. eCollection 2021 Aug.

Zorzano and Herrera. 1989. "Disposition of ethanol and acetaldehyde in late pregnant rats and their fetuses." *Pediatr Res* 25: 102-106.

Protocols

DFG method 2 (1995): Schmitz, P. and Tschickardt, M. 2012. Aldehydes (Formaldehyde, Acetaldehyde, Propionaldehyde, Butyraldehyde, Glutaraldehyde, Pentanal, Hexanal, Heptanal, Octanal, Nonanal) [Air Monitoring Methods, 2002]. The MAK Collection for Occupational Health and Safety. 3–13.(http://onlinelibrary.wiley.com/doi/10.1002/3527600418.am5000e0005/full)

DFG method 1 (1990): Wolf, D. and Hahn, J. U. 2012. Aldehyde (Formaldehyd, Acetaldehyd, Propionaldehyd, Butyraldehyd, Glutaraldehyd) [Air Monitoring Methods in German language, 1992]. The MAK Collection for Occupational Health and Safety. 1–11. (http://onlinelibrary.wiley.com/doi/10.1002/3527600418.am5000d0007/full)

HSE MDHS 102 (2010). Methods for the Determination of Hazardous Substances. Health and Safety Laboratory. Aldehydes in air Laboratory method using high performance liquid chromatography

INRS M-66 (2016). Métropol. Acétaldéhyde M-66.

IRSST 385. Méthode analytique pour la mesure de l'acétaldéhyde. (Consulté en juin 2024 : Acétaldéhyde)

LCSQA (2007). Mesure du formaldéhyde. Comparaison de différentes méthodes de prélèvement des aldéhydes, en présence d'ozone, en conditions réelles et simulées.

LCSQA (2013). Air intérieur – Etude 8/3. L'acétaldéhyde : métrologie et état des lieux des niveaux de concentration en air intérieur.

NF X 43 264 (2011): Air des lieux de travail : Prélèvement et dosage d'aldéhydes par pompage sur supports imprégnés de DNPH et dosage par chromatographie en phase liquide CLPH.

NIOSH 2018 (2013): Aliphatic aldehydes. NIOSH Manual of Analytical Methods (NMAM), Fourth Edition.

NIOSH 2538 (1994): Acetaldehyde by GC. NIOSH Manual of Analytical Methods (NMAM), Fourth Edition.

NIOSH 3507 (1994): Acetaldehyde by HPLC. NIOSH Manual of Analytical Methods (NMAM), Fourth Edition.

OSHA 68 (1988), Acetaldehyde. T-68-FV-01-8801-M

US EPA TO 11A (1999). Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air. Second Edition. Compendium Method TO-11A. Determination of Formaldehyde in Ambient Air Using Adsorbent Cartridge Followed by High Performance Liquid Chromatography (HPLC) [Active Sampling Methodology].

US-EPA TO 15 (1999) Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air. Second Edition. Compendium Method TO-15. Determination Of Volatile Organic Compounds (VOCs) In Air Collected In Specially Prepared Canisters And Analyzed By Gas Chromatography/ Mass Spectrometry (GC/MS).

Annex 1: Literature search

To identify relevant references, the lexical query begins with the identification of keywords and the formalisation of a search strategy (e.g. period, scope, search equation, database, etc.). It is based in particular on a translation of the parameters of the PECO structure (Table 27) (Anses 2023 - ACCMER).

Table 27 : PECO structure linked to a reference value proposal

Population (or subjects studied)	General population /workers/sensitive population
Exposure	Acetaldehyde
Comparator	Non exposed vs exposed
***Outcome** (result of interest, outcome measured, criteria of judgement. i.e. : mortality; effects on health, psychosocial effects, perceptions)**	All effects observed in human health in particular following inhalation exposure

Study question: Identification of the toxicological studies performed with acetaldehyde following inhalation

The goal of this review was to derive occupational exposure limit values for acetaldehyde by inhalation.

Description of the review method

The literature search was conducted in the Pubmed and SCOPUS databases.

Secondary literature was also considered to check for potential additional research studies or mechanistic data not captured during the literature search: IPCS-INCHEM 1995; INRS, 2022; DFG, 1982, 2013; ACGIH® 2014; Anses, 2014.

Titles, key words and abstracts were screened with the following key words (#1 AND #2 AND #3 NOT #4):

Bibliography review 10 October 2022

PUBMED

Key words: acetaldehyde + toxicity + inhalation, last 10 years

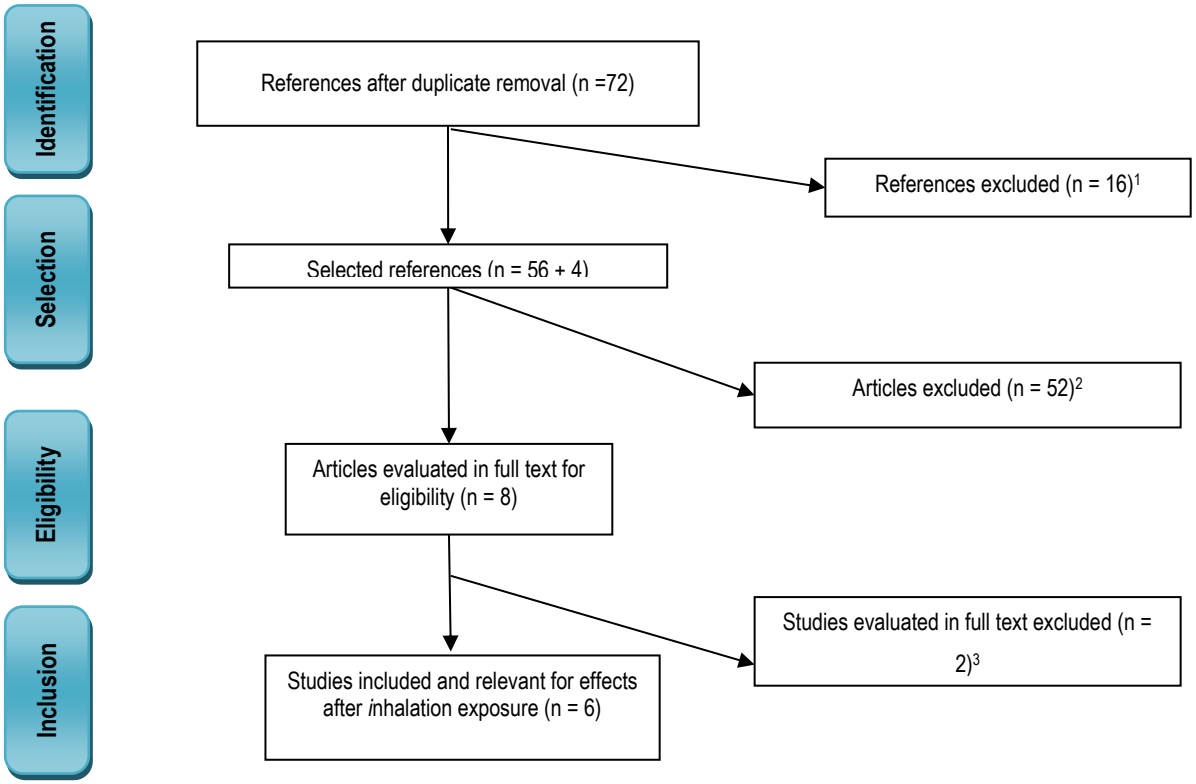
Filter: in the last 10 years: 36 results

SCOPUS

Equation: (TITLE-ABS-KEY (acetaldehyde) AND TITLE-ABS-KEY (inhalation)) AND (LIMIT-TO (PUBYEAR , 2022) OR LIMIT-TO (PUBYEAR , 2021) OR LIMIT-TO (PUBYEAR , 2020) OR LIMIT-TO (PUBYEAR , 2019) OR LIMIT-TO (PUBYEAR , 2018) OR LIMIT-TO (PUBYEAR , 2017) OR LIMIT-TO (PUBYEAR , 2016) OR LIMIT-TO (PUBYEAR , 2015) OR LIMIT-TO (PUBYEAR , 2014))

36 publications

In total, 72 publications (after duplicate removal) were identified based on a combined search in PubMed and Scopus databases. 60 references (toxicological / research reports, journal / articles, book section) were retained based on titles and abstracts. See the following diagram (8).



[1] Articles excluded did not provide data for acetaldehyde by inhalation exposure. Articles in French and English included covered the period 2002 to 2022.

[2] Articles evaluated based on titles and abstracts that were excluded did not provide adverse effect relevant data for acetaldehyde in humans after inhalation exposure, or provided acetaldehyde-related source data.

[3] Articles evaluated in full text for eligibility that were excluded did not provide relevant data for acetaldehyde in humans.

Figure 8 : Diagram PRISMA

Annex 2: Summary of *in vivo* and *in vitro* results performed with acetaldehyde

Table 28 : **Results of *in vivo* results in somatic and germ cells**

Test system	Species, strain, number per group	Exposure	Results	References
Somatic cells				
Sister Chromatid Exchange, bone marrow non-standardised test; toxicity not specified	mouse, CBA, 1 ♂/dose	single, intraperitoneal, 1 or 0.5 mL of a 10^{-4} % (v/v) solution;	After 28 hours: Positive	(Obe and Beek 1979)
Sister Chromatid Exchange, bone marrow non-standardised test; mortality at 0.6 mg/kg bw and above	hamster, Chinese, 6–7 ♂/dose	single, intraperitoneal, 0, 0.01, 0.1, 0.5 mg/kg bw	Positive at 0.5 mg/kg bw	(Korte et al. 1981)
Micronuclei, bone marrow, peripheral blood	mouse, CD-1, 6 ♂/dose	single, intraperitoneal, 0, 95, 190, 380 mg/kg bw in sodium chloride solution, purity: 89.4%	after 24 hours: Positive at 190 mg/kg bw and above note : LD50 = 470 mg/kg bw	(Morita et al. 1997)

<table>
<tr>
<td>Micronuclei, bone marrow, peripheral blood</td>
<td>mouse, CD-1, 5 ♂</td>
<td>single, intraperitoneal, 0, 100, 200, 300, 400 mg/kg bw in sodium chloride solution, purity: > 99.5%
3 dose levels ; tests on acute toxicity performed</td>
<td>after 24 hours:
Positive at 200 mg/kg bw and above (dose-related increase)

LD50 = 338 mg/kg bw
<table>
<tr><th>Manufact. lot</th><th>LD50 mg/kg bw</th><th>Dose mg/kg bw</th><th>Percentage of micronuclei in bone marrow cells mean</th><th>SD</th><th>p-value*</th></tr>
<tr><td>Wako</td><td>470</td><td>0</td><td>0.12</td><td>0.08</td><td>-</td></tr>
<tr><td></td><td></td><td>95</td><td>0.22</td><td>0.15</td><td>0.132</td></tr>
<tr><td></td><td></td><td>190</td><td>0.33</td><td>0.10</td><td>0.010</td></tr>
<tr><td></td><td></td><td>380</td><td>0.85</td><td>0.21</td><td>0.000</td></tr>
<tr><td>Merck</td><td>338</td><td>0</td><td>0.12</td><td>0.08</td><td>-</td></tr>
<tr><td></td><td></td><td>100</td><td>0.10</td><td>0.07</td><td>0.726</td></tr>
<tr><td></td><td></td><td>200</td><td>0.44</td><td>0.11</td><td>0.002</td></tr>
<tr><td></td><td></td><td>300</td><td>0.62</td><td>0.16</td><td>0.000</td></tr>
<tr><td></td><td></td><td>400</td><td>1.10</td><td>0.25</td><td>0.000</td></tr>
</table>
* P-value of pairwise comparisons.</td>
<td>(Morita et al. 1997)</td>
</tr>
<tr>
<td>Micronuclei, peripheral blood</td>
<td>mouse, C57BL/6J, 4 ♂, control animals: 2 ♂</td>
<td>5 days, intraperitoneal, 0, 6, 12 mg/kg bw in sodium chloride solution, purity: not specified</td>
<td>Positive at the low dose (6 mg/kg), but not at the high dose (12 mg/kg)

toxicity not specified
Low number of mice/group</td>
<td>(Ma T 1985)</td>
</tr>
<tr>
<td>Micronuclei</td>
<td>Male Han rats, 5 animals/group</td>
<td>single, intraperitoneal injection of 125 or 250 mg/ kg bw; blood samples collected after 0, 24, 48 and 72 hours</td>
<td>Positive at 24 and 48 hours; dose related increase;
No data at 72 hours due to toxicity
<table>
<tr><th>Dose (mg/kg bw)</th><th>Time (h)</th><th>Laboratory*</th><th>Mean RET** ± SD</th><th>Mean MNRET** per 20,000 RET ± SD</th><th>Mean MNNCE** ± SD</th></tr>
<tr><td>0</td><td>0</td><td>GW</td><td>1.29 ± 0.29</td><td>0.13 ± 0.06</td><td>0.01 ± 0.00</td></tr>
<tr><td></td><td></td><td>LL</td><td>1.47</td><td>0.14</td><td>0.01</td></tr>
<tr><td>125</td><td>24</td><td>GW</td><td>0.80 ± 0.12</td><td>0.21 ± 0.07</td><td>0.01 ± 0.00</td></tr>
<tr><td></td><td></td><td>LL</td><td>0.91</td><td>0.19</td><td>0.01</td></tr>
<tr><td></td><td>48</td><td>GW</td><td>1.32 ± 0.21</td><td>0.30 ± 0.09</td><td>0.01 ± 0.00</td></tr>
<tr><td></td><td></td><td>LL</td><td>1.37</td><td>0.19</td><td>0.01</td></tr>
<tr><td></td><td>72</td><td>GW</td><td>1.82 ± 0.18</td><td>0.14 ± 0.05</td><td>0.01 ± 0.00</td></tr>
<tr><td></td><td></td><td>LL</td><td>1.65</td><td>0.18</td><td>0.01</td></tr>
<tr><td>250</td><td>24</td><td>GW</td><td>1.00 ± 0.42</td><td>0.28 ± 0.07</td><td>0.02 ± 0.01</td></tr>
<tr><td></td><td></td><td>LL</td><td>0.99</td><td>0.32</td><td>0.01</td></tr>
</table></td>
<td>(Hynes et al. 2002)</td>
</tr>
</table>

<table>
<tr><td></td><td></td><td></td><td>
<table>
<tr><td>48</td><td>GW</td><td>1.31 ± 0.25</td><td>0.33 ± 0.11</td><td>0.02 ± 0.01</td></tr>
<tr><td></td><td>LL</td><td>1.14</td><td>0.39</td><td>0.01</td></tr>
<tr><td>72</td><td>GW</td><td>1.90 ± 0.42</td><td>0.14 ± 0.05</td><td>0.01 ± 0.01</td></tr>
<tr><td></td><td>LL</td><td>1.42</td><td>0.16</td><td>0.01</td></tr>
</table>
* GW, GlaxoWellcome; LL, Litron Laboratories. ** RET, reticulocytes; MNRET, micronucleated reticulocytes; MNNCE, micronucleated monochromatic erythrocytes. No data on statistical significance presented.
</td><td></td></tr>
<tr><td>Gene mutation and micronuclei</td><td>Wild type and knock-out mice with inactive ALDH2 gene;
Micronuclei determined in reticulocytes;
Mutations determined by T-cell receptor (TCR) gene mutation assay</td><td>Inhalation, 125 and 500 ppm vapour, continuously for 2 weeks

Negative control was inhalation of clean air

Oral, 100 mg/kg bw/d, once a day for 2 weeks continuously</td><td>Micronuclei:
Positive in knock-out mice (p<0.05)
Negative in wild-type mice
Mutations (TCR mutant frequency):
Positive in knock –out mice (p<0.05)
Negative in wild-type mice

No positive controls were used
<table>
<tr><th>Exposure route</th><th>Exposure level</th><th>Micronuclei in reticulocytes</th><th>Mutant frequency in T-cell receptor gene</th></tr>
<tr><td>Knock-out mice (Aldh2 -/-)</td><td></td><td></td><td></td></tr>
<tr><td>Inhalation</td><td>0 (control)</td><td>-</td><td>-</td></tr>
<tr><td></td><td>225 mg/m^3</td><td>1.8 *</td><td>Not determined</td></tr>
<tr><td></td><td>900 mg/m^3</td><td>1.9/unspecified **/***</td><td>1.7**</td></tr>
<tr><td>Oral administration</td><td>0 (control)</td><td>-</td><td>-</td></tr>
<tr><td></td><td>100 mg/kg bw</td><td>2/1.7 **/***</td><td>2.4/1.6 **/***</td></tr>
<tr><td>Wildtype mice (Aldh2 +/+)</td><td></td><td></td><td></td></tr>
<tr><td>Inhalation</td><td>0 (control)</td><td>-</td><td>-</td></tr>
<tr><td></td><td>225 mg/m^3</td><td>-</td><td>-</td></tr>
<tr><td></td><td>900 mg/m^3</td><td>-</td><td>-</td></tr>
<tr><td>Oral administration</td><td>0 (control)</td><td>-</td><td>-</td></tr>
<tr><td></td><td>100 mg/kg bw</td><td>-</td><td>-</td></tr>
</table>
* compared to Aldh2 +/+ control mice (p<0.05); ** compared to Aldh2 +/+ control mice (p<0.01); *** compared to Aldh2 -/- control mice (p<0.05).
</td><td>(Kunugita et al. 2008)</td></tr>
<tr><td>Micronuclei</td><td>Male Sprague Dawley and F-344 rats, bone marrow erythrocytes</td><td>0, 500, 1000, 2000 mg/kg bw, intraperitoneal injection;</td><td>Positive (both cell types)</td><td>(Wakata et al. 1998)</td></tr>
</table>

		highest dose tested was maximum tolerated dose; at least 4 animals/group		
Chromosome aberration	Rat embryos	Single intra-amniotic injection of 7800 mg/kg bw	Positive original publication available in Russian only	(Bariliak and Kozachuk 1983)
Germ cells				
Micronuclei, early spermatids	mouse, hybrid (C57BL/6J×C3H/He) 4 ♂per group, mouse early spermatids control animals: 7 ♂	Single dose, Intraperitoneal injection, 0, 125, 250, 375, or 500 mg/kg bw/day in sodium chloride solution	after 13 days: negative No increase in MN frequency in early spermatids following IP injection at doses up to 500 mg/kg/bw survival rate significantly decreased in highest exposure group only one specific stage investigated, not the entire spermatogenesis line age	(Lahdetie 1988)
Sex-linked recessive lethal mutations; multisubstances study	Drosophila melanogaster	1) single injection of 22,500 ppm; 2) 25,000 ppm in feed; data presented on mortality and sterility	Positive (injection) Negative (feed)	(Woodruff et al. 1985)
Sister chromatid exchange	Mouse spermatogonial cells	Single intraperitoneal injection, 0, 0.4, 4.0, 40 and 400 mg/kg bw; 4-5 animals/concentration, cells isolated, 53 hours after injection	Positive : all doses applied, p< 0.05 no clear exposure-response relationship observed Inhibition of aldehyde dehydrogenase resulted in an increase in SCEs at normally non-genotoxic doses (0.004 and 0.04 mg/kg bw). Authors did not test for intoxication, concentrations used were considered non-toxic/-lethal	(Madrigal-Bujaidar et al. 2002)

			Dose (mg/kg bw) / SCE/cell ± SD / SCE increase 0 / 1.9 ± 0.16 0.4 / 2.9 ± 0.33* / 1.1 4 / 4.1 ± 0.34* / 2.2 40 / 4.6 ± 0.51* / 2.7 400 / 5.1 ± 0.8* / 3.2 50 (cyclophosphamide) / 6.0 ± 0.1* / 4.1 SCE, sister chromatid exchange. * Statistically significant different compared to control, $p < 0.05$.	

SMART: somatic mutagenicity and recombination test, MN: micronucleus test, SCE: sister chromatid exchange

Table 29 : Results of in vitro cell transformation tests using rodent cells treated with acetaldehyde

Test system	Exposure	Result	References
C3H/10Tl/2 cells, mouse	10–100 µg/mL	Negative Positive at 10 µg/mL together with 0.25 µg/ml TPA and above LC50: 25 µg/mL	(Abernethy, Frazelle, and Boreiko 1982)
cell line, HRRT, kidney, rat	up to 3 mM (132 µg/mL)	Positive at 3 mM with 1.1 µg/mL TPA 1.2 no cytotoxicity up to 3 mM (132 µg/mL)	(Eker and Sanner 1986)

HRRT: hereditary renal rat tumour; m. a.: metabolic activation; n.d.: not determined; TPA: 12-O-tetradecanoyl phorbol-13-acetate

Table 30 : Summary of *in vitro* genotoxicity/mutagenicity studies in mammalian cells performed with acetaldehyde

Test system	Species and/or cell line type	Exposure	Results	References
Mammalian cells				

Comet assay	Human lymphocytes	1.56–100 mM (69–4400 µg/mL)	+S9: Positive at 1.56 mM Single strand breaks, at 100 mM; double Strand breaks-S9: no data 100 mM (4400 µg/ ml) (1 hour): < 20% surviving cells	(Singh and Khan 1995)
Comet assay	Human lymphocytes Mucosa cells, stomach, colon, human	3–200 mM (132–8800 µg/mL)	+S9: Positive at 3 mM (132 µg/mL) and above > 70% viability at 200 mM (8800 µg/mL	(Blasiak et al. 2000)
Alkaline elution	Human lymphocytes	10, 20 mM (440, 880 µg/mL)	Negative strand breaks Positive: DNA crosslinks at 10 mM (440 µg/mL) and above no other details	(Lambert et al. 1985)
Alkaline elution	Bronchial epithelial cells, human	Up to 1 mM (44 µg/mL)	Negative	(Saladino 1985)
Alkaline elution	Bronchial epithelial cells, human	1–100 mM (44–4400 µg/mL) for 5 hours Positive and negative control included, cell vaibility tests performed	Negative: single strand breaks; positive: DNA crosslinks at 3 mM (132 µg/mL) and above IC50 (concentrations which lead to 50% inhibition): 25 mM (1100 µg/ mL) (colony forming efficiency); 125 mM (5500 µg/ mL) viability	(Grafstrom et al. 1994)
Alkaline elution	CHO cells	0.5–4.5 mM (22–198 µg/mL)	Negative: single strand breaks; Positive: DNA cross-links at 1.5 mM (66 µg/mL) and above viability (% of controls) over the entire concentration range 100%	(Marinari et al. 1984)
Alkaline elution	Rat hepatocytes	0.03–3 mM (1.3–132 µg/mL)	Negative viability (% of controls) > 58%	(Sina et al. 1983)

Alkaline elution	Mouse lymphocyte cells	1.5–44 mM (66–1900 µg/mL)	Negative 84% not viable at 44 mM (1900 µg/mL)	(Garberg, Åkerblom and Bolcsfoldi 1988) quoted by (DFG 2013b)
Alkaline elution	Male Fisher-344 rats; DNA protein cross-links studies homogenates of nasal respiratory mucosa were incubated with acetaldehyde – There was also in vivo results as part of this study	0,180, 540, 1800 and 5400 $mg.m^{-3}$ (0, 100, 300, 1000 and 3000 ppm), 1) for a single 6 hours exposure, or 2) to 5400 $mg.m^{-3}$ (3000 ppm), 6 hours a day for 5 consecutive days	1) positive in the respiratory mucosa (dose-dependent increase), but negative in the olfactive mucosa 2) positive in the respiratory mucosa and in the olfactive mucosa	(Lam Casanova and Heck 1986)
Chromosome aberration	Primary rat skin fibroblasts	0.1–10 mM (4.4–440 µg/mL) for 12 and 24 hours ; 50 metaphases analysed / doses	positive at 0.1 mM (4.4 µg/mL) and above 12 hours : negative 24 hours : positive ($p<0.05$, except lowest dose concentration-related increase in aneuploidy cytotoxicity not specified no positive controls	(Bird, Draper and Basrur 1982)
Chromosome aberration	Human peripheral lymphocytes from 3 healthy volunteers	0 ; 0.001; 0.002% (v/v) 0.18–0.36 mM (7.8–15 µg/mL) 100 or 200 mitoses scored / sample	Negative in samples from healthy volunteers Positive dose-dependent in human lymphocytes of 1 person with Fanconi's anaemia cytotoxicity not specified, no positive control	(Obe and Beek 1979)
Chromosome aberration	Human lymphocytes	0.02 mM (7.8 µg/mL), 2×/day, 4 days	Positive at the only one concentration tested cytotoxicity not specified	(Obe, Ristow and Herha 1978)

<table>
<tr><td></td><td></td><td></td><td></td><td>quoted in (WHO. 1995)</td></tr>
<tr><td>Chromosome aberration</td><td>Different DNA repair deficient Chinese hamster ovary cells</td><td>0.3,0.3,1.0,1.8,2.5 and 3.6 mM
for 2 hours;
100 metaphases scored/ group</td><td><table>
<tr><th>Cell line</th><th>CAs</th><th></th><th>Abnormal cells or SCEs</th><th></th></tr>
<tr><th></th><th>1 mM</th><th>1.8 mM</th><th>F_{abs} 0.6 mM</th><th>F_{SCE} 0.6 mM</th></tr>
<tr><td>AA8</td><td>1</td><td>1</td><td>1</td><td>1</td></tr>
<tr><td>EM9</td><td>1.43</td><td>2.50</td><td>1</td><td>1.25</td></tr>
<tr><td>V3-3</td><td>1.78</td><td>0</td><td>1.29</td><td>1.29</td></tr>
<tr><td>KO40</td><td>2.96</td><td>6.70</td><td>2.36</td><td>1.21</td></tr>
<tr><td>51D1</td><td>31.9</td><td>67.1</td><td>27.28</td><td>0.93</td></tr>
<tr><td>irs-1SF</td><td>9.52</td><td>0</td><td>3.50</td><td>0.70</td></tr>
<tr><td>UV61</td><td>0.42</td><td>0.94</td><td>0.36</td><td>1.68</td></tr>
<tr><td>UV4</td><td>2.6</td><td>4.40</td><td>2.36</td><td>0.68</td></tr>
<tr><td>UV5</td><td>0.27</td><td>0.63</td><td>0.21</td><td>1.20</td></tr>
</table>No positive control</td><td>(Mechilli et al. 2008)</td></tr>
<tr><td>Chromosome aberration</td><td>Human lymphocytes, human fibroblasts</td><td>40, 400 and 800 µM (1.8–35 µg/mL)</td><td>Positive at 0.4 mM (70 µg/ mL) and above

cytotoxicity at 800 µM (35 µg/mL) and above</td><td>(Veghelyi and Osztovics 1978)</td></tr>
<tr><td>Chromosome aberration</td><td>Human lymphocytes</td><td>90–1080 µM (4–48 µg/mL)</td><td>Positive at 0.72 mM (33 µg/ mL) and above

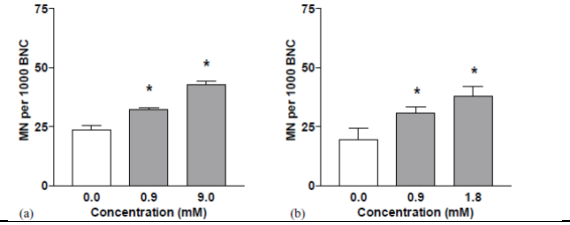
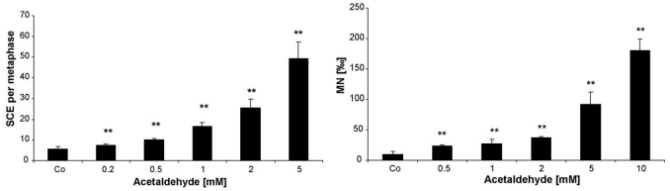
cytotoxicity at > 1080 µM (48 µg/mL)</td><td>(Bohlke, Singh, and Goedde 1983)</td></tr>
<tr><td>Chromosome aberration</td><td>Chinese hamster embryonic diploid fibroblast</td><td>0.002–0.006% (0,20,40 and 60 µg/mL, -S9 (0.35–1.1 mM; 16–48 µg/mL)</td><td>Positive at 0.006% (1.1 mM)
aneuploidy (usually hypodiploidy, but also hyperdiploidy)

highest concentration: mitosis index decreased

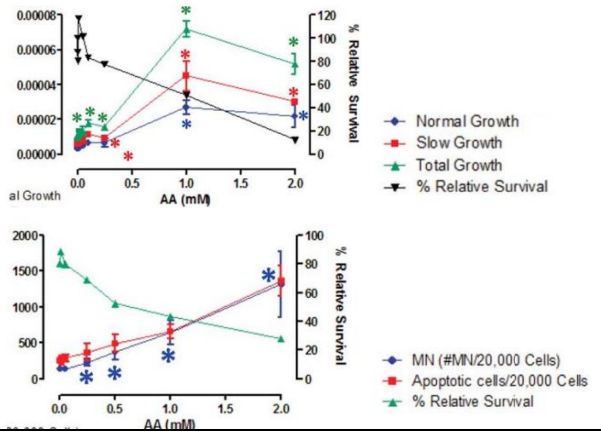
no data on cytotoxicity, no positive control</td><td>(Dulout and Furnus 1988)</td></tr>
<tr><td>Chromosome aberration</td><td>CHO cells</td><td>88–5000 µg/mL (2–114 mM)</td><td>Positive at 2 mM (88 µg/ mL) and above

cytotoxicity
not specified</td><td>(WHO 1995)</td></tr>
<tr><td>Micronucleus assay</td><td>Human lymphocytes</td><td>0.2–2.0 mM (8.8–88 µg/mL)</td><td>Positive at 0.8 mM (35 µg/mL) and above

cytotoxicity at > 1 mM</td><td>(Migliore and Nieri 1991)</td></tr>
</table>

Micronucleus assay	Human lymphocytes isolated from peripheral blood from one healthy non smoking donor	0.6–1 mM (26–44 µg/mL)	Positive at 0.6 mM (26 µg/mL) and above (dose-related increase, p<0.05) no cytotoxicity; additional test (FISH) showed possible aneugenic effects	(Migliore, Cocchi, and Scarpato 1996)
Micronucleus assay	Primary rat skin fibroblasts	0.1–10 mM (4.4–440 µg/mL) for 12, 24 or 48 hours ; less than 1000 cells analysed/dose	Positive at 0.5 mM (22 µg/mL) and above (p<0.05) except lowest dose tested cytotoxicity not specified, no positive controls	(Bird, Draper and Basrur 1982)
Micronucleus assay	Human derived hepatoma (hepG2, Hep3B)	0.0, 0.9, 9.0 mM for 24 hours	Positive (concentrations-related increase in bi-/mono-nucleated ratio  	(Majer et al. 2004 quoted by ECHA 2016)
Micronucleus assay	V79 Chinese hamster ovary cells	0.2-10 mM (MN)	Positive (dose-dependent increase)  No positive control	(Speit et al. 2008 quoted by ECHA 2016)

<table>
<tr>
<td>Cytokinesis blocked micronucleus assay (CBMN)</td>
<td>MCL-5 Human fibroblast cell line</td>
<td>0, 0.1, 0.2, 0.4, 0.8, 1.0, 2% (0, 0.79, 1.58, 3.16, 6.32, 7.9, 15.8 mg.LmL^{-1}). (v/v; a range of 6 different concentrations) for 22 hours; > 4000 cells per dose examined</td>
<td>Positive (from 0.4% onwards, p<0.05), dose dependent increase in MN induction vs control over the dose range tested.
aneuploidy
<table>
<tr><th>Dose (% v/v)</th><th>Number of cells scored</th><th>CBPI</th><th>% Cytostasis</th><th>BN cells with micronuclei (MNBn) (%)</th><th>Apoptosis (%)</th><th>Necrosis (%)</th><th colspan="2">Relative proportions of kinetochore positive</th></tr>
<tr><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th>K$^+$</th><th>K$^-$</th></tr>
<tr><td>00</td><td>4036</td><td>1.55</td><td>0</td><td>0.85</td><td>0.37</td><td>7.84</td><td>0.47</td><td>0.53</td></tr>
<tr><td>0.005</td><td>5097</td><td>1.22</td><td>60</td><td>1.86*</td><td>3.53**</td><td>8.74</td><td>nt</td><td>nt</td></tr>
<tr><td>0.010</td><td>5044</td><td>1.21</td><td>61.81</td><td>2.08*</td><td>2.60**</td><td>13.29**</td><td>0.46</td><td>0.54</td></tr>
<tr><td>0.015</td><td>5043</td><td>1.21</td><td>61.81</td><td>2.28*</td><td>2.47**</td><td>10.82**</td><td>0.33</td><td>0.67</td></tr>
<tr><td>0.020</td><td>4906</td><td>1.19</td><td>65.45</td><td>2.60*</td><td>1.85**</td><td>11.69**</td><td>0.34</td><td>0.66</td></tr>
<tr><td>0.025</td><td>4919</td><td>1.19</td><td>65.45</td><td>3.73*</td><td>1.70**</td><td>17.78**</td><td>0.32</td><td>0.68</td></tr>
</table>
CBPI – Cytokinesis Blocked Proliferation Index.
nt: not tested.
MN = micronuclei, K$^+$ = kinetochore positive, K$^-$ = kinetochore negative.
* Significant increase P < 0.05 compared with control cultures.
** Significant increase P < 0.01 compared with control cultures.</td>
<td>(Kayani and Parry 2010 quoted by ECHA 2016)</td>
</tr>
<tr>
<td>micronuclei</td>
<td>Human lymphoblastoid TK6 cells</td>
<td>8 different concentrations tested; between 0.005 and 4 mM; negative and positive controls included, only data analysed when cytotoxicity was below 55%</td>
<td>Positive : 0.25,0.5 and 1.0 mM</td>
<td>ECHA registration data, in vitro.002, study report 2010 (echa.europe.eu) quoted by (ECHA 2016)</td>
</tr>
<tr>
<td>Gene mutation HPRT</td>
<td>Human lymphocytes, hprt locus</td>
<td>0.6–2.4 mM (27–106 µg/mL) (24 hours);
0.2–0.6 mM (8.8–27 µg/mL) (48 hours)</td>
<td>Positive at 1.2 mM and above (after 24 hours); at 0.2 mM and above (after 48 hours)
Dose related increase in number of mutants
relative survivors: 0.6% at 3.6 mM (158 µg/mL) (24 hours)</td>
<td>(He and Lambert 1990)</td>
</tr>
</table>

Gene mutation HPRT	Human fibroblasts	1–12 mM (44–528 µg/mL)	Positive at 5 mM (220 µg/mL) and above survival at 5 mM (220 µg/mL) = 50% cells treated with 8 and 10 mM showed delayed recovery of the growth rate	(Grafstrom et al. 1994)
Gene mutation TK+/-	L5178Y mouse lymphoma cells	4–8 mM (176–352 µg/mL)	Positive at 4 mM (176 µg/ mL) and above Growth reduces with increasing exposure toxic at highest concentration, colony size not specified	(Wangenheim. 1988)
Gene mutation	Human TK cells ; mutants determined at the hprt and tk locus	0.001, 0.005, 0.01, 0.05, 0.25, 0.5, 1.0, 2 and 4 mM for 24 hours	Negative : hprt locus Positive : TK locus (dose-dependent increase, starting at 0.05 mM) 	(Budinsky et al. 2013 quoted by (ECHA 2016)

Annex 3: Reliability assessment of in vivo toxicity studies

Reliability assessment of in vivo toxicity studies			
Study under evaluation			
Authors:			
	Dorman D.C., Struve M.F., Wong B.A., Gross E.A., Parkinson C., Willson G.A., Tan Y-M., Campball J.L., Teeguarden J.G., Clewel III H.J., Andersen M.E.		
Title:			
	Derivation of an inhalation reference concentration based upon olfactive neuronal loss in male rats following sub chronic acetaldehyde inhalation		
Testing facility, year, sponsor, study no. or bibliographic reference:			
	Inhalation Toxicology. 2008, 20:245-256. The majority of the authors come from Hamner Institute for Health Sciences. The study was funded by American Forest and Paper Association.		
Explanations are available for most criteria and show up, when the cursor is moved over the criteria field. Please read carefully! Red criteria: the maximum score is needed for these criteria to achieve reliability category 1 or 2 (see worksheet Explanations): Please evaluate with special care!			
Criteria			**Evaluator's explanations, comments on criteria, etc.**
No.	**Criteria Group I: Test substance identification**	**Score**	
1	Was the test substance identified?	1	acetaldehyde
2	Is the purity of the substance given?	0	
3	Is information on the source/origin of the substance given?	1	
4	Is all information on the nature and/or physic-chemical properties of the test item given, which you deem indispensable for judging the data (see explanation for examples)?	1	
		3	

	Criteria Group II: Test organism characterization		
5	Is the species given?	1	F-344 rat
6	Is the sex of the test organism given?	1	male
7	Is information given on the strain of test animals plus, if considered necessary to judge the study, other specifications (see explanation for examples)?	1	
8	Is age or body weight of the test organisms at the start of the study given?	1	
9	For repeated dose toxicity studies only (give point for other study types): Is information given on the housing or feeding conditions?	1	
		5	
	Criteria Group III: Study design description		
10	Is the administration route given?	1	
11	Are doses administered or concentrations in application media given?	1	
12	Are frequency and duration of exposure as well as time-points of observations explained?	1	
13	Were negative (where required) and positive controls (where required) included (give point also, when absent but not required, see explanations for study types and their respective requirements on controls)?	1	No control but not necessary in this type of study
14	Is the number of animals (in case of experimental human studies: number of test persons) per group given?	1	
15	Are sufficient details of the administration scheme given to judge the study (see explanation for examples)?	1	
16	For inhalation studies and repeated dose toxicity studies only (give point for other study types): Were achieved concentrations analytically verified or was stability of the test substance otherwise ensured or made plausible?	1	
		7	
	Criteria Group IV: Study results documentation		
17	Are the study endpoint(s) and their method(s) of determination clearly described?	1	
18	Is the description of the study results for all endpoints investigated transparent and complete?	1	

19	Are the statistical methods applied for data analysis given and applied in a transparent manner (give also point, if not necessary/applicable, see explanations)?	1	
		3	
	Criteria Group V: Plausibility of study design and results		
20	Is the study design chosen appropriate for obtaining the substance-specific data aimed at (see explanations for details)?	1	
21	Are the quantitative study results reliable (see explanations for arguments)?	1	
		2	
		21	
	A Numerical result leads to initial Category:	**1**	
	B Checking red scores leads to revised Category:	**1**	
	C Evaluator's proposal: Category:		
	D Justification in case evaluator deviates from B:		
Optional documentation of observations with importance to relevance (not part of the reliability assessment)			
	During the course of the quality assessment observations may be made which are important for discussing the **relevance** of the data for specific purposes. The optional possibility is provided here to document these observations for future use.		
	What is the purpose of this quality evaluation (data documentation for use under REACH, classification activity under GHS, ECVAM validation activities, other)?		
	other OEL elaboration		
	Study conducted according to recent OECD or EU guidelines (or other, e.g. national guidelines)? If yes, which ones? Study conducted under GLP conditions?		

	NO		
	(If not a guideline study): Does a guideline exist for the study endpoint(s) under investigation?		
	no		
	Are you aware of relevant deviations from the guideline(s) in the study evaluated? If yes, which one?		
	Did you make observations with importance to the regulatory use of the data (example 1: evaluator may hint that a whole body inhalation study was performed with a substance, for which profound percutaneous absorption is expected or known, leading to substantial percutaneous uptake in addition to inhalation uptake; example 2: an Ames reversion assay was performed with strains able to identify frame-shift mutations only or without external metabolic activation; example 3: evaluator is in possession of positive evidence that the results obtained with the in vitro study under evaluation, in conjunction with known toxic kinetic data, are useful to assess the nephrotoxicity of the substance in humans)?		
	Would you like to make other/general comments on the usability of the data?		

Annexe 4: Technical support: detailed presentation of workplace air acetaldehyde monitoring methods classified in categories 1B and 2.

Annex 4.1: Method A - Sampling on silica gel cartridge/tube or glass fibre filter coated with 2,4 DNPH, acetonitrile elution followed by HPLC analysis and UV detection

Table 31 : Method A: Descriptive parameters

Method A - Sampling on silica gel cartridge/tube or glass fibre filter coated with 2,4 DNPH, acetonitrile elution followed by HPLC analysis and UV detection							
		NF ISO 16000-3	NIOSH 2018	INRS M-66 NF X 43-264	DFG method 2	HSE MDHS 102	DFG method 1
Gas/vapour - Aerosol - Mix		Gas					
Sampling	Active/passive sampling	Active					
	Sampler	Silica gel cartridge coated with 2.4 DNPH, 1 or 2 mg for commercial cartridges(Waters Sep PAk, Supelco LpDNPH)	Silica gel cartridge coated with 2.4 DNPH, 1 or 2 mg for commercial cartridges (Waters Sep PAk, Supelco LpDNPH)	Silica gel cartridge (500 mg) coated with 2.4 DNPH 1 %	Waters Sep-Pak cartridge, 350 mg silica gel coated with 2,4 DNPH 0,29 %	2,4 DNPH-coated glass filbre filter in a suitable personal inhalable dust sampler or 2,4 DNPH-coated silica gel cartridge	2,4 DNPH-coated 37 mm glass filbre filter, 1 to 3 associated in a suitable personal inhalable dust sampler
	Sampling rate ($L.min^{-1}$)	0.5 to 1.2	0.1 to 1.5	0.2 to 1	0.1	0.1 to 1	0.333
	Air volume (L)	Sample size less than 75% of permissible DNPH load	1 to 15	3 to 480 L	6	1.5 to 480	20
	Sampling time	5 min to 24h	10 to 150min	15 to 480min	1h	15 to 480min	1h
Measurement	Sample preparation	Elution 5 mL carbonyl free acetonitrile, in a vial or by percolation	Elution 10 mL carbonyl free acetonitrile	Elution 5mL acetonitrile	Elution 5 mL acetonitrile	Acetonitrile : Filter 2 mL Commercial cartridge volume specified in manufacturer's instructions	Elution 10 mL acetonitrile
	Analysis technique	Liquid chromatograph, UV detector					

Method A - Sampling on silica gel cartridge/tube or glass fibre filter coated with 2,4 DNPH, acetonitrile elution followed by HPLC analysis and UV detection							
		NF ISO 16000-3	NIOSH 2018	INRS M-66 NF X 43-264	DFG method 2	HSE MDHS 102	DFG method 1
	Analytical parameters	**Column:** C18 silica column **Mobile phase :**:acetonitrile /water, isocratic or gradient (NF ISO 16000-3, INRS M-66, NIOSH 2018, DFG method 2) ; methanol/water/acetonitrile (DFG Méthode 1), acetonitrile 55%/water+acetonitrile with NaH_2PO_4 0, 01M. (MDHS 102) **Flow rate :** 1.0 mL.min^{-1} (NF ISO 16000-3, MDHS 102), 1.3 mL.min^{-1} (NIOSH 2018), 0.8 mL.min^{-1} (DFG method 1), 0.4 mL.min^{-1} (DFG method 2) **Injection volume :** 25 µL (NF ISO 16000-3), 20 µL (NIOSH 2018), 10 µL (DFG method 2), **Column temperature** : 40°C (MTA/MA-062/A08); room T°C (NF ISO 16000-3, DFG method 2 and method 1); 50°C (MDHS 102) **Detector :** UV- Λ= 360 – 365 nm.					

Table 32 : Method A: Validation data

	NF ISO 16000-3	NIOSH 2018	INRS M-66 NF X 43-264	DFG method 2	HSE MDHS 102	DFG method 1
Validated range ($mg.m^{-3}$)	0.001 à 1 $mg.m^{-3}$ depending on duration and volume, not to exceed 75% of the DNPH load Working range : 0.008 à 3.48 $mg.m^{-3}$ for 1mg DNPH @ 48 L air sampling	Working range are approximately 0.05 à 15 $mg.m^{-3}$ (V= 15 L)	NR	NR	Applicable to both 15 min to 8 h sampling in airborne concentrations in the range 0,01–10 $mg.m^{-3}$. Recommended : For pumped samples on filters with expected exposures up to 2 $mg.m^{-3}$: max sampling time 8 h;sampling rate : 0.1 to 1.0 $L.min^{-1}$; sampled volume : 1.5 to 480 L.	Analytical recovery 50 to 500 µg, 2.5 to 25 $mg.m^{-3}$ and quantification limit 0.167 $mg.m^{-3}$ @ 20 L (10/3 DL, DL = 0.05 $mg.m^{-3}$ @ 20 L)
Elution coefficient / Elution efficiency / Analytical recovery	NR	Fortification of Supelco samplers with free acetaldehyde in solution by spiking : 1.5 ; 3 ; 6 ; 12 and 20 µg, equal to 0.1 to 1.33 $mg.m^{-3}$ @ 15 L, average recovery at the level : 99 to 108 %, pooled RSD 3.1 %	NR	NR	"Sampling and analytical recovery of the aldehydes from the samplers is typically in excess of 99 %."	between 88.8 and 93.3% for 2.5 < [C] < 25$mg.m^{-3}$ @ 20 L
Experimental validation data for diffusive sampling rate	Not applicable					
Diffusive sampling rate stability data	Not applicable					
Backdiffusion	Not applicable					

	NF ISO 16000-3	NIOSH 2018	INRS M-66 NF X 43-264	DFG method 2	HSE MDHS 102	DFG method 1
Breakthrough and capacity data	1 mg DNPH, 75% of the mass consumed -> 0.167 µg of acetaldehyde	For 1 mg DNPH, at room temperature, µg/sample : in dry air 10 % HR 178, in Lab air 173, in humid air 85 % HR 158.	NR	NR	NR	NR
Detector response linearity	NR	linear response between 0.68 and at least 150 µg/sample, 0,045 to over 10 $mg.m^{-3}$ (15 L and 20 µL inject)	NR	Supposed to be linear, without precision	NR	Checked between 0.1 and 2.5 $µg.mL^{-1}$
Storage studies	Reduce non-refrigeration period to a minimum, less than 2 days. If refrigerated, less than 30 days	Storage of sample cartridges and solutions after desorption at 5°C in the dark. Storage efficiency 102% for cartridges after 30 days (n=6). Solutions remain stable beyond 36 days ([acetaldehyde] = 1.25 $µg.mL^{-1}$).	8 days refrigerated INRS M-66	NR	NR	NR Before sampling, coated filters stable for 15 days
Environmental conditions	Sampling at temperatures above 10°C	slight drop in humid air capacity, less than 10%.	NR.	NR	NR	NR
Selectivity / Interference	Identified interferents: ozone carbonyl compounds	Identified interferents: Ozone, ketones and other aldehydes can react with DNPH. Ozone may degrade the DNPH derivative of acetaldehyde.	NR	Carbonyl compounds. For concentrations < 2 $mg.m^{-3}$ no interference	Identified interferents: ozone, carbonyl compounds, NO_2	Interferent: carbonyl compounds, furfuryl alcohol No interference up to a concentration of 500 mg per 10 mL acetonitrile: hydrogen peroxide, ammonia, toluene, dichloroethane, ethyl acetate, 2,2,4-

		NF ISO 16000-3	NIOSH 2018	INRS M-66 NF X 43-264	DFG method 2	HSE MDHS 102	DFG method 1
					from NO_2 (formaldehyde)		methylpentane, methanol, ethanol, isopropanol, chloroform, MIBK, nitric oxide and ozone
Speciation		Adjustment of chromatographic conditions enables separation of the DNPH derivative of acetaldehyde.					
8h-OEL	**Expanded uncertainty**	Appendix A: Intercomparison circuit, 14 cities 16 laboratories, n=346, absolute difference between sample sets 14.5% for acetaldehyde. Cartridges spiked at 0.5 5 and 10 µg, average uncertainty in one laboratory 13.8%, in a second, 30 cartridges 5.1%.	For 5 batches of 6 tubes spiked with acetaldehyde in solution at various concentration levels between 1.5 and 20 µg per sample, 0.1 to 1.33 $mg.m^{-3}$ for 15 L sampled, mean recovery at each level varies from 99 to 108% and relative standard deviation is homogeneous by Bartlett's test at 3.1%.	NR	NR	NR	Reproducibility 4.3 % at [C] = 2.5$mg.m^{-3}$ @ 20L with an average Cv of 9.6
	Detection limit	NR	0.2 µg on Supelco™ LpDNPH cartridge, 1 mg DNPH, 13.3 $µg.m^{-3}$ @15 L, elution in 10 mL acetonitrile.	NR	0.024 µg/sample, -> 0.004 $mg.m^{-3}$ @ 6 L	NR	0.05 $mg.m^{-3}$ @ 20 L 1 µg/sampler
	Quantification limit	NR	0.68 µg on a Supelco™ LpDNPH cartridge, 1 mg DNPH, 45.3 $µg.m^{-3}$ @ 15 L, elution 10 mL, 0.34 µg for 5 mL	NR	NR	NR	NR
15min-STEL	**Expanded uncertainty**	NR	For 5 batches of 6 tubes spiked with acetaldehyde in solution at various concentration levels between 1.5 and 20 µg per sample, 0.1 to 1.33 $mg.m^{-3}$ for 15 L sampled, mean recovery at	NR	NR	NR	NR

		NF ISO 16000-3	NIOSH 2018	INRS M-66 NF X 43-264	DFG method 2	HSE MDHS 102	DFG method 1
			each level varies from 99 to 108% and relative standard deviation is homogeneous by Bartlett's test at 3.1%.				
	Detection limit	NR	0.2 µg on Supelco™ LpDNPH cartridge, 1 mg DNPH, 13.3 µg.m^{-3} @15 L, elution in 10 mL acetonitrile.	NR	NR	0.5 to 3 µg.m^{-3}	NR
	Quantification limit	NR	0.68 µg on a Supelco™ LpDNPH cartridge, 1 mg DNPH, 45.3 µg.m^{-3} @ 15 L, elution 10 mL, 0.34 µg for 5 mL	NR	NR	NR	NR
Additional information		-					

NR : Not reported

Annex 4.2: Method B - Sampling on sorbent tube containing XAD-2 resin coated with 2-(hydroxymethyl)piperidine, toluene elution followed by GC analysis and NPD or FID detector

Table 33 : Method B: Descriptive parameters

Méthode B – Sampling on sorbent tube containing XAD-2 resin coated with 2-(hydroxymethyl)piperidine, toluene elution followed by GC analysis and NPD or FID detector			
		OSHA 68	**NIOSH 2538**
Gas/vapour - Aerosol - Mix		Gas	
Sampling	**Active/passive sampling**	Active	
	Sampler	Solid sorbent tube, 2-HMP coated on XAD-2 resin, 2 beds 450 mg/225 mg	
	Sampling rate ($L.min^{-1}$)	0.05	0.01 to 0.05
	Air volume (L)	3	1 to12
	Sampling time	60 min	10 to 240 min
Measurement	**Sample preparation**	Elution with 5 mL toluene, mix using a vortex several times	Elution with 5 mL toluene, ultrasonic bath 60 min
	Analysis technique	GC with NPD detector	GC with FID detector
	Analytical parameters	GC column : Glass column chromosorb W AW 80/100 with 10% de UCON 50-HB 5100 Eluent gas ::helium He flow rate : 30 $mL.min^{-1}$ Column T°C : 100 to 140°C at 4°$C.min^{-1}$ upon injection, 140 to 180°C at 20°$C.min^{-1}$, hold at 180°C, 25 min. Injection size : 0,9 µL Injection T°C : 180°C Detector T°C : 275°C NPD conditions : H_2 3 $mL.min^{-1}$, air 50 mL.min	GC column : wide-bore, fused-silica capillary, 15 m x 0,32 mm, 1 µm DB 1301 film DB-Wax or equivalent Eluent gas ::helium He flow rate : 1 $mL.min^{-1}$, makeup 29 $mL.min^{-1}$ Column T°C : 70°C 1 min, 6°$C.min^{-1}$ to 110°C, hold 2 min, 30°$C.min^{-1}$ to 260°C, hold 1 min Injection size : 1 µL Injection T°C : 250°C Detector T°C : 300°C

Table 34 : Method B: Validation data

Méthode B – Sampling on sorbent tube containing XAD-2 resin coated with 2-(hydroxymethyl)piperidine, toluene elution followed by GC analysis and NPD or FID detector		
	OSHA 68	**NIOSH 2538**
Validated range ($mg.m^{-3}$)	108 to 840 $mg.m^{-3}$ @ 3 L	Working range : 1.3 à 730 $mg.m^{-3}$ @ 3 L
Elution coefficient / Elution efficiency / Analytical recovery	102 to 106 % in the validated range	NR
Experimental validation data for diffusive sampling rate	Not applicable	
Diffusive sampling rate stability data	Not applicable	
Backdiffusion	Not applicable	
Breakthrough and capacity data	Samples were collected in controlled test atmosphere containing acetaldehyde 840 $mg.m^{-3}$ in air for increasing time. At 78% HR and 28°C, 5% breakthrough occur after 304 min, 15.2 L air sampled and 12.8 mg of acetaldehyde had been collected. In a controlled test atmosphere containing acetaldehyde 823 $mg.m^{-3}$, at 19% HR and 27°C, 14% breakthrough occur after 60 min, 3 L air sampled.	NR.
Detector response linearity	Checked on validated range, 542.5 to 2170 µg/sample, 0.9 µL inj.	NR
Storage studies	Samples stored at 23 °C were collected from a controlled test atmosphere containing 416 $mg.m^{-3}$ of acetaldehyde at 80% HR and 28°C, recovery more than 95% after 16 days. Same test 380 $mg.m^{-1}$ acetaldehyde, 81 % HR and 27°C but refrigerated storage at -20°C, recovery more than 98% at 16 days.	Recovery (26.8 and 107 µg per sample) was 100% after 21 days of refrigerated storage (8.9 to 35.7 $mg.m^{-3}$ @ 3 L).
Environmental conditions	Influence of dry air (see breakthrough study)	NR
Selectivity / Interference	Any collected substance that is capable of reacting with 2-HMP is a potential interference, mineral acids may neutralize 2-HMP and carbonyl compounds, such as acetone, may be capable of reacting with 2-HMP.	Non identified
Speciation	Adjustment of chromatographic conditions enables separation of the DNPH derivative of acetaldehyde.	

Méthode B – Sampling on sorbent tube containing XAD-2 resin coated with 2-(hydroxymethyl)piperidine, toluene elution followed by GC analysis and NPD or FID detector			
		OSHA 68	**NIOSH 2538**
8h-OEL	**Expanded uncertainty**	"Overall precision at 95 % confidence level for the 23 day ambient temperature storage test is ± 11.9%, this includes an additional ± 5% for sampling error. The overall procedure must provide results at 360 $mg.m^{-3}$ that are ± 25% or better at the 95% confidence level."	NR
	Detection limit	NPD detection : 595 pg, 0.9 µL injected, elution 5 mL,	FID detection : 2 µg, 1 µL injected, elution 5 mL,
	Quantification limit	3.14 µg per sample, 1.05 $mg.m^{-3}$ @ 3 L	4 µg per sample, 1.33 $mg.m^{-3}$ @ 3 L
15min-STEL	**Expanded uncertainty**	N.R.	Not determined
	Detection limit	NPD detection : 595 pg, 0.9 µL injected, elution 5 mL,	FID detection : 2 µg, 1 µL injected, elution 5 mL,
	Quantification limit	3.14 µg per sample, 4.53 $mg.m^{-3}$ @ 0.75 L (0.05 mL.min-1 x 15 min)	4 µg per sample, 5.33 $mg.m^{-3}$ @ 0.75 L (0.05 mL.min-1 x 15 min)
Additional information		-	Refers to OSHA 68 protocol for validation data..

NR: Not reported

Annexe 5: Minority opinions

One expert from the HRV Committee abstained on the collective expert appraisal report during the validation.

Marc BARIL : “As I was not a member of the HRV Committee when part A “health effects” was validated, I do not wish to make a statement”.

AGENCE NATIONALE DE SÉCURITÉ SANITAIRE
de l'alimentation, de l'environnement et du travail

14 rue Pierre et Marie Curie 94701 Maisons-Alfort Cedex
www.anses.fr

ISBN 979-10-286-0560-5 - Dépôt légal : Septembre 2025