anses

Valeurs limites d'exposition en milieu professionnel

Diisocyanate de toluène (TDI) Évaluation des effets sur la santé et des méthodes de mesure

Rapport d'expertise collective

Décembre 2020

Connaître, évaluer, protéger

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 Toluene diisocyanate mixed isomers (TDI)

CAS N° 26471-62-5

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 le diisocyanate de toluène (TDI)

CAS N° 26471-62-5

Mission permanente VLEP / OEL Permanent Mission
Saisine n° 2012-SA-0080 / Request n°2012-SA-0080

RAPPORT d'expertise collective
Collective expert appraisal REPORT

Comité d'experts spécialisé « Valeurs sanitaires de référence »
Expert Committee on « health reference values »

Groupe de travail « métrologie » / Working group on « metrology »

December 2020 / Décembre 2020

AGENCE NATIONALE DE SÉCURITÉ SANITAIRE de l'alimentation, de l'environnement et du travail
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Maisons-Alfort, le 28 Novembre 2025

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

Considérant :

- la VLEP européenne établie dans la directive (UE) 2024/869, suite à l'avis de l'Agence européenne des produits chimiques (ECHA)
- la prise en compte du présent rapport du CES Valeurs sanitaires de référence, validé en décembre 2020, dans l'avis du comité d'évaluation des risques de l'ECHA ayant conduit à la VLEP indiquée dans la directive préalablement citée,
- l'avis de l'Anses relatif à l'évaluation des méthodes de mesure pour la famille des diisocyanates (saisine n°2025-MPEX-0105)
- l'analyse scientifique complémentaire nécessaire pour l'actualisation des connaissances qu'il faudrait mener pour endosser en 2025 ce rapport par un avis de l'Anses,
- les ressources limitées pour mener l'ensemble des actions de son programme de travail,

l'Anses a retenu de ne pas finaliser le présent rapport du CES VSR de décembre 2020 par la rédaction d'un avis.

Pour autant, en cohérence avec l'avis n°2025-1 de son Comité de déontologie et de prévention des conflits d'intérêts de l'Anses, elle procède à la diffusion des travaux menés.

Citation suggérée

Anses. (2020). Collective expert appraisal report on recommending occupational exposure limits for chemical agents. Assessment of health effects and methods for the measurement of exposure levels in workplace atmospheres for toluene diisocyanate CAS N° 26471-62-5. (request 2012-SA-0080). Maisons-Alfort : Anses, 161 p.

Anses. (2020). Rapport d'expertise collective. 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 le diisocyanate de toluène (TDI). Saisine 2012-SA-0080. Maisons-Alfort : Anses, 161 p.

Mots clés

VLEP, valeurs limites, niveaux d'exposition, milieu professionnel, agents chimiques, effets sur la santé, métrologie, méthodes de mesure, lieux de travail, valeur référence, Diisocyanate de Toluène, TDI, 2,4-TDI, 2,6-TDI

Key words

OEL, limit values, exposure levels, occupational, chemical agents, health effects, metrology, measurement methods, workplaces, reference value, Toluene Diisocyanate, TDI, 2,4-TDI, 2,6-TDI.

Presentation of participants

PREAMBLE: EXPERT MEMBERS OF EXPERT COMMITTEES, WORKING GROUPS OR REVIEWERS ARE ALL APPOINTED IN A PERSONAL CAPACITY, INTUITU PERSONAE, AND DO NOT REPRESENT THEIR AFFILIATED ORGANIZATION.

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Expertise collective : synthèse de l'argumentaire et conclusions

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

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

le diisocyanate de toluène (TDI)

CAS n°26471-62-5

Ce document synthétise les travaux des comités d'experts spécialisés « valeurs sanitaires de référence » (CES VSR), « Caractérisation des dangers des substances et valeurs toxicologiques de référence » (CES « Substances ») et « Expertise en vue de la fixation de valeurs limites à des agents chimiques en milieu professionnel » (CES VLEP) et du groupe de travail « métrologie ».

Présentation de la question posée

L'Anses a été saisie le 3 février 2012 par la direction générale du travail (DGT) afin de mener les travaux d'expertise nécessaires à la fixation de valeurs limites d'exposition professionnelle pour le diisocyanate de toluène (TDI).

La France dispose, à travers une circulaire de 1986[1], d'une valeur moyenne d'exposition sur 8 heures (VME) pour le TDI de 0,08 $mg.m^{-3}$ (soit 0,01 ppm) et d'une valeur limite court terme sur 5 minutes (VLE) de 0,16 $mg.m^{-3}$ (soit 0,02 ppm).

La DGT a demandé à l'Anses de réévaluer cette valeur et de proposer le cas échéant, de nouvelles valeurs d'exposition en milieu professionnel fondées sur des considérations sanitaires pour le TDI.

Contexte scientifique

Le dispositif français d'établissement des VLEP comporte trois phases clairement distinctes :

- une phase d'expertise scientifique indépendante (seule phase confiée à l'agence) ;
- une phase d'établissement d'un projet réglementaire de valeur limite contraignante ou indicative par le ministère chargé du travail ;
- une phase de concertation sociale lors de la présentation du projet réglementaire au sein du Conseil d'Orientation sur les Conditions de Travail (COCT). L'objectif de cette phase étant de discuter de l'effectivité des valeurs limites et de déterminer d'éventuels délais d'application, en fonction de considérations de faisabilité technico-économique.

La phase d'expertise scientifique nécessaire à la fixation des valeurs limites d'exposition professionnelle (VLEP) est confiée à l'Anses.

[1] Circulaire du 12 mai 1986 (non parue au Journal Officiel)

Les VLEP telles que recommandées par le CES sont des niveaux de concentration en polluants 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 négligeable. Même si des modifications physiologiques réversibles sont parfois tolérées, aucune 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 niveaux de concentrations sont déterminés par les experts du CES à partir des informations disponibles dans des études épidémiologiques, cliniques, de toxicologie animale, etc. L'identification de ces concentrations sécuritaires pour la santé humaine nécessite généralement d'appliquer des facteurs d'ajustement aux valeurs identifiées directement par les études. Ces facteurs permettent de prendre en compte un certain nombre d'éléments d'incertitude inhérents à la démarche d'extrapolation conduite dans le cadre d'une évaluation des effets sanitaires des substances chimiques sur l'Homme.

Trois types de valeurs sont recommandées par le CES :

- valeur limite d'exposition 8 heures : il s'agit de la limite de la moyenne pondérée en fonction du temps de la concentration atmosphérique d'un agent chimique dans la zone de respiration d'un travailleur au cours d'un poste de 8 heures. Dans l'état actuel des connaissances scientifiques (en toxicologie, médecine, épidémiologie, etc.), 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 ;
- valeur limite d'exposition à court terme (VLCT) : il s'agit de la limite de la moyenne pondérée en fonction du temps de la concentration atmosphérique d'un agent chimique dans la zone de respiration d'un travailleurs sur une période de référence de 15 minutes pendant le pic d'exposition quelle que soit sa durée. Elle vise à protéger les travailleurs des effets néfastes sur la santé (effets toxiques immédiats ou à court terme, tels que des phénomènes d'irritation), dus à des pics d'exposition ;
- valeur plafond : il s'agit de la 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. Cette valeur est appliquée aux substances reconnues comme irritant fort ou corrosif ou pouvant causer un effet grave potentiellement irréversible, à très court terme.

Ces trois types de valeurs sont exprimés :

- soit en $mg.m^{-3}$, c'est-à-dire en milligrammes d'agent chimique par mètre cube d'air et en ppm (parties par million), c'est-à-dire en centimètres cube d'agent chimique par mètre cube d'air, pour les gaz et les vapeurs ;
- soit en $mg.m^{-3}$ uniquement, pour les aérosols liquides et solides ;
- soit en $f.cm^{-3}$, c'est-à-dire en fibres par cm^3 pour les matériaux fibreux.

La valeur de la VLEP-8h peut être dépassée sur de courtes périodes pendant la journée de travail à condition toutefois :

- que la moyenne pondérée des valeurs sur l'ensemble de la journée de travail ne soit pas dépassée ;
- de ne pas dépasser la valeur de la VLCT si elle existe.

En plus des VLEP, le CES évalue la nécessité d'attribuer ou non une mention « peau », lorsqu'une pénétration cutanée significative a été identifiée (Anses 2017). Cette mention

indique la nécessité de prendre en compte la voie d'exposition cutanée dans l'évaluation de l'exposition et, le cas échéant, de mettre en œuvre des mesures de prévention appropriées (telles que le port de gants de protection). En effet, la pénétration cutanée des substances n'est pas prise en compte pour la détermination des niveaux de valeurs limites atmosphériques et peut donc potentiellement entraîner des effets sanitaires indépendamment du respect de ces dernières.

Le CES évalue également la nécessité d'attribuer ou non une mention « bruit » signalant un risque d'atteinte auditive en cas de co-exposition au bruit et à la substance en dessous des limites d'exposition recommandées afin que les préventeurs mettent en place des mesures appropriées (collectives, individuelles et médicales) (Anses 2017).

Le CES évalue également 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 ont été évaluées notamment sur leur conformité aux exigences de performance de la NF EN 482 et de leur niveau de validation.

Organisation de l'expertise

L'Anses a confié aux comités d'experts spécialisés (CES) « caractérisation des substances chimiques » (CES « Substances »), « Expertise en vue de la fixation de valeurs limites à des agents chimiques en milieu professionnel » (CES VLEP) puis « Valeurs Sanitaires de référence » (CES VSR), l'instruction de cette saisine. Le groupe de travail « Métrologie » a été mandaté pour l'évaluation des méthodes de mesures atmosphériques dans l'air des lieux du travail.

Plusieurs agents de l'Anses ont contribué à ces travaux et se sont chargés de la coordination scientifique des différents groupes d'experts.

Les travaux d'expertise ont été soumis régulièrement aux CES tant sur les aspects méthodologiques que scientifiques. Le rapport produit tient compte des observations et éléments complémentaires transmis par les membres du CES.

Durant l'expertise, des échanges ont eu lieu avec les membres du comité d'experts du DECOS (Dutch Expert Committee on Occupational Safety) du Conseil de santé des Pays-Bas (*Health Council of the Netherlands*) dans le cadre d'une collaboration scientifique sur les isocyanates. Les approches des deux comités (CES de l'Anses et DECOS) n'ayant pu converger, les travaux n'ont pas permis d'aboutir aux mêmes conclusions.

Ces travaux d'expertise sont ainsi issus d'un collectif d'experts aux compétences complémentaires. Ils ont été réalisés dans le respect de la norme NF X 50-110 « qualité en expertise ».

Prévention des risques de conflits d'intérêts

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

Pour l'évaluation des effets sur la santé :

Un profil toxicologique a été élaboré par le CES « caractérisation des dangers des substances » ou CES « Substances » (mandat 2014-2017) et soumis au CES « Expertise en vue de la fixation de valeurs limites à des agents chimiques en milieu professionnel » ou CES « VLEP » pour la dérivation des valeurs en milieu professionnel (mandat 2014-2017).

Le rapport de synthèse est issu d'éléments bibliographiques prenant en compte la littérature scientifique parue sur cette substance jusqu'en 2018. La recherche bibliographique a été effectuée à partir des articles recensés dans les bases de données Medline, Toxline et HSBD et ToxNet.

Pour l'évaluation des méthodes de mesure des niveaux d'exposition sur le lieu de travail :

Un rapport de synthèse a été élaboré par le GT « métrologie » et soumis au CES VSR (mandat 2017-2020) qui l'a commenté.

Le rapport de synthèse présente les différents protocoles de mesure du TDI dans l'air des lieux de travail recensés et regroupés en fonction des méthodes mises en œuvre. Ces dernières ont ensuite été évaluées et classées au regard des exigences de performances indiquées notamment dans la norme NF EN 482 : « 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 méthodologie (Anses 2017).

La liste des principales sources consultées est précisée dans le rapport méthodologie (Anses 2017).

Le classement de ces méthodes est réalisé selon la manière suivante :

- catégorie 1A : la méthode est reconnue et validée (l'ensemble des critères de performance de la norme NF-EN 482 sont satisfaits) ;
- catégorie 1B : la méthode est partiellement validée (les critères essentiels de performance de la norme NF EN 482 sont satisfaits) ;
- catégorie 2 : la méthode est indicative (des critères essentiels de validation ne sont pas suffisamment explicités) ;
- catégorie 3 : la méthode n'est pas recommandée (des critères essentiels de validation sont absents ou inappropriés).

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.

Il est à noter que seule l'évaluation des méthodes de mesure au regard de la VLCT-15min avait été réalisée pour la phase de consultation publique. L'évaluation au regard de la VLEP-8h a ainsi été ajoutée après la phase de consultation publique**.**

La méthodologie d'évaluation des méthodes de mesure a été mise à jour au cours de cette expertise impliquant notamment une classification différenciée de la méthode d'échantillonnage et de la méthode d'analyse dans le cas de substances devant être prélevées à la fois sous forme vapeur et sous forme particulaire (Anses 2020).

L'évaluation des méthodes de mesure du TDI ne prend pas en compte cette mise à jour. Cependant, la non actualisation n'a pas d'impact sur le classement global des méthodes de

mesure en raison d'une surestimation de la fraction inhalable par les dispositifs de prélèvement.

Le rapport ainsi que la synthèse et les conclusions de l'expertise collective ont été adoptées par le CES « Valeurs sanitaires de référence » le 21/03/2019.

Ce rapport et les conclusions ont fait l'objet d'une consultation publique du 18/08/2019 au 18/10/2019. Les personnes ou organismes ayant contribué à la consultation publique sont listés en annexe 8. Les commentaires reçus ont été examinés et discutés par le CES VSR qui a adopté la version post-consultation le 25/06/2020.

Les compléments concernant l'évaluation des méthodes de mesure au regard de la VLEP-8h ont été examinés et discutés par le CES VSR qui a adopté cette version finalisée du rapport et des conclusions le 10 décembre 2020.

Résultat de l'expertise collective concernant les effets sur la santé

Information générale

Le TDI est commercialisé sous la forme d'un liquide composé d'un mélange d'isomères, le 2,4-TDI et le 2,6-TDI généralement présents dans les proportions suivantes : 80/20 (2,4-TDI/2,6-TDI). Bien que la quantité de 2,4-TDI soit supérieure à celle du 2,6-TDI dans les mélanges commerciaux, des études en milieu professionnel ou sur volontaires montrent que les niveaux atmosphériques de 2,6-TDI sont plus élevés que ceux du 2,4-TDI (Saunders et Frisch 1962; Skarping et al. 1991; Tinnerberg et al. 1997)

Le TDI est principalement utilisé pour la production de mousse polyuréthane mais aussi dans les mastics, colles, élastomères (ECHA 2016).

En milieu de travail, l'exposition se fait principalement par inhalation sous forme d'aérosols (étant donné le point de fusion élevé et la faible volatilité du TDI à température ambiante).

Revue des recommandations récentes en matière de valeurs limites d'exposition professionnelle

Le SCOEL (Scientific Committee on Occupational Exposure Limits) ne recommande, actuellement, aucune valeur limite en milieu professionnel pour le TDI.

Récemment l'ACGIH[2] a recommandé une valeur 8h-TWA de 1 ppb et 5 ppb pour la valeur 15min-STEL (concernant chacun des isomères et le mélange) (ACGIH 2016).

L'OEHHA[3] a calculé une valeur limite sur 8 heures ou 8-h REL (Reference exposure level) de 0,002 ppb basée sur le déclin du volume expiratoire maximal par seconde (VEMS) (en l'absence d'asthme au TDI) (OEHHA 2016).

En 2018, Daniels et al. (affiliés au NIOSH) ont calculé une OEL de 0,3 ppb (correspondant à un excès de risque d'asthme professionnel de 1 pour 1000) (Daniels et al. 2018).

[2] American Conference of Governmental Industrial Hygienists

[3] Office of Environmental Health Hazard Assessment

En novembre 2018, le DECOS (Dutch Expert Committee on Occupational Safety) du Health Council des Pays-Bas a publié un rapport recommandant une 8h-TWA[4] de 0,1 µg NCO/m^3 pour les di et triisocyanates (ce qui correspond à une valeur de 0,04 ppb pour le TDI), basée sur un excès de risque de déclenchement d'une hyperréactivité bronchique (HRB) de 1% chez les travailleurs (Health Council of the Netherlands 2018). Les études retenues sont celles de Pronk et al. 2007 et Collins et al. 2017.

Données de toxicocinétique

Selon les données de la littérature, le TDI n'est pas ou est très peu absorbé sous sa forme inchangée quelle que soit la voie d'exposition. Les trois mécanismes pouvant limiter l'absorption et la distribution du TDI sont : la polymérisation (*in situ*), les propriétés physico-chimiques du TDI qui conduisent à son hydrolyse en toluène diamine (TDA), et des réactions chimiques qui peuvent se dérouler, au niveau atmosphérique mais aussi avec les fluides et tissus humains lors du contact.

Ces mécanismes sont appuyés par différents éléments (DFG 2003, Bonnard et al. 2006) :

- la présence d'une fonction NCO, hautement réactive

- l'absence de TDI sous sa forme inchangée dans le sang, les tissus ou les excrétions aussi bien chez l'Homme que chez l'animal ; seuls ont été identifiés les métabolites du TDI, le TDA et ses dérivés acétylés, ou les adduits du TDI ou TDA (adduits à l'albumine ou l'hémoglobine)

- la présence de TDI sous forme polymère dans l'estomac et de TDA dans les urines chez le rat après ingestion de TDI

- la différence de proportion d'isomères entre le mélange commercial et le mélange retrouvé sur les lieux de travail (diminution de la proportion de 2,4-TDI).

Absorption

Inhalation

Chez le travailleur, le TDI est principalement absorbé par inhalation. Les études menées chez l'Homme (chez des volontaires ou en milieu de travail) ont démontré l'absorption du TDI après inhalation en raison de la présence de métabolites dans les urines. Dans une étude sur volontaires (n=5) exposés à 40 µg/m^3 de TDI pendant 7h30, les auteurs ont rapporté des concentrations plasmatiques de TDI de 2,2 µg/L après 8h et 2,4 µg/L après 24h (Saunders and Frisch, 1962 cité par Prueittet al. 2013, Skarping et al. 1991; Tinnerberg et al. 1997). Chez les travailleurs, 9 sujets ont été exposés dans une usine de production de mousse polyuréthane à base de TDI (80:20 de 2,4-TDI et 2,6-TDI) à des concentrations comprises entre 9,5 et 94 µg/m^3 (la proportion de 2,6-TDI variait de 44 à 87%). Les auteurs ont rapporté des concentrations de TDA urinaire qui variaient de 6,5 à 31,7 µg/g créatinine (avec une relation linéaire avec les concentrations atmosphériques). Selon Maître et al., environ 20% de TDI est métabolisé en TDA dans les urines (Maître et al. 1993).

Chez l'animal, Timchalk et al. ont exposé des rats mâles (F344) à du 2,4-TDI marqué au ^{14}C sous forme vapeur (2 ppm durant 4h). Les résultats ont montré qu'environ 15% de TDI étaient

[4] time-weighted average

excrétés dans les urines mais les auteurs n'ont détecté aucune radioactivité dans l'air exhalé (Timchalk et al. 1994).

Voie cutanée

Une étude de terrain réalisée dans une usine de production de mousse, à base de polyuréthane, a été menée chez l'Homme afin d'évaluer la contribution de l'absorption cutanée vis-à-vis de la charge corporelle (Austin, 2007). Les auteurs ont comparé les résultats de prélèvements urinaires chez deux groupes de sujets travaillant dans un environnement similaire (avec le même niveau d'exposition atmosphérique au TDI), mais avec pour l'un des deux groupes un contact physique avec des mousses (polyuréthane non polymérisé). Sur les 13 sujets exposés par voie cutanée au TDI, 10 ont présenté, en fin de poste, des niveaux de TDA urinaire supérieurs à la limite de détection (contre 2 sur 10 dans le groupe témoin). Les résultats de cette étude suggèrent la possibilité que l'absorption cutanée explique la différence de niveaux de TDA urinaire entre les deux groupes. Cependant les auteurs n'ont pu établir de relation claire entre les concentrations atmosphériques de TDI et les concentrations urinaires de TDA en fin de poste. De plus, selon Maître et al. le ratio d'isomères présent dans l'air (TDI) et dans l'urine (TDA) pourrait être un indicateur pertinent du contact cutané (Maître et al. 1993).

Chez l'animal, Rosenberg et Savolainen ont mesuré la concentration de TDA dans les urines de rats exposés à une solution de 40% de 2,4-TDI (3h par jour et 4 jours consécutifs). Afin de mesurer le TDA (libre et conjugué) dans les urines, les auteurs ont procédé à une hydrolyse acide (6N HCL, 100°C, 45 min). Ils n'ont pas détecté de TDA libre ou conjugué mais la concentration de TDA après hydrolyse était de 1,5 µg/mL. Le TDI réagit rapidement avec la peau chez le rat mais il est faiblement absorbé (Rosenberg et Savolainen 1985).

Voie orale

Aucune donnée n'a été retrouvée chez l'Homme.

Chez le rat, trois études ont montré que le TDI n'était pas bien absorbé (IPCS 1987, Stoltz et al. 1988 and Timchalk et al. 1994). Les études animales ont également montré que plus la concentration de TDI était élevée, moins le TDI était absorbé dans la circulation systémique (en raison de la polymérisation plus importante du TDI). Le taux d'absorption, par ingestion, a été estimé entre 12 et 20% chez le rat.

Distribution

Inhalation

Les seules études disponibles sur la distribution du TDI ont été réalisées sur le rat. Les animaux ont été exposés à du 2,4-TDI marqué au ^{14}C (0,026 ppm ; 0,143 ppm ; 0,821 ppm durant 4h). Les plus hauts niveaux de radioactivité mesurée dans les tissus (à savoir voies aériennes, système digestif, sang) augmentaient avec le niveau d'exposition (Kennedy et al. 1994). La radioactivité dans les tissus n'augmentait pas de façon proportionnelle avec la concentration atmosphérique. De la même façon, la fraction estimée de la dose inhalée chez les rats exposés à 0,6 ppm (1%) était 2 fois plus élevée que chez les rats exposés à 2 ppm (0,5%) (Stoltz, Czarnecki et al. 1987 cité par ECB 2000). Chez des rats exposés à 2 ppm de 2,4-TDI marqué au ^{14}C pendant 4h, les résultats ont montré que 10% de la quantité totale de

radioactivité étaient retrouvés dans la peau et seulement 0,02% dans les graisses (Timchalk et al. 1994).

Voie cutanée

Il y a très peu de données disponibles. Une étude chez le rat montre une absorption cutanée faible et relativement lente avec une quantité significative de la dose appliquée retenue sur le site d'application (ou autour de celui-ci).

Voie orale

Il n'y a pas de données chez l'Homme.

Chez le rat, une étude a montré que, après ingestion de 6 mg/kg pc de TDI marqué au ^{14}C, la concentration sanguine atteignait un maximum d'environ 1% de la dose ingérée au bout d'une heure et de 0,5% au bout de 2h (Stoltz et al. 1988). Timchalk et al. ont montré que la biodisponibilité du TDI augmentait lorsque la dose administrée diminuait. Les auteurs expliquent ces résultats par la polymérisation partielle du TDI dans l'estomac aux fortes doses (Timchalk et al. 1994).

Métabolisation

Il n'y a pas de données chez l'Homme.

Le schéma métabolique suivant (figure 1) est proposé sur les bases de données expérimentales chez l'animal (OEHHA 2016).

Le TDI réagit facilement avec les groupements hydroxyle, sulfhydryle, amines des macromolécules présentes dans les cellules épithéliales des voies aériennes, du sérum, et de la peau (Brown et Burkert 2002, Bello et al. 2004). Dans l'intestin, le TDI est hydrolysé en TDA qui peut être absorbé et métabolisé ou peut former des polymères polyurées (peu absorbé et donc éliminé dans les fèces).

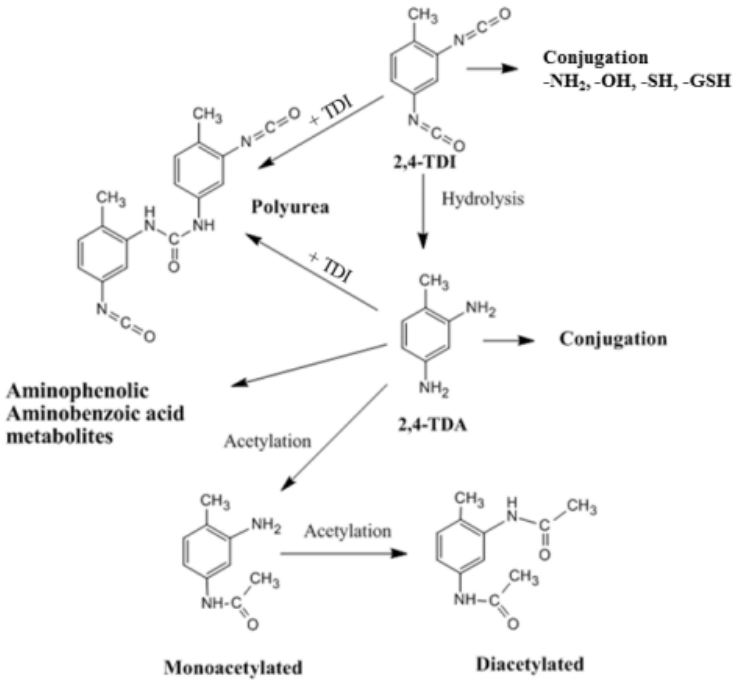

Figure 1 : Schéma métabolique du 2,4- TDI chez le rat (OEHHA 2016)

Inhalation

Une étude chez le rat a montré que seulement 10 % de 2,4-TDA étaient formés après inhalation de 2,4-TDI. La majorité (90%) des métabolites sont retrouvés sous forme de conjugués de TDI/TDA acido-labiles (et 10% sous forme de TDA acétylés) (Timchalk et al. 1994).

Voie cutanée

Dans une étude chez le rat, après l'application d'une solution de 2,4-TDI (40% dans du dibutyl éther) sur la peau durant 3 h/j pendant 4 jours consécutifs, les auteurs n'ont pas détecté de TDA libre ou de TDI libre dans les urines (18h après la fin de l'exposition). Mais après hydrolyse acide, la concentration de TDA urinaire était de 1,5 µg/L (Rosenberg et Savolainen 1985). Ces résultats suggèrent que seule la forme conjuguée est présente dans les urines.

De la même façon, Hoffmann et al. ont montré la présence de métabolites dans les urines (radioactivité mesurée) sans les identifier. Les auteurs n'avaient pas détecté de radioactivité dans les fèces (Hoffmann et al. 2010).

Voie orale

La formation des métabolites du TDI dépend fortement des conditions physiologiques et donc de la voie d'administration. A pH 7, le TDI se lie très facilement aux protéines alors qu'en milieu acide, le TDI sera plus facilement hydrolysé pour former du TDA ou des dérivés polyurées (Doe and Hoffmann 1995).

Excrétion

Inhalation

Chez le rat, l'excrétion du TDI absorbé par inhalation est lente. Quarante-huit heures après une exposition à 2 ppm de 2,4-TDI marqué au ^{14}C, 34% de la radioactivité étaient encore présents dans les tissus et la carcasse des rats exposés (ECB 2000).

Voie cutanée

Il n'y a pas de donnée disponible concernant cette voie d'exposition.

Voie orale

Les études réalisées chez le rat ont montré que le TDI absorbé par voie orale générait principalement du TDI sous forme polyurée qui est ensuite excrété dans les fèces (80% de la dose ingérée mais ce pourcentage dépend de la concentration) et une faible quantité de TDA libre, acétylé ou conjugué qui est excrétée dans les urines.

Données de toxicité

Les données de toxicité du TDI présentées dans ce rapport sont issues de revues ou rapports d'agence (DFG 2003, USEPA 2012, ATSDR 2015, ECHA 2016, OEHHA 2016, Health Council of the Netherlands 2018). Seules les études et les effets les plus pertinents sont décrits.

Lorsque les données humaines sont jugées suffisantes, les études animales ne sont pas décrites.

Les données de toxicité du TDI chez l'Homme sont basées sur des études anciennes (études sur volontaires, études de cas et études épidémiologiques).

Le TDI est le principal agent chimique responsable d'asthme en milieu professionnel (il est la cause de 5-15% des asthmes professionnels) (OEHHA 2016).

Toxicité aiguë

L'inhalation de faibles concentrations d'isocyanates peut entrainer des maux de tête, des nausées, ou vomissements (DFG 2003). Aux fortes concentrations, des expositions sur de courtes durées peuvent causer des symptômes broncho-pulmonaires (toux, impression de suffocation, dyspnée et asthme). L'inhalation de diisocyanates à fortes concentrations cause des lésions immédiates au niveau du tractus respiratoire et des poumons (via l'activation des macrophages et des cellules éosinophiles) (Nakashima et al. 2002).

En 1961, 222 cas d'exposition accidentelle au TDI ont été rapportés dans la littérature américaine, 54 d'entre eux ont été considérés comme sévères (Elkins et al. 1962). Après 1960, les mesures de prévention mises en place ont permis de faire chuter ce nombre. Le lien de causalité entre l'exposition au TDI et les symptômes décrits est difficile à établir en raison des co-expositions professionnelles. L'exposition accidentelle au TDI peut engendrer un syndrome de détresse respiratoire aiguë mais aussi des maladies obstructives chroniques telles que l'asthme (Moller et al. 1986). Aucune relation dose réponse n'a été décrite (DFG, 2003).

Dans une revue sur l'exposition professionnelle au TDI, Nakashima et al. ont fait valoir que la formation d'adduits avec les protéines épithéliales pouvait provoquer des lésions épithéliales, induire des modifications de la perméabilité des voies respiratoires et de la signalisation cellulaire (Nakashima et al. 2002).

Irritation

Irritation pulmonaire

Certaines études menées sur des volontaires sains ont montré une légère irritation des voies respiratoires lors d'une exposition aiguë de 50 ppb de TDI (Henschler et al. 1962). Chez des sujets asthmatiques des effets plus sévères (toux et oppression thoracique) ont été rapportés pour des expositions aiguës (10 ppb pendant une heure suivie d'une pause de 45 min et d'une exposition de 20 ppb pendant une heure) (Baur et al. 1985 quoted by OEHHA 2016). D'autres études n'ont rapporté aucun effet (irritation, réponse inflammatoire, modification du VEMS) chez les sujets présentant une HRB ou un asthme ou chez les contrôles après exposition au TDI (Chester et al. 1979; Fabbri et al. 1987; Moller et al. 1986).

Vandenplas et al., après avoir exposé au TDI 17 sujets, sains et non exposés professionnellement, à 5 ppb pendant 6h et, 4 semaines après à 20 ppb pendant 20 min, n'ont pas observé de symptômes respiratoires ni de diminution du VEMS. Ils ont cependant mesuré une légère diminution de la conductance spécifique des voies aériennes (SGVa), paramètre qui reflète la conductance des voies respiratoires indépendamment des volumes respiratoires. Cette observation suggère donc une association entre l'exposition au TDI et un effet sur les voies aériennes. Une diminution du débit expiratoire maximal à 25% (DEM25%) de la capacité vitale forcée (CVF) est également mesurée. Ce dernier paramètre reflèterait le débit des voies aériennes les plus distales mais sa fiabilité est discutable. Ils n'ont pas observé d'augmentation de la concentration de la protéine des cellules de Clara (CC16) ni des cytokines pro-inflammatoires (TNF-α, IL-4, IL-5, IL-6 et IL-8). Cependant, une légère augmentation de la concentration d'albumine dans le lavage broncho-alvéolaire (LBA) est mesurée chez les exposés par rapport aux non-exposés (26,4+/-12,5 mg/L après exposition au TDI comparé à 21,8+/-8,6 mg/L avant l'exposition, p=0.044). Ceci suggère une perte de l'intégrité de la barrière alvéolo-capillaire (Vandenplas et al. 1999).

Il a été observé que les sujets présentant un asthme au TDI étaient plus sensibles à l'exposition au TDI en réagissant à de plus faibles concentrations atmosphériques de TDI (O'Brien et al. 1979 a, b).

Chester et al., ne retrouvent pas d'altération dans la résistance spécifique des voies aériennes (RVA) chez les sujets (sains et asthmatiques) exposés à 20 ppb de TDI durant 20 min (Chester et al. 1979).

Irritation cutanée

Le TDI peut causer de sévères irritations de la peau, des brûlures du second degré et des dermatites mais aussi de sévères irritations des yeux.

Des cas de dermatites causées par contact avec du TDI ont été rapportés (Rothe 1976). Des kératopathies atypiques ont été observées chez les travailleurs d'une usine de production de mousse, à base de polyuréthane, ces effets n'ont pas été attribués au TDI mais à d'autres agents.

Des études ont rapporté des effets sur la peau suite à une exposition au TDI en milieu professionnel (Daftarian et al. 2002 et Huang et al. 1991). Il est difficile d'attribuer ces effets à une irritation ou une sensibilisation mais il peut être suggéré que la dermatite d'irritation est plus fréquente que la dermatite de contact allergique.

Sensibilisation

Sensibilisation respiratoire

L'exposition au TDI déclenche une hypersensibilisation de l'arbre trachéo-bronchique aussi appelée « asthme aux isocyanates ». Les études montrent que cette réaction pourrait être due aussi bien à des mécanismes immunologiques passant par des IgE spécifiques qu'à des mécanismes non spécifiques. L'asthme déclenché par le TDI est accompagné d'une HRB spécifique ou non. Des cas d'hypersensibilité ont été décrits après une seule exposition à de fortes doses de TDI et des cas d'alvéolites allergiques extrinsèques (forme rare de pneumonite interstitielle consécutive à une sensibilisation respiratoire) ont été rapportés suite à l'exposition aux diisocyanates (DFG 2003).

De plus, le déclenchement d'une sensibilisation bronchique consécutive à un contact cutané a également été mentionné (DFG 2003). Il a été montré que le principal facteur déclenchant la réaction allergique chez des volontaires sensibilisés au TDI n'était pas la concentration administrée (C) ou la durée (T) mais la dose totale administrée (CxT).

L'induction de la sensibilisation respiratoire a été évaluée chez des cobayes à des concentrations de 20 ppb durant 70 jours ou 120-7600 ppb durant 1 semaines (Karol 1983). La réponse respiratoire a été mesurée avec l'augmentation du taux respiratoire et la production d'anticorps après exposition à 1% de TDI-GSA (TDI-guinea pig serum albumin conjugates). Les auteurs ne rapportent aucun effet à 120 ppb (sur la base de la production d'anticorps) mais montrent une sensibilisation pulmonaire à partir d'une exposition de 360 ppb. Matheson et al. ont exposé des souris à 20 ppb durant 6 semaines tous les jours et après 2 semaines sans exposition, les animaux ont été exposés à 20 ppb durant 1h. Les résultats ont montré une inflammation des voies aériennes et une hyperactivité à la méthacholine, une augmentation des anticorps IgE et IgG et une augmentation de l'expression des cytokines Th1/Th2 dans le tissu pulmonaire. Ces données permettent de fixer un LOEL de 20 ppb pour l'induction chez la souris (Matheson et al. 2005).

Dans une étude de 2014, Pauluhn et al. , ont développé un protocole chez le rat (Brown Norway), pour déterminer la valeur seuil pour l'élicitation dans le déclenchement de réponses asthmatiformes chez des rats sensibilisés et exposés de nouveau. Les résultats de l'étude (exprimés en dose totale administrée, CxT) ont permis de suggérer que la réponse primaire et l'élicitation étaient liées à l'irritation/inflammation des tissus pulmonaires (Pauluhn et al. 2014).

Sensibilisation cutanée

Malgré l'augmentation de l'utilisation du TDI en milieu professionnel, la probabilité de développer une allergie cutanée suite à l'exposition répétée au TDI semble relativement faible. Selon Daftarian et al., il n'est pas clairement établi que les effets cutanés observés soient attribuables à une irritation ou sensibilisation primaire (Daftarian et al. 2002).

Toxicité chronique respiratoire

Ce paragraphe présente les revues existantes, les plus pertinentes en milieu professionnel, provenant d'agences ou d'organismes internationaux.

Rapport de la DFG[5] (DFG 2003)

De nombreuses études ont montré que le TDI pouvait déclencher une détérioration de la fonction pulmonaire.

La DFG a identifié l'étude de Jones et al. de 1992, étude longitudinale sur 5 ans dans une usine de production de mousse polyuréthane. Cette étude ne rapportait pas de lien entre le niveau d'exposition au TDI (5 ppb) et la diminution annuelle du VEMS. Cependant, une corrélation statistiquement significative a été observée entre la prévalence de bronchites chroniques et l'exposition cumulée au TDI. Selon Clark et al., les symptômes décrits dans l'étude de Jones et al. de 1992 pourraient être causés par l'exposition à d'autres agents (tels que des colles utilisées dans les processus de production) (Clark et al. 2003). Jones et al. ont rapporté des pics d'exposition pouvant dépasser 0,02 ppm (Jones 1992). Selon certains auteurs, les pics d'exposition sont plus prédictifs des effets respiratoires que les expositions cumulées sur 8 heures (Omae 1992b).

Rapport de l'US EPA[6] (US EPA 2012)

Dans sa revue de la littérature, l'US EPA a comparé les études présentant des résultats négatifs et celles qui présentaient des résultats positifs concernant les effets de l'exposition au TDI. Certaines études (réalisées sur plusieurs années) montraient une relation dose-réponse entre la concentration atmosphérique et les variations annuelles du VEMS (Wegman et al. 1974, 1977, 1982). Les études de Peters et al. ont montré une corrélation entre la modification du VEMS sur la journée de travail et la diminution annuelle de VEMS (Peters 1974; Peters et al. 1969, 1968, 1975). Musk et al. (1982, 1985, 1988) ont proposé un NOAEL basé sur la relation concentration-réponse établie par Wegman et al. (1974, 1977, 1982).

L'US EPA a retenu comme étude clé, l'étude de Diem et al. de 1982, réalisée sur 143 travailleurs (2093 échantillons recueillis sur tous les postes de travail). Les sujets sont divisés en 2 groupes d'exposition cumulée : inférieure et supérieure à 68,2 ppb x mois (0,486 $mg.m^{-3}$). Les 2 groupes ont ensuite été subdivisés en 6 sous-groupes en fonction du statut tabagique des travailleurs. Les postes de travail ont été classés sur la base de la moyenne des niveaux d'exposition d'un côté et de la durée d'exposition aux différents niveaux (20, 40, 60 et 80 ppb).

Selon l'US EPA, les résultats de cette étude ont montré :

- une forte corrélation entre la réduction du VEMS et l'exposition cumulée du TDI (après ajustement sur le tabagisme) ;
- une plus forte diminution (significative) de la moyenne annuelle du VEMS chez le groupe des non-fumeurs exposés à plus de 68,2 ppb comparativement au groupe faiblement exposé, cette différence n'étant pas retrouvée chez les fumeurs (actifs ou anciens) ;
- une corrélation entre la durée d'exposition au-dessus de 20 ppb et la diminution de la moyenne annuelle du VEMS.

Dans cette étude, les données recueillies chez les sujets n'ayant jamais fumé, suggèrent que la diminution annuelle du VEMS et du débit expiratoire forcé à 25-75% (DEF25-75%) est causé par une exposition long-terme à des concentrations moyennes supérieures à 1,9 ppb de TDI (moyenne arithmétique).

[5] Deutsche Forschungsgemeinschaft
[6] United States Environmental Protection Agency

L'US EPA a fixé un LOAEL à 1,9 ppb et un NOAEL à 0,9 ppb avec comme effet critique la réduction chronique des paramètres ventilatoires tels que le VEMS. Dans l'étude de Diem et al. (1982), les données chez les individus qui n'ont jamais fumé suggèrent que les effets sont causés par une exposition long-terme à plus de 1,9 ppb en moyenne (chute du VEMS annuel et du débit expiratoire entre 25-75%). Cette étude a été retenue en raison de différents éléments tels que la puissance statistique, la prise en compte des facteurs de confusion ainsi que des variations inter et intra-individuelles.

Les experts du CES VSR ont émis des doutes concernant cette étude, des limites au niveau méthodologique ayant été mises en avant (cf. Annex 4).

Health Council of the Netherlands – DECOS (2018)

Le DECOS dans son rapport publié en novembre 2018 a analysé des études épidémiologiques rapportant les effets respiratoires concernant des expositions à des di- et tri-isocyanates. Les experts du DECOS ont ainsi analysé 42 études rapportant une concentration critique associée à un effet sur la fonction respiratoire (Health Council of the Netherlands 2018).

Dans le cadre de la collaboration avec le DECOS, l'Anses a analysé ces 42 études via une approche adaptée de la méthode OHAT7 (NTP 2015) pour analyser la qualité des études. Seules 6 études ont été sélectionnées pour une analyse approfondie (les critères de sélection étaient : plus de 100 sujets, travailleurs exposés uniquement au TDI et études rapportant une diminution du VEMS). Ces études sont celles de Diem et al. 1982, Bodner et al. 2001, Littorin et al. 2007, Ott et al. 2000, Wegman et al. 1977 et 1982. Le CES a estimé qu'aucune de ces études ne permettait d'élaborer une valeur limite d'exposition professionnelle, en raison des limites méthodologiques de ces études mais aussi en raison du mécanisme d'action.

Mécanisme d'action sensibilisation/irritation

Données cliniques

L'asthme au TDI se présente cliniquement comme un asthme classique (inflammation, bronchoconstriction, et hypersécrétion de mucus). Comme dans tout asthme professionnel, le seul traitement efficace reste le retrait de l'exposition mais si après ce retrait, certaines personnes deviennent asymptomatiques, d'autres peuvent présenter des symptômes d'asthme persistants. L'asthme au TDI se développe après une période de latence assez variable qui peut aller de quelques mois à plusieurs années après l'exposition (Mapp et al. 1985). La réaction asthmatiforme peut être très rapide (<1h), retardée (de 2 à 4h plus tard) ou à la fois immédiate et retardée. Elle se produit souvent après une exposition aux faibles concentrations d'isocyanates (Wegman et al. 1982) et présente une reconnaissance et un diagnostic complexe.

Exceptionnellement, l'asthme peut se déclencher après une exposition à de fortes concentrations et peut-être lié aux effets irritants des isocyanates. Le TDI présente des effets irritants se produisant sans période de latence sous la forme d'HRB non-spécifique due à une toxicité directe (Shin et al. 2013). Certains individus peuvent conserver une sensibilisation à la suite d'une seule exposition de ce type (Moller et al. 1986 ; Leroyer et al. 1998).

Mécanismes physiopathologiques

[7] Office of Health Assessment and Translation

Les mécanismes physiopathologiques responsables de la réponse asthmatique au TDI restent toutefois encore peu élucidés et une réponse univoque paraît peu probable. Un certain nombre d'arguments permettent de justifier un mécanisme immunologique pour la sensibilisation respiratoire du TDI : la période de latence entre le premier contact et la survenue de la réponse asthmatique, la faible incidence par rapport au grand nombre de sujets exposés (5 à 10% des travailleurs), la similitude des symptômes observés lors de l'asthme au TDI avec ceux déclenchés par les allergènes inhalés et la présence d'IgE dans le sérum de certains sujets présentant une sensibilisation pulmonaire (Karol et Jin 1991).

Cependant, il existe des différences entre la sensibilisation immunologique pulmonaire classique médiée par les IgE et la sensibilisation au TDI telles que la réponse retardée (Finotto et al. 1991), les réactions atypiques aux tests de challenge bronchique (Perrin et al. 1991) ou encore la faible détection d'IgE spécifiques chez les sujets diagnostiqués pour un asthme au TDI (Son et al. 1998). L'intérêt limité de la détection des IgE dans les cas de sensibilisation au TDI pourrait tenir au manque d'informations sur les complexes conjugués et donc sur les anticorps formés contre ces formes conjuguées (Karol et Jin 1991). La sensibilisation pulmonaire pourrait intervenir pour une exposition répétée au TDI (dès 0,05 ppm en chronique, Bonnard et al. 2006) permettant de stimuler les mécanismes immunitaires des individus exposés qui réagiraient ensuite à des doses plus faibles de TDI par déclenchement de la réaction immunitaire.

Le TDI peut se comporter comme un haptène c'est-à-dire une substance de faible poids moléculaire, non immunogène en elle-même qui, une fois captée par les cellules épithéliales pulmonaires, se lie aux protéines pour former des adduits.

Ces formes conjuguées (dont la structure n'est pas clairement définie) sont capables d'initier une réponse immunologique après capture par les cellules dendritiques immatures des voies aériennes. Après maturation, ces cellules apprêtent le TDI conjugué et le présente aux lymphocytes T naïfs et polarisent les lymphocytes T en les dirigeant vers la voie de différenciation la plus adaptée à l'agression (Raulf-Heimsoth et Baur, 1998). L'immunité adaptative dépend de l'activation, l'expansion clonale et la différenciation des lymphocytes T et B spécifiques d'un antigène donné à l'aide de lymphocytes T auxilliaire ou T helper (Th) à savoir les Th1, Th2, Th17 et les lymphocytes T régulateurs (Treg).

Dans le cas d'une sensibilisation pulmonaire au TDI, il a été rapporté une prédominance de la réponse de type Th2 (orientant vers l'immunité à médiation humorale faisant intervenir la lignée B et la production d'anticorps à haute affinité) avec une sécrétion des IL-4, IL 5 et IL-13 qui est suivie par une production d'IgE spécifique. Suite à une exposition ultérieure au TDI, ces IgE se fixent eux-mêmes aux mastocytes pour entrainer leur dégranulation et la libération d'histamine responsable de la bronchoconstriction (Maestrelli et al. 1997; Ban et al. 2006). Il s'agit d'une réaction d'hypersensibilisation de type I associée à une hypersensibilisation respiratoire classique.

Cependant, l'apparition de réactions retardées et de symptômes chroniques associés à l'asthme au TDI, ainsi que le fait que l'atopie ne soit pas un facteur de risque reconnu de survenue de l'asthme au TDI, semblent impliquer une réaction d'hypersensibilisation de Type IV. Des réponses immunes de nature cellulaire impliquant les lymphocytes T CD8+ ont aussi été décrites (expliquant les manifestations retardées observées dans la survenue de l'asthme au TDI). Dans un modèle de souris exposées par inhalation au TDI, il a été rapporté non seulement un rôle prédominant des lymphocytes T CD4+ sécrétant des cytokines Th2 mais aussi une coopération interactive avec les lymphocytes T CD8+ sécrétant les cytokines Th1 telles que l'IFNγ (Matheson et al. 2005). La présence de lymphocytes T CD8+ produisant des

IL-5 et IFNγ a été identifiée dans le mucus bronchique des sujets sensibilisés au TDI (Maestrelli et al. 1994). Ces cytokines sont ensuite capables d'attirer et d'activer des cellules inflammatoires tels que les neutrophiles et éosinophiles (Bentley et al. 1992; Sun et al. 2006; Świerczyńska-Machura et al. 2012; 2014) qui sécrètent à leur tour les médiateurs de l'inflammation responsables de la réaction asthmatiforme.

Les études rapportant des sensibilisations croisées entre isocyanates sont contradictoires (Malo et al. 1983 ; Hettick and Siegel 2011 ; Pollaris et al. 2015).

Les mécanismes d'action directe des isocyanates sur les bronches ont également été rapportés. Certaines études ont montré que l'action d'inhibition du TDI sur les acétylcholinestérases au niveau des parois bronchiques pourrait jouer un rôle dans le développement de la bronchoconstriction (Brown et al. 1982; Dewair et al. 1983; Brondeau et al. 1990).

Les variations génétiques (génotypes sur les protéines du complexe majeur d'histocompatibilité, de la gluthation transférase ou de la N-acétyltransférase) peuvent prédisposer les individus au développement d'un asthme professionnel lié à l'exposition aux isocyanates (Mapp et al. 2005).

Tous ces mécanismes montrent que la sensibilisation pulmonaire au TDI conduisant au développement d'un asthme professionnel résulte de mécanismes plus complexes que ceux observés dans la sensibilisation immunologique pulmonaire classique.

Lien entre la sensibilisation cutanée et respiratoire

Chez les travailleurs, les cas de sensibilisation cutanée (sous la forme de dermatites de contact ou urticaire allergique) suite à une exposition au TDI sont rares (Goossens et al. 2002).

Plus récemment, les études ont montré que l'exposition cutanée au TDI suivie par une exposition par inhalation influençait la sensibilisation allergique au TDI (Vanoirbeek et al. 2004) et induisait une réponse locale et systémique à Th2 (Ban et al. 2006) chez la souris.

Un certain nombre d'études s'intéressent au fait que cette exposition cutanée au TDI et plus globalement aux isocyanates pourrait déclencher une sensibilisation allergique responsable d'asthme professionnel au TDI comme il a été, très récemment, mis en évidence par Pauluhn chez des rats ; l'étude montre que l'allergie respiratoire au TDI impliquerait 2 mécanismes séquentiels : une exposition dermique capable d'entraîner une sensibilisation systémique qui, suivie d'une exposition par inhalation, initierait et amplifierait l'inflammation allergique et la progression vers l'asthme (Pauluhn 2014). Une autre étude très récente chez la souris suggère que les follicules pileux et les glandes sébacées pourraient constituer un réservoir d'entrée du TDI et de déclenchement des réponses immunes à celui-ci (Nayak et al. 2014).

La revue concernant les études animales de Schupp et Collins (2012) montre que les résultats des études actuelles chez l'animal sont en cohérence avec celles qui sont réalisées chez l'Homme.

Génotoxicité

Etudes in vitro

Dans une étude in vitro (lymphocytes humains) des aberrations chromosomiques et des échanges de chromatides sœur ont été observés (après 24h d'exposition et en l'absence d'un système d'activation métabolique) (Maki-Paakkanen et al. 1987 cité par IARC 1999).

Les études *in vitro* ont montré que le TDI pouvait induire des effets au niveau de l'ADN (cassures double brin, échange de chromatides sœurs) (Marczynski et al. 1993 et Gulati et al. 1989). Cependant les études conduites sur les micronoyaux étaient négatives (Lindberg et al. 2011). Le TDI induit également des mutations en présence d'un système d'activation métabolique sur TA 98, TA 100 et TA 1538 (IARC 1999). Dans les études, les tests sont menés avec du diméthylsulfoxyde (DMSO) ou de l'éther diméthylique de l'éthylèneglycol. (EDGE). Lorsque le DMSO est remplacé par l'EDGE, le TDI devient mutagène sur TA 1537 et TA 98 (Seel et al. 1999). Des études ont montré l'induction de mutations sur des cellules lymphatiques de souris (locus $Tk^{+/-}$) (McGregor et al. 1991). Chez la drosophile, des mutations létales récessives liées au sexe ont été observées (Foureman et al. 1994).

Des études ont été menées sur les effets génotoxiques du TDA (Prueitt et al. 2013 et 2017). Les auteurs ont montré que le TDA avait des effets mutagènes après activation métabolique et que le 2,6-TDA était mutagène uniquement sur les souches *S. typhimurium* TA 98 et TA 1538 en présence d'activation métabolique.

Etudes animales

Les diisocyanates peuvent former des adduits aux protéines mais aussi à l'ADN. Leurs métabolites peuvent également former des adduits à l'ADN. Bien qu'il n'ait pas été retrouvé d'étude concernant la formation d'adduits à l'ADN avec le TDI, il peut être envisagé que des adduits puissent se former par liaison covalente avec l'ADN (comme pour le MDI décrit par Vock et al. 1995).

Les études réalisées chez l'animal montrent, après inhalation de TDI marqué au ^{14}C, la présence de radioactivité dans l'épithélium des voies aériennes supérieures et la formation d'adduit aux protéines (principalement albumine). Bien que les adduits aux protéines ne soient pas considérés comme l'expression d'une génotoxicité, ils sont souvent considérés comme biomarqueurs d'exposition et de toxicité.

Etudes humaines

Une étude menée sur des travailleurs (n=26) a montré une augmentation de la fréquence des aberrations chromosomiques et des échanges de chromatides sœurs dans les lymphocytes (concentrations de TDI entre 0,007 et 0,016 mg/m^3) (Bilban 2004). Marczynski et al. a montré une fragmentation de l'ADN dans les globules blancs de travailleurs exposés au MDI (et non TDI) par inhalation (5 à 20 ppb) (Marczynski et al. 1992b).

Marczynski et al. ont réalisé un test des comètes sur des lymphocytes de travailleurs ayant présenté des symptômes respiratoires et avec une exposition connue aux diisocyanates. Les résultats obtenus avant et après exposition mais aussi entre les différents groupes d'exposition (des 5 sujets volontaires de l'étude) ne différaient pas de façon significative (Marczynski et al. 2005).

Les adduits aux protéines (albumine) sont de bons marqueurs de l'exposition en milieu professionnel. Dans une étude, Mhike et al. ont comparé les adduits au TDI et HDI (par liaison covalente) dans le sang humain. Les résultats ont montré une plus grande réactivité du TDI avec l'albumine et l'hémoglobine comparativement au HDI à pH 7,4 (Mhike et al. 2016).

En conclusion, sur la base des différentes études (et résultats contradictoires), les résultats ne permettent pas de conclure sur les effets mutagènes et génotoxiques du TDI.

Cancérogénicité

En 1999, le CIRC a classé le TDI comme cancérogène possible chez l'Homme (Groupe 2B). Les experts du CIRC ont estimé que les preuves étaient insuffisantes chez l'Homme et suffisantes chez l'animal (IARC 1999). Le NTP a considéré que, sur la base des preuves suffisantes chez l'animal, l'effet cancérogène du TDI pouvait être raisonnablement envisagé chez l'Homme (NTP 2016).

L'hydrolyse du TDI génère au niveau du système digestif du TDA qui est cancérogène (classé catégorie 1B selon le CLP). L'exposition par inhalation entraine la formation de TDI conjugué et peu de TDA. Pour l'OEHHA, cette différence peut expliquer les effets cancérogènes du TDI observés lors de l'exposition par voie orale (OEHHA 2016).

Chez l'Homme

Chez l'Homme, il existe 3 grandes études de cohortes menées en Suède, au Royaume-Uni (Angleterre et Pays de Galles) et aux USA.

Dans la cohorte suédoise (Hagmar et al. 1993), les résultats n'ont pas montré de lien entre l'exposition aux isocyanates et le risque de cancer du poumon. Les résultats ont été confirmés par une étude portant sur la même cohorte 11 ans plus tard (Mikoczy et al. 2004).

Au Royaume-Uni, 20% des individus suivis au sein de la cohorte sont décédés. Mais l'étude n'a pas retrouvé de lien entre les travailleurs exposés aux isocyanates par inhalation et le risque de cancer du poumon ou avec d'autres types d'effets respiratoires (International Isocyanate Institute 1992b cité par DFG 2003), Sorahan and Pope 1993, Sorahan et Nichols 2002). Selon les auteurs, l'augmentation de cancers du poumon observée chez la femme a été attribuée au tabac.

Pinkerton et al. ont étudié les sujets de la cohorte et ont ajouté que la mortalité par cancer du poumon n'était pas reliée à la durée de l'exposition ou à l'exposition cumulée au TDI mais à la durée de l'emploi sur les postes de finition (Pinkerton et al. 2016).

Dans la cohorte américaine, les auteurs ont retrouvé une relation possible entre le temps écoulé entre le premier emploi dans une usine de production de mousse à base de polyuréthane et l'apparition de lymphomes non-hodgkiniens et de maladies de Hodgkin (Schnorr et al. 1996). L'exposition au TDI dans cette étude était évaluée uniquement par le biais des concentrations atmosphériques.

En conclusion sur la base de ces études, et en l'absence de cas recensés de cancer, il s'avère que l'exposition au TDI par inhalation ne semble pas induire d'excès de risque de cancer chez l'Homme.

Chez l'animal

L'administration de TDI (85/15 de 2,4-TDI et 2,6-TDI) par gavage chez des rats et des souris femelles, a donné naissance à des tumeurs dans le foie (adénomes hépatocellulaires), des tumeurs bénignes dans les glandes mammaires (fibroadénomes) et des tumeurs bénignes dans le pancréas chez le rat mâle. L'exposition au TDI par voie orale entraine une augmentation de l'incidence de tumeurs malignes et bénignes des tissus sous-cutanés chez le rat mâle et des tumeurs des vaisseaux sanguins chez la souris femelle. Il semble que le TDA induise le même type de tumeurs (Dieter et al., 1990, cité par IARC 1999).

Aucun effet cancérogène n'a été observé dans une étude exposant les rats et souris à des concentrations de 0,05 et 0,15 ppm de TDI 6 heures par jour 5 jours par semaine pendant 2 ans (Bonnard et al. 2006 et Löser et al. 1983).

Concernant le TDA, le National Cancer Institute (NCI) a mené une étude sur des souris (50 mâles et 50 femelles) exposées à du 2,4 TDA par voie orale à des concentrations de 100 et 200 ppm pendant 101 semaines. Les résultats ont montré une augmentation statistiquement significative de l'incidence de carcinomes hépatocellulaires aux deux doses mais aussi de l'incidence de lymphomes chez les souris femelles exposées à la faible dose (NCI 1979). Le NCI a également mené une étude sur des rats (50 mâles et 50 femelles) exposés à du 2,4 TDA par voie orale à des concentrations de 125, 250 ppm durant 40 jours. En combinant l'incidence de survenue de carcinomes hépatocellulaires et de nodules néoplasiques, les résultats ont montré une relation dose-réponse chez les mâles ($p = 0,014$) et femelles ($p = 0,008$). De plus, l'incidence des fibroadénomes des glandes mammaires était dose-dépendante chez les femelles (et statistiquement significative aux doses les plus fortes avec $p<0,001$).

En conclusion

La transformation de TDI en TDA (cancérogène connu chez l'animal) est l'explication la plus plausible de tumeurs observées après administration par voie orale de TDI. La différence de formation de TDA selon la voie d'exposition explique que le TDI soit cancérogène par voie orale mais pas par inhalation.

Concernant les données épidémiologiques, elles ne permettent pas de démontrer la cancérogénicité du TDI chez l'Homme.

Construction des VLEP

Choix de l'effet critique

Les données disponibles chez l'Homme permettent de montrer les effets respiratoires (asthme, hyperréactivité bronchique, sensibilisation pulmonaire, atteinte de la fonction respiratoire) suite à une exposition au TDI. Néanmoins les études chez l'Homme concernant ces effets présentent des limites pour l'établissement d'une relation dose-réponse (pics d'exposition et exposition cutanée peu quantifiables, port de protections individuelles, nombre de sujets dans les études, manque de données sur les indicateurs de la fonction respiratoire, la mesure du VEMS, seule, ne pouvant suffire pour évaluer l'altération de la fonction respiratoire).

Les données chez l'animal indiquent que l'induction de la sensibilisation respiratoire est à considérer comme effet à seuil. La revue de Schupp et Collins de 2012 rapporte que les NOAEL et LOAEL pour l'irritation et la sensibilisation sont du même ordre de grandeur chez les animaux. Selon les auteurs, les données disponibles dans la littérature permettent de conclure que l'irritation et la sensibilisation peuvent être interdépendantes. Pauluhn a développé un modèle animal qui montrait que les effets respiratoires produits par le TDI étaient des effets à seuil et qu'une valeur protégeant de l'irritation permettait de protéger de la sensibilisation (Pauluhn 2014).

Les experts ont donc retenu l'irritation pulmonaire comme effet critique. Selon les experts, protéger contre l'irritation permet de protéger contre la sensibilisation. Mais cette valeur ne permet pas de protéger les personnes déjà sensibilisées des réactions allergiques. En effet, en raison de la grande variabilité interindividuelle chez les individus sensibilisés, il paraît peu prudent de recommander une valeur limite pouvant prévenir de l'élicitation chez ces personnes (Dotson et al. 2015). De plus, Banks et al. ont démontré que l'élicitation de symptômes cliniques pouvaient survenir à des niveaux égaux ou inférieurs à la VLEP-8h dérivée pour prévenir de l'induction de la sensibilisation chez les sujets naïfs (Banks et al. 1989).

Construction de la valeur limite court terme sur 15 min (VLCT-15 min)

L'étude retenue pour la construction de la VLCT-15 min est l'étude de Vandenplas et al. de 1999. Dans cette étude , les auteurs ont exposé 17 sujets volontaires (8 fumeurs et 9 non-fumeurs). Ces sujets non exposés professionnellement aux isocyanates et ne présentant pas de symptômes respiratoires (asthmes et bronchites chroniques) ont été exposés à 5 ppb pendant 6 h et, après 4 semaines d'intervalle, à 20 ppb pendant 20 min. Les auteurs ont mesuré les paramètres infra-cliniques et estimé le risque d'irritation respiratoire induite par l'exposition au TDI.

Les auteurs ont observé une augmentation faible du niveau d'albumine dans le lavage bronchique (p=0,044). Les résultats de l'étude ont également montré une diminution faible de la conductance spécifique des voies aériennes (p=0,053), ainsi que du DEM25% (p=0,015).

L'augmentation de l'albumine dans le BAL est susceptible de représenter indirectement des preuves de modification de la perméabilité de la barrière épithéliale et de légères fuites de composants plasmatiques sanguins dans le compartiment alvéolaire (Vandenplas et al. 1999).

Les experts considèrent que la concentration de 20 ppb comme un LOAEC.

A ce LOAEC de 20 ppb, il est proposé d'appliquer les facteurs d'ajustement (FA) suivants :

- un facteur d'ajustement pour le passage de LOAEC à NOAEC de 3

- selon la méthodologie habituelle, l'application d'un facteur d'ajustement interindividuel ne serait pas justifié pour les effets irritants (locaux). Cependant le TDI peut induire des effets plus sévères selon les individus pour des expositions de même niveau. Comme décrit par Vogelmeier et al. (1991) et Baur et al. (1994), l'exposition au TDI a induit une irritation sensorielle chez 3 sujets sains exposés à 20 ppb durant 2h mais une réponse pulmonaire sévère chez des asthmatiques exposés à 10 ppb durant 1h. Les experts appliquent donc un facteur de 5 pour tenir compte des variabilités interindividuelles.

Soit : 20 ppb/ 15 = 1,3 ppb ou 9,4 µg.m^{-3} (facteur de conversion[8] à 20°C et 1023 hPa)

La VLCT-15min proposée par les experts est donc de 1,3 ppb.

Les experts considèrent que cette valeur protège des effets sensibilisants mais pas des réactions allergiques chez les individus déjà « sensibilisés ».

Construction d'une valeur limite d'exposition professionnelle sur 8 heures pragmatique (VLEP-8h pragmatique)

En raison de la sévérité des effets du TDI, et dans la mesure où le contrôle d'une VLCT-15min implique un mesurage lors de la survenue des pics d'exposition et que cela peut parfois

[8] 1 ppm = 7.24 mg.m^{-3}

s'avérer difficile à contrôler sur la durée d'un poste de travail de 8 heures, les experts estiment important de pouvoir recommander, en sus de la VLCT-15 min, une valeur limite d'exposition ne devant pas être dépassée sur une période de 8 heures.

En l'absence de données robustes et adéquates, les experts proposent de déterminer une VLEP-8h pragmatique, l'objectif n'étant pas de fixer une valeur en dessous de laquelle il n'y a pas de risque sanitaire mais de mettre à disposition des préventeurs un outil de gestion des risques afin de limiter les expositions sur le lieu de travail (Anses 2017).

Afin de minimiser le risque de dépasser la VLCT-15 min sur la durée d'un poste de travail de 8 heures (soit 32 fois 15 minutes) la concentration atmosphérique de TDI ne devrait pas dépasser la VLCT-15 min/32 sur une journée de travail de 8 heures.

Soit :

VLEP-8h pragmatique = VLCT-15 min / 32 = 1,3 / 32 = 0,04 ppb

Les experts recommandent une VLEP-8h pragmatique de **0,04 ppb** ce qui est en accord avec la valeur recommandée par le DECOS dans son rapport de 2018 (soit 0,1 µg NCO/m^3 correspondant à 0,04 ppb de TDI). Cette valeur ne protège pas les personnes sensibilisées des réactions allergiques.

Mention « Peau »

Bien que la sensibilisation cutanée ne soit pas un critère pour la recommandation de la mention « peau », les experts considèrent que la pénétration cutanée de TDI est susceptible d'entraîner une sensibilisation respiratoire.

Les experts recommandent donc la mention « Peau ».

Mention « Bruit »

En l'absence de donnée sur les effets ototoxiques du TDI, aucune mention « Bruit » n'est recommandée pour le TDI.

Résultat de l'expertise collective concernant les méthodes de mesure atmosphériques dans les lieux de travail

Évaluation des méthodes de mesure du TDI dans l'air des lieux de travail

L'exposition au TDI se produit sous forme gazeuse et/ou particulaire. Elle est liée à la pression de vapeur : à température ambiante, le TDI n'a pas tendance à se volatiliser mais s'il est chauffé ou aérosolisé, la volatilité augmente rapidement. Par conséquent, le premier point critique pour la mesure du TDI dans l'air est la nature du dispositif d'échantillonnage. Les experts ont décidé de ne conserver que les dispositifs capables de collecter simultanément les phases gazeuse et particulaire du TDI. Il a estimé que la faible pression de vapeur du TDI induit une forte probabilité qu'une partie du TDI généré sous forme gazeuse soit condensée en très fines gouttelettes ou adsorbée à la surface des particules. Par conséquent, les protocoles basés sur les badges d'échantillonnage par diffusion gazeuse n'ont pas été évalués car ils ne sont pas en mesure de collecter la phase particulaire.

Des instruments permettant le suivi du niveau d'exposition aux isocyanates en temps réel sont disponibles dans le commerce. Ils sont basés sur la détection et la mesure par spectrométrie

à mobilité d'ions ou des produits de réaction résultant de la réaction de l'isocyanate avec un papier imprégné de réactif. Ces instruments ne prennent en compte que la phase vapeur et n'ont donc pas été évalués.

Pour évaluer l'exposition individuelle aux vapeurs et aérosols de TDI dans l'atmosphère des lieux de travail, de nombreuses méthodes de mesure indirectes (méthodes d'échantillonnage actif) sont disponibles. Ces méthodes suivent les mêmes étapes : échantillonnage de la phase vapeur et de l'aérosol, dissolution dans un solvant contenu dans un barboteur et/ou adsorption sur un filtre imprégné; réaction avec un réactif de dérivation pour créer un dérivé non volatil qui stabilise les espèces intéressantes, puis analyse par chromatographie liquide couplée à une détection spectrométrique.

Remarques générales sur les méthodes d'échantillonnage actives

Le choix de l'échantillonneur - filtre, barboteur ou barboteur et filtre en série - est dicté par le scénario d'exposition et dépend de l'environnement où les échantillons sont prélevés :

- les barboteurs sont habituellement efficaces pour échantillonner les aérosols mais des particules de moins de 2 µm de diamètre peuvent les traverser. Les filtres en fibre de verre imprégnés sont efficaces pour recueillir des vapeurs et des particules de tailles très variables.
- les filtres à fibres imprégnés d'un réactif de dérivation peuvent être utilisés pour échantillonner des isocyanates en phase gazeuse, des isocyanates aliphatiques en aérosol, des isocyanates aromatiques en aérosol avec un diamètre de particules < 2 µm.
- les barboteurs peuvent être utilisés pour échantillonner des aérosols d'isocyanates aromatiques avec un diamètre de particules > 2 µm ;
- la combinaison d'un barboteur et d'un filtre permet de prélever des vapeurs et des aérosols d'isocyanates aliphatiques ou aromatiques (particules d'un diamètre supérieur ou inférieur à 2 µm).

Les méthodes utilisant un barboteur suivi d'un filtre n'ont pas été exclues même si elles ne mettent pas en œuvre un dispositif de prélèvement de la fraction inhalable. En effet, ces échantillonneurs (barboteur suivi de filtre) surestiment la fraction collectée ou peuvent avoir une efficacité de collecte similaire à celle d'un filtre imprégné dans un échantillonneur de la fraction inhalable. Ces méthodes ont été considérées au mieux comme indicatives.

Remarques générales sur la dérivation

Toutes les méthodes sont basées sur la dérivation des groupes isocyanates réactifs pour conduire à des produits qui peuvent être analysés par chromatographie liquide. Ces méthodes ont plusieurs objectifs : 1) piéger l'isocyanate en phase gazeuse sous forme non volatile, 2) bloquer la fonction isocyanate pour éviter les réactions secondaires de polymérisation ou de dégradation et greffer un groupe chromophore pour favoriser la détection UV.

Selon la méthode utilisée, différents agents de dérivation peuvent être choisis[9] :

- Les réactifs 1,2-MPP et 1,2-PP réagissent quantitativement avec les isocyanates aromatiques et sont facilement solubles dans de nombreux solvants. Ils sont également résistants aux UV et stables en solution.
- Les réactifs MAMA et MAP donnent des produits dérivés très sensibles à la détection UV, c'est-à-dire environ 2 à 3 fois plus sensibles que 1,2-MPP ou 1,2-PP. Au contraire,

[9] Agents dérivatifs et abréviations :
1-(2-méthoxyphényl)pipérazine : 1,2-MPP ; 1-(2-pyridyl)pipérazine : 1,2-PP ; dibutylamine : DBA ; 9-(méthylaminométhyl)-anthracène : MAMA ; 1-(9-anthracénylméthyl)pipérazine : MAP ; N-[(4-nitrophényl)méthyl]propylamine : Réactif nitré.

les composés dérivés formés sont sensibles aux UV et leur solubilité est faible dans de nombreux éluants organiques courants utilisés en chromatographie liquide.

- La DBA est très réactive en solution, c'est pourquoi elle est souvent utilisée en barboteur.

Évaluation des méthodes de mesure du TDI dans l'air des lieux de travail

Sept méthodes de mesure du TDI dans l'air des lieux de travail ont été recensées et évaluées (Cf. Tableau 1)

Tableau 1 : Recensement et classement des méthodes de mesure du TDI dans l'air des lieux de travail au regard de la VLEP-8h et de la VLCT-15min recommandées

	Méthodes	Protocoles	VLEP-8h	VLCT-15min	
			Contrôle technique réglementaire	Contrôle technique réglementaire	Suivi des expositions court terme
1	Barboteur (1,2-MPP dans toluène) suivi par un filtre en fibres de verre imprégné de 1,2-MPP	ISO 16702 (2008) ; HSE-MDHS 25/4 (2014)	**3**	**3**	**3**
2	Barboteur (DBA dans toluène) suivi par un filtre en fibres de verre non-imprégné	ISO 17734-1 (2013)	**3**(*)	**3**(*)	**3**(*)
3	Barboteur (MAP dans benzoate de butyle) suivi par un filtre en fibres de verre imprégné de MAP	ISO 17735 (2009) ; NIOSH 5525 (2003)	**3**	**2**	**2**
4	Filtre PTFE associé à un filtre en fibres de verre imprégné de MAMA ou MAP dans une cassette fermée ou un IOM	ISO 17736 (2010), ISO 17735 (2009), NIOSH 5525 (2003), IRSST 376	**3**	**3**	**3**
5	1 ou 2 filtres en fibres de verre imprégnés de 1,2-MPP ou de 1,2-PP	IFA 7120 (2010) ; IFA 7670 (2004) ; MAK Diisocyanate (2006) ; INRS MétroPol 245, 246, 249, 250 (2003) ; ISO 14382 ; OSHA 42 (1983)	**3**	**3**	**2**
6	Barboteur avec un agent de dérivation (1,2-MPP, MAP ou réactif nitré)	NIOSH 5521 (1994) ; 5525 (2003) ; INSHT MTA/MA-034/A95 (1995) ; MAK HDI	**3**	**3**	**3**

		TDI (1985); OSHA 18 (1981)			
7	Tube “Denuder” imprégné de DBA, terminé par un filtre en fibre de verre imprégné de DBA	ISO 17734-1 (2013)	**3**	**3**	**3**
(*) méthode classée en catégorie 3 du fait d’une absence de données de validation					

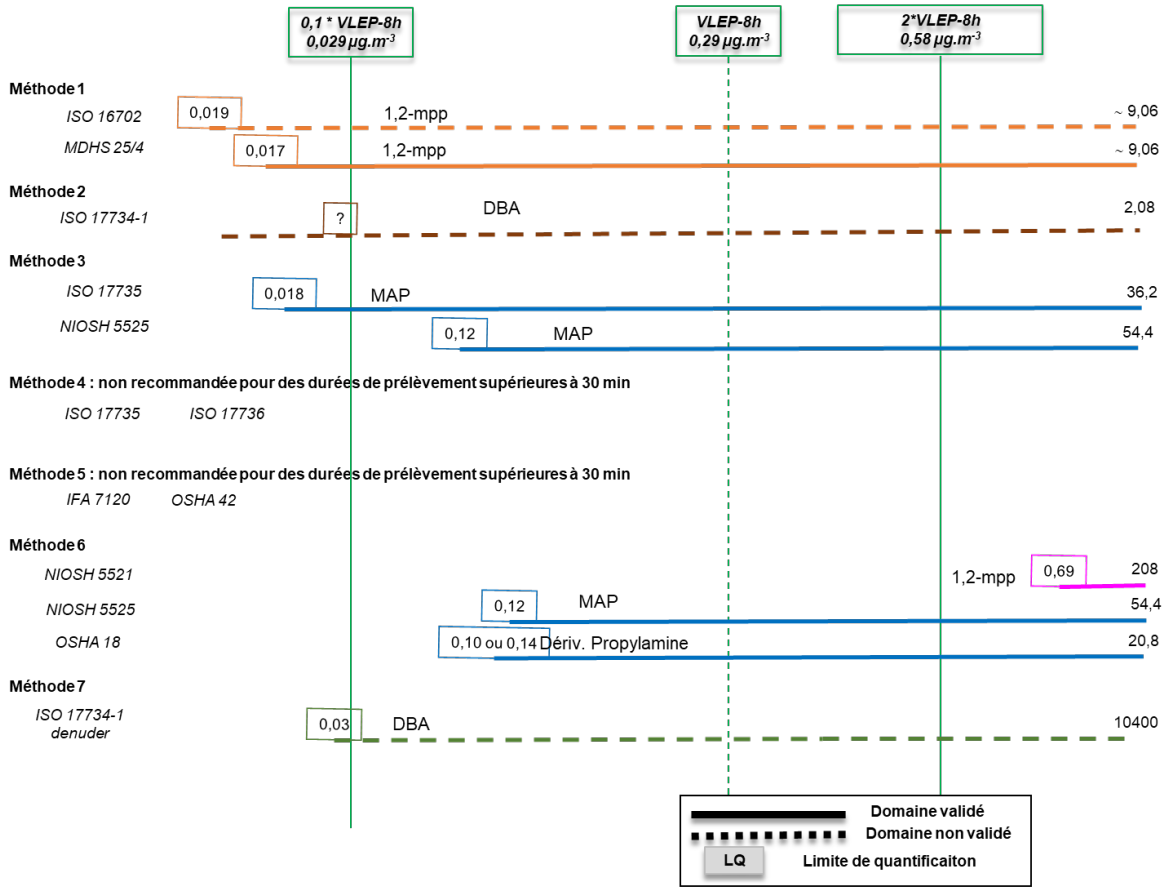

Figure 2 : Domaine de validité et limite de quantification des méthodes comparés au domaine 0,1 à 2 fois la VLEP-8h proposée par le CES.

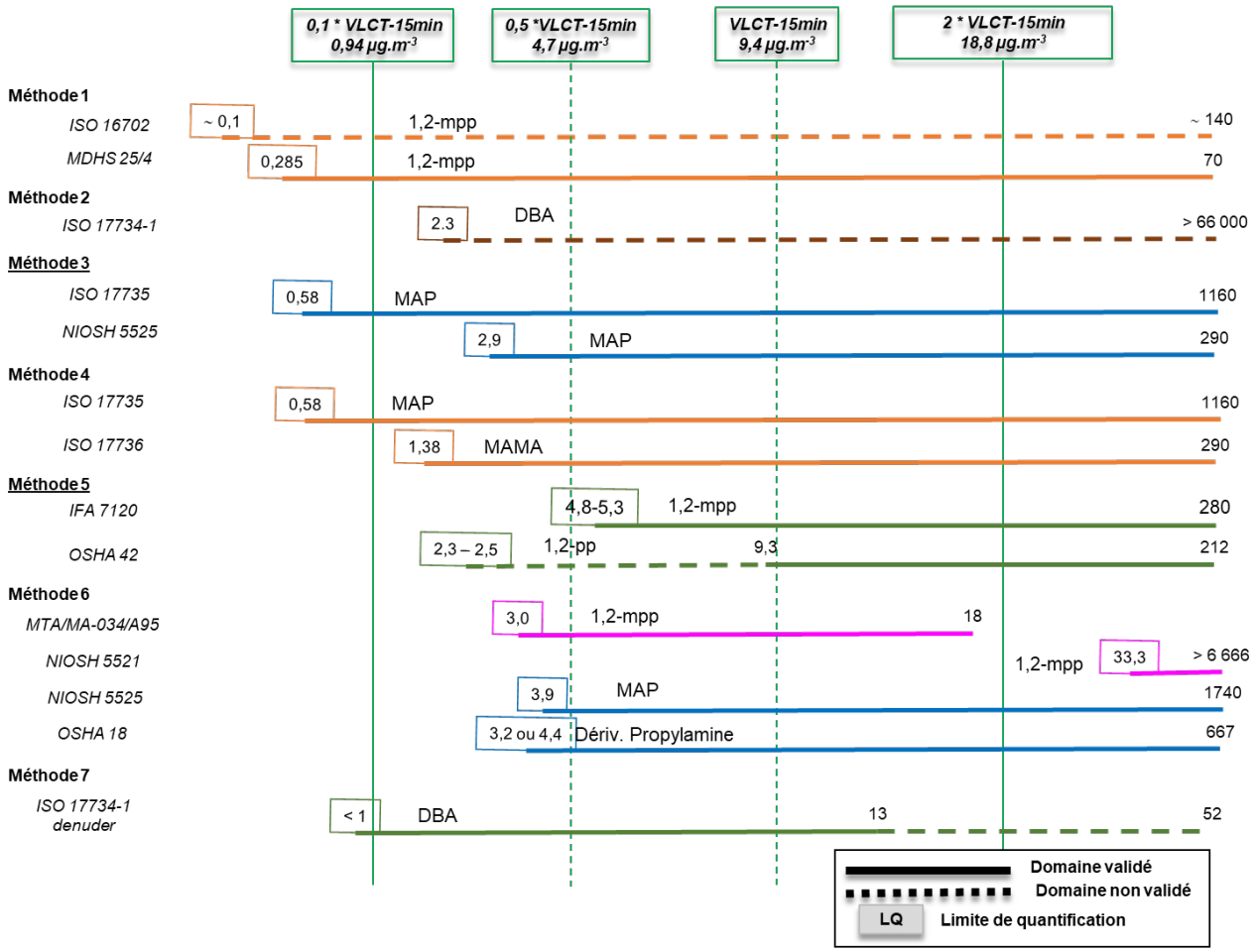

Figure 3 : Domaine de validité et limite de quantification des méthodes comparés au domaine 0,1 à 2 fois la VLCT-15min proposée par le CES.

Méthode 1 :

La méthode consiste à effectuer un échantillonnage actif à travers un barboteur contenant de la 1,2-MPP dans du toluène puis un filtre en fibre de verre imprégné de 1,2-MPP. L'analyse est ensuite réalisée par chromatographie liquide avec détection ultraviolette, électrochimique ou spectrométrie de masse. La méthode est décrite dans la norme ISO 16702 (2011) et le protocole MDHS 25/4 (2014).

L'objectif de la méthode étant de déterminer la concentration totale d'isocyanates dans l'air (µg NCO.m^{-3}), peu de données de validation pour le TDI sont disponibles. Une plage de travail et une limite de quantification sont mentionnées, mais pas spécifiquement pour le TDI. L'efficacité de l'échantillonnage n'est pas mentionnée. L'incertitude étendue de la méthode est estimée entre 47 et 50% pour le TDI (dans la plage de 0,1 à 2 µg NCO), ce qui est élevé, proche du maximum autorisé par la norme NF EN 482 que ce soit pour des périodes de référence de 8 heures ou 15 minutes. Pour ces raisons, la méthode 1 est classée dans la catégorie 3 et n'est pas recommandée pour le contrôle technique réglementaire de la VLEP-8h, de la VLCT-15min ni pour le suivi des expositions court terme.

Par ailleurs, compte tenu des effets sanitaires du toluène, le prélèvement individuel à l'aide d'un barboteur contenant une solution absorbante à base de toluène doit être évitée afin d'éviter un risque d'exposition des travailleurs.

Méthode 2

La méthode consiste à effectuer un échantillonnage actif à travers un barboteur contenant du DBA dans du toluène suivi d'un filtre en fibre de verre non imprégné, analyse par chromatographie liquide avec détection ultraviolette, azote ou masse. La méthode est décrite dans la norme ISO 17734-1 (2013).

La méthode est classée en catégorie 3(*) pour le contrôle technique réglementaire de la VLEP-8h, de la VLCT-15min et le suivi des expositions court terme en raison du manque d'information concernant l'efficacité de l'échantillonnage et de la récupération.

Par ailleurs, comme pour la méthode 1, compte tenu des effets sanitaires potentiels du toluène, le prélèvement individuel à l'aide d'un barboteur contenant une solution absorbante à base de toluène doit être évitée afin d'éviter un risque d'exposition des travailleurs.

Méthode 3

La méthode consiste à effectuer un échantillonnage actif à travers un barboteur contenant du MAP dans du benzoate de butyle suivi d'un filtre en fibre de verre imprégné de MAP, puis une analyse par chromatographie liquide avec détection ultraviolette ou fluorimétrique. La méthode est décrite dans la norme ISO 17735 (2009) et le protocole NIOSH 5525 (2003).

De nombreuses données de validation disponibles dans les protocoles mettant en œuvre cette méthode sont conformes aux exigences de la norme NF EN 482 et aux critères d'évaluation, en particulier les données relatives à la plage de travail, la capacité maximale, la stabilité au stockage, les interférences et l'incertitude élargie de la méthode.

Malgré cela, la méthode n'est pas validée pour une durée d'échantillonnage de 480 minutes et un volume d'air de 480 L en raison de :

- une incertitude élargie de la méthode estimée supérieure au maximum autorisé par les exigences de la norme NF EN 482
- une évaluation non conventionnelle de l'échantillonnage et de la récupération (avec des solutions de TDI dérivées liquides dopées sur filtre) ;
- l'absence d'études environnementales.

La méthode 3 est classée en catégorie 3 et n'est pas recommandée pour le contrôle réglementaire technique de la VLEP-8h.

La méthode est validée pour un temps d'échantillonnage de 15 minutes et un échantillon d'air de 15 litres. Toutefois, en raison de l'évaluation non conventionnelle de l'échantillonnage et de la récupération (dopage avec des solutions de dérivés de TDI) et de l'absence d'étude de l'influence des conditions environnementales, la méthode est classée en catégorie 2 pour le contrôle technique réglementaire de la VLCT-15min et le suivi des expositions court terme.

Méthode 4

La méthode consiste à effectuer un échantillonnage actif à travers un filtre en PTFE suivi d'un filtre en fibres de verre imprégné ou extrait avec MAMA ou MAP ; filtre PTFE extrait avec 1,2-MPP ; analyse avec chromatographie liquide, détection ultraviolette ou fluorimétrique. La méthode est décrite dans les normes ISO 17736 (2010) et ISO 17735 (2009) ainsi que les protocoles NIOSH 5525 (2003) et IRSST 376.

Les données sur l'incertitude globale sont disponibles dans les normes ISO 17735 et 17736. Les valeurs d'incertitude élargies sont élevées, proches ou supérieures aux exigences de la norme NF EN 482 : 36% sans tenir compte de l'apport d'incertitude lié à l'efficacité de la

collecte d'après la norme ISO 17735 ou 50% pour la mesure du TDI sous forme vapeur et 90% pour la mesure du TDI sous forme aérosol selon la norme ISO 17736.

Compte tenu de ces valeurs d'incertitudes élevées, la méthode 4 est classée en catégorie 3 pour le contrôle technique réglementaire de la VLEP-8h et de la VLCT-15min ainsi que pour le suivi des expositions court terme

Méthode 5

La méthode consiste à effectuer un échantillonnage actif à travers un ou deux filtres en fibres de verre imprégné(s) de 1,2-MPP ou 1,2-PP, puis une analyse par chromatographie liquide avec détection ultraviolette ou fluorimétrique. La méthode est décrite dans plusieurs protocoles et normes similaires : IFA 7120 (2010), IFA 7670 (2004), MAK diisocyanate (2006), INRS MetroPol 245 - 246 - 249 - 250 (2003), OSHA 42 (1983) et ISO 14382 Standard (2012). Les différents protocoles utilisent différents dispositifs d'échantillonnage : échantillonneur à cassette à face ouverte, échantillonneur à cassette à face fermée ou échantillonneur GSP. Toutefois, cela ne modifie pas les données de validation attachées à cette méthode puisque l'efficacité de la collecte n'est pas étudiée. L'échantillonnage doit être effectué à l'aide d'un dispositif capable de recueillir la fraction inhalable.

Des données de validation conformes aux exigences métrologiques sont disponibles au travers des différents protocoles, notamment en ce qui concerne la plage de travail, la capacité maximale, la stabilité au stockage, l'efficacité d'échantillonnage et de la récupération, l'influence des conditions environnementales et l'incertitude élargie de la méthode. La limite de quantification ne permet pas d'atteindre le dixième de la VLCT-15min.

Pour le TDI, et plus généralement pour les isocyanates aromatiques, la littérature rapporte que l'échantillonnage par filtre n'est pas aussi efficace que l'échantillonnage par barboteur pour les raisons suivantes :

- l'isocyanate adsorbé sur les particules réagit sur la surface du filtre avec le réactif au point d'impact. Pour les particules de grande taille, le filtre est localement appauvri en réactif et l'isocyanate ne sera que partiellement dérivé. Le même phénomène s'applique lorsque l'isocyanate couvre toute la surface de la particule (Streicher et al. 2000).
- en présence de polyalcools et/ou d'agents durcisseurs dans l'atmosphère, la réaction de polymérisation avec l'isocyanate est en concurrence directe avec la réaction de dérivation. Un certain nombre d'articles mettent en évidence la différence de concentration mesurée avec un échantillonnage par filtre en laboratoire à l'aide d'un générateur d'atmosphère d'isocyanate par rapport à l'atmosphère réelle dans des ateliers de production de mousse de polyuréthane (Mattsson et al. 2008, Streicher et al. 2000, Guglya 2000). L'échantillonnage par filtre sous-estime systématiquement le niveau de TDI. L'hypothèse la plus souvent formulée est que la cinétique de la réaction de dérivation est trop faible par rapport à la cinétique de la réaction de polymérisation en phase vapeur et qu'il y a également une dégradation chimique des composés dérivés dans le temps.

Une période d'échantillonnage inférieure à 20 minutes et une désorption du filtre dans une solution contenant le réactif sur le terrain juste après l'échantillonnage, permettent de réduire considérablement et de minimiser ce biais.

La méthode n'est pas validée pour un échantillonnage long, au-delà de 20 minutes, en raison de la sous-estimation due à la dégradation chimique des composés dérivés au fil du temps.

La méthode 5 est classée en catégorie 3 pour le contrôle technique réglementaire de la VLEP-8h.

Compte tenu de ces éléments et malgré des informations limitées sur les interférences, la méthode est classée en catégorie 2 pour le suivi des expositions court terme et en catégorie 3 pour le contrôle technique réglementaire de la VLCT-15min.

Méthode 6

La méthode consiste à effectuer un échantillonnage actif par un ou plusieurs barboteurs en série contenant un réactif de dérivation (1,2-MPP, 1,2-PP ou MAP) puis une analyse par chromatographie liquide avec détection ultraviolette, fluorimétrique ou électrochimique. La méthode est décrite dans les protocoles NIOSH 5521 (1994), NIOSH 5525 (2003) et OSHA 18 (1981).

Cette méthode est classée en catégorie 3 pour le contrôle technique réglementaire de la VLEP-8h et de la VLCT-15min ainsi que pour le suivi des expositions court terme pour les raisons suivantes :

- lorsqu'un seul barboteur est utilisé, la fraction d'aérosol échantillonnée n'est pas conforme à la fraction inhalable conventionnelle : les particules de moins de 2 µm de diamètre peuvent passer à travers le barboteur ;
- La limite de quantification n'est pas suffisante pour atteindre le dixième de la VLCT-15min.

En outre, il convient d'éviter l'exposition des travailleurs par échantillonnage individuel avec un barboteur rempli d'une solution absorbante à base de toluène ou de xylène, car ce sont des agents chimiques dangereux.

Méthode 7

La méthode consiste à effectuer un échantillonnage actif au travers d'un tube « denuder » imprégné de DBA terminé par un filtre en fibre de verre de 13 mm imprégné de DBA. Les filtres sont ensuite extraits consécutivement avec de l'acide sulfurique, du méthanol et du toluène ; la phase toluène est centrifugée trois fois avant évaporation et, enfin, reprise par acétonitrile. L'analyse est réalisée par chromatographie en phase liquide couplée à une détection de masse ou de masse/masse. Cette méthode est décrite dans la norme ISO 17734-1.

La fraction prélevée à l'aide de ce dispositif est inconnue (trou d'entrée d'un diamètre de 8 mm et d'un débit de 0,2 $L.min^{-1}$). De plus de nombreuses données de validation font défaut, comme la capacité maximale du préleveur, l'efficacité d'échantillonnage et de la récupération, ainsi que les données d'incertitude de la méthode. Pour toutes ces raisons, cette méthode est classée en catégorie 3 pour le contrôle technique réglementaire de la VLEP-8h et de la VLCT-15min ainsi que pour le suivi des expositions court terme.

Conclusions et recommandations

Sept méthodes de mesure du 2,4- et 2,6-TDI dans l'air des lieux de travail ont été recensées et évaluées notamment selon les critères définis par la norme NF EN 482.

Les méthodes ont été regroupées en fonction du dispositif d'échantillonnage (barboteur, filtre, dénuder) et pour certaines d'entre elles en fonction du réactif de dérivation utilisé :

- Méthode 1 : Barboteur contenant de la 1,2-MPP dans du toluène suivi d'un filtre en fibres de verre imprégné de 1,2-MPP ;

- Méthode 2 : Barboteur contenant de la DBA en solution dans du toluène suivi d'un filtre en fibres de verre non imprégné ;
- Méthode 3 : Barboteur contenant du MAP en solution dans du benzoate de butyle suivi d'un filtre en fibres de verre imprégné de MAP ;
- Méthode 4 : Filtre PTFE associé à un filtre en fibres de verre imprégné de MAMA dans un échantillonneur de la fraction inhalable ;
- Méthode 5 : 1 ou 2 filtres en fibre de verre imprégnés de 1,2-MPP ou 1,2-PP dans un échantillonneur de la fraction inhalable
- Méthode 6 : Barboteur seul contenant un réactif de dérivation (,2-MPP, 1,2-PP ou MAP)
- Méthode 7 : Tube denuder imprégné de DBA terminé par un filtre en fibre de verre imprégné de DBA.

Pour toutes les méthodes, l'analyse est similaire : chromatographie en phase liquide avec phase normale ou inverse, UV, fluorimétrie, électrochimie, détection d'azote ou de masse.

L'échantillonnage doit être effectué à l'aide d'un dispositif capable de recueillir la fraction inhalable.

Néanmoins, même si les barboteurs ne sont pas des échantillonneurs de la fraction inhalable, les méthodes utilisant ces dispositifs combinés à un filtre en aval pour recueillir les particules < 2 µm n'ont pas été exclues parce qu'elles peuvent avoir une efficacité de collecte similaire (NIOSH 5525) ou bien surestimer la fraction recueillie.

Le choix de l'échantillonneur - filtre, barboteur ou barboteur et filtre en série - est dicté par le scénario d'exposition et dépend de l'environnement où les échantillons sont prélevés.

Considérant les données de validation fournies dans les différents protocoles :

- La méthode 1 a été classée dans la catégorie 3 pour le contrôle réglementaire de la VLEP-8h et de la VLCT-15min, ainsi que pour le suivi des expositions court terme parce que les critères essentiels de validation sont absents ou inappropriés et que l'incertitude de mesure élargie est proche du maximum autorisé par les exigences de la norme NF EN 482.
- La méthode 2 a été classée dans la catégorie 3* pour le contrôle réglementaire de la VLEP-8h et de la VLCT-15min, ainsi que pour le suivi des expositions court terme e raison de l'absence de critères essentiels de validation.
- La méthode 3 a été classée en :
    - Catégorie 3 pour le contrôle réglementaire de la VLEP-8h car l'incertitude élargie est supérieure au maximum autorisé par les exigences de la norme NF EN 482, en raison d'une évaluation non conventionnelle de certains critères et de l'absence d'études environnementales.
    - Catégorie 2 pour le contrôle technique réglementaire de la VLCT-15min et le suivi des expositions court terme, compte tenu d'une évaluation non conventionnelle de l'échantillonnage, de la récupération et de l'absence d'étude sur les interférences et l'influence des conditions environnementales. La méthode couvre la plage de concentration de 0,1 à 2*VLCT-15min soit 0,94 à 18,8 µg.m^{-3}.
- La méthode 4 a été classée en :
    - Catégorie 3 pour le contrôle réglementaire de la VLEP-8h car la méthode du filtre est uniquement adaptée pour déterminer les concentrations de TDI sous forme de vapeur et d'aérosols avec des prélèvement de courte durée (15 min)

car une dégradation chimique des composés dérivés au fil du temps peut conduire à une sous-estimation.

- Catégorie 3 pour le contrôle technique réglementaire de la VLCT-15min et pour le suivi des expositions à court terme car l'incertitude élargie de la méthode est proche ou supérieure au maximum autorisé par les exigences de la norme NF EN 482.

- La méthode 5 a été classée en :
  - Catégorie 3 pour le contrôle technique réglementaire de la VLEP-8h car la méthode du filtre est uniquement adaptée pour déterminer les concentrations de TDI sous forme de vapeur et d'aérosols avec des prélèvements de courte durée (15 min) car une dégradation chimique des composés dérivés au fil du temps peut conduire à une sous-estimation.
  - Catégorie 2 pour le suivi des expositions court terme et en catégorie 3 pour le contrôle technique réglementaire de la VLCT-15min. La méthode couvre la plage de concentration de 0,5 à 2 * VLCT-15min, soit 4,7 à 18,8 $\mu g.m^{-3}$, mais ne permet pas d'atteindre le dixième de la VLCT-15min.

Cette méthode, qui consiste à prélever des échantillons par filtration sans l'aide d'un barboteur, offre l'avantage d'être plus pratique, mais elle exige de réaliser l'extraction sur le terrain du ou des filtres avec le solvant immédiatement après le prélèvement. Les dérivés TDI générés à partir de 1,2-MPP et 1,2-PP sont stables dans le temps et insensibles à la lumière. Les étalons 2,4- et 2,6-TDI dérivés en solution sont disponibles dans le commerce pour étalonner les détecteurs. Ils produisent une réponse appropriée avec détecteur UV, fluorimètre, détecteur électrochimique ou spectromètre de masse. Certains protocoles, les normes ISO 17736 et ISO 17737, HSE MDHS 25/4 et NIOSH 5525, indiquent que, pour un échantillonnage de 15 minutes, cette méthode est la plus appropriée.

- La méthode 6 a été classée en catégorie 3 pour le contrôle technique réglementaire de la VLEP-8h et de la VLCT-15min ainsi que pour le suivi des expositions court terme car la fraction d'aérosol échantillonnée n'est pas conforme à la fraction inhalable classique et la limite de quantification n'est pas suffisante.
- La méthode 7 a été classée en catégorie 3 pour le contrôle technique réglementaire de la VLEP-8h et de la VLCT-15min ainsi que pour le suivi des expositions court terme car la fraction d'aérosol échantillonnée est inconnue et les critères essentiels de validation font défaut.

En conclusion, les experts recommandent la méthode 3 comme méthode indicative pour le contrôle technique réglementaire de la VLCT-15min et les méthodes 3 et 5 comme méthodes indicatives pour le suivi des expositions court terme.

La mise en œuvre de la méthode 3, impliquant le port individuel de barboteur en verre, peut être problématique sur le terrain compte tenu du risque d'exposition par inhalation du travailleur, du risque de casse du barboteur et de la gêne occasionnée par le port du barboteur. L'utilisation d'un micro-impinger destiné au prélèvement individuel permet de réduire ces risques.

Les experts recommandent le développement d'une méthode validée satisfaisant toutes ses exigences métrologiques pour le contrôle technique réglementaire de la VLEP-8h et de la VLCT-15min.

Tableau 2 : Méthodes recommandées pour la mesure du TDI dans l'air des lieux de travail au regard de la VLEP-8h et de la VLCT-15 min

Méthodes		Protocoles	VLEP-8h	VLCT-15min	
			Contrôle technique réglementaire	Contrôle technique réglementaire	Suivi des expositions court terme
3	Barboteur (MAP dans benzoate de butyle) suivi par un filtre en fibres de verre imprégné de MAP Analyse par HPLC/ UV / fluorescence	ISO 17735 (2009) ; NIOSH 5525 (2003)	**3** **(non recommandée)**	**2**	**2**
5	1 ou 2 filtres en fibres de verre imprégnés de 1,2-MPP ou de 1,2-PP Analyse par HPLC/UV / fluorescence	IFA 7120 (2010) ; IFA 7670 (2004) ; MAK Diisocyanate (2006) ; INRS MétroPol 245, 246, 249, 250 (2003) ; ISO 14382 ; OSHA 42 (1983)	**3** **(non recommandée)**	**3** **(non recommandée)**	**2**

Conclusions de l'expertise collective

Sur la base des données actuellement disponibles pour le TDI, les experts recommandent de fixer une VLCT-15min de 1,3 ppb (soit 9,4 µg.m^{-3}) et une VLEP-8h pragmatique de 0,04 ppb.

Les experts recommandent une mention « Peau ».

Les experts ne recommandent pas de mention « Bruit ».

Les experts recommandent d'éviter tout contact cutané.

Les experts soulignent que la VLCT-15 min et la VLEP-8h pragmatique recommandées ne protègent pas les personnes déjà « sensibilisées »

Les experts précisent que pour les sensibilisants le principe ALARA[10] (aussi bas que raisonnablement possible) doit être appliqué.

Concernant les méthodes de mesure du TDI sur les lieux de travail, aucune méthode de mesure n'est recommandée pour le contrôle technique réglementaire de la VLEP-8h. Les experts recommandent pour le contrôle technique réglementaire de la VLCT-15min la mise en œuvre de la méthode indicative consistant à effectuer un prélèvement actif au travers d'un barboteur contenant une solution de MAP dans du benzoate de butyle suivi d'un filtre en fibres de verre imprégné de MAP, puis une analyse par HPLC/UV/Fluorescence.

Les experts recommandent également de développer et valider une méthode de mesure satisfaisant toutes ses exigences métrologiques que ce soit pour le contrôle technique réglementaire de la VLEP-8h ou bien de la VLCT-15min.

[10] As Low As Reasonably Achievable

Collective expert appraisal report

Acronyms and abbreviations

1,2-MPP	1-(2-methoxyphenyl)piperazine)
1,2-PP	1-(2-pyridyl)piperazine)
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 & Safety)
BAL	bronchoalveolar lavage
BHR	bronchial hyperreactivity
BL	Bronchial lavage
CAS	Chemical Abstracts Service
CLP	Classification, Labelling and Packaging of substances and mixtures
COCT	Conseil d'Orientation sur les Conditions de Travail - French Steering Committee on Working Conditions
DAD	Diode Array Detector
DBA	Dibutylamine
DECOS	Dutch expert Committee on Occupational Safety of the health council of the Netherlands
FEV1	forced expiratory volume
DFG	Deutsche Forschungsgemeinschaft
EC	European Commission
ECD	Electrochemical detection
ECHA	European chemicals agency
EINECS	European Inventory of Existing Commercial Chemical Substances
FEV_1	Forced Expiratory Volume in 1 second
FID	Flame Ionization Detector
Fluo	Fluorimetric detection
FVC	forced vital capacity
GSA	guinea pig serum
HCSP	Haut conseil de la santé publique (High Council of Public Health)
HPLC	High pressure liquid chromatography

HRV Committee	Health reference values" Committee
HSDB	Hazardous Substances Data Bank
HSE	Health and safety executive
IAQV	Indoor air quality guide values
IgE	Immunoglobulin E antibody type
INERIS	Institut National de l'Environnement Industriel et des Risques
INRS	Institut National de Recherche et de Sécurité
ISO	International Standard Organization
IUPAC	International Union of Pure and Applied Chemistry
LC	liquid chromatography
MAMA	9-(methylaminomethyl)-anthracene
MAP	1-(9-anthracenylmethyl)piperazine
MDHS	Methods for the Determination of Hazardous Substances
MEF25%	maximal expiratory flow at 25%
MS	mass spectrometry
ND	nitrogen detection
NIOSH	National Institute for Occupational Safety and Health - USA
Nitro reagent	N-[(4-nitrophenyl) methyl] propylamine
NTP	National Toxicology Program
OEHHA	Office of Environmental Health Hazard Assessment
OEL	Occupational Exposure Limits
OELV	Occupational Exposure Limit Values
OSHA	Occupational Safety and Health Administration - USA
ppb	parts per billion
ppm	parts per million
RADS	reactive airway dysfunction syndrome
RAST	radioallergosorbent test
REACh:	Registration, Evaluation and Autorisation of CHemicals
SCOEL	Scientific Committee on Occupational Exposure Limits
sGAW	specific airway conductance
sRAW	specific airway resistance
STEL	Short Term Exposure Level

TDA	Toluene diamine
TDAh	Toluene diamine after acid hydrolysis
TDI	Toluene diisocyanate
TWA	time-weighted average
UV	Ultraviolet dectection
VLCT	Valeur limite court terme
VLEP	Valeur limite d'exposition professionnelle
VME	Valeur moyenne d'exposition
WG	Working group

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Preambule

The French system for establishing Occupational Exposure Limits OELVs 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 (OELVs) was entrusted to the agency in the framework of the French 2005-2009 Occupational Health Plan (PST).

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.

These concentration levels are determined by the HRV Committee experts based on information available from epidemiological, clinical and animal toxicology studies. Identifying concentrations that are safe for human health are the results of correction factors applied to the values identified directly by the studies. These corrections factors take into account a number of uncertainties inherent to the extrapolation process conducted as part of an assessment of the health effects of chemicals on humans.

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.

These three types of values are expressed:

- in $mg.m^{-3}$, i.e. in milligrams of chemical per cubic metre of air, or in ppm (parts per million), i.e. in cubic centimetres of chemical per cubic metre of air, for gases and vapours;

- or in $mg.m^{-3}$ only for liquid (fog) and solid (fumes) aerosols;
- or in $f.cm^{-3}$, i.e. in fibres per cubic centimetre for fibrous materials.

The 8h-OELV 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 (STELV), when one exists, is not exceeded.

In addition to the OELs, the HRV Committee assesses the need to assign a “skin” notation, when significant penetration through the skin 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 taken into account when determining the atmospheric limit levels, even it can potentially cause health effects even when the atmospheric levels are respected.

The HRV Committee 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[11]. 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);
- 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)

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

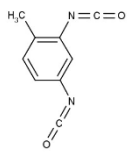
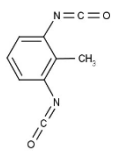
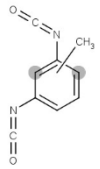
Part A – Report on assessment of health effects

1 General information

1.1 Substance identification

TDI is marketed in the form of liquid mixtures containing 2,4- and 2,6-TDI isomers. The most common mixtures are based on 80/20 and 65/35 v/v 2,4-TDI/2,6-TDI, but other mixtures are possible.

Table 3: Substance identification

Substance identification				Sources consulted
IUPAC Name	2,4-diisocyanato-1-methylbenzene	1,3-diisocyanato-2-methylbenzene	No name because it is a mixture	*INRS, 2017* *Pubchem, 2018* *(https://pubchem.ncbi.nlm.nih.gov/)*
Others names	2,4 Toluenediisocyanate Toluene 2,4 Diisocyanate, Toluene Diisocyanate, Tolylene diisocyanate 2,4-TDI	2,6-Toluene Diisocyanate, 2,6-Diisocyanatotoluene, 2-Methyl-m-phenylene diisocyanate 2,6-TDI	Generic TDI* :	
CAS Number	584-84-9	91-08-7	26471-62-5*	
EINECS Number	202-039-0	209-544-5	247-722-4	
Formula	$C_9H_6N_2O_2$			
Semi developed formula	$CH_3C_6H_3(NCO)_2$			
Developed formula				
Chemical family :	Isocyanate			

** For purposes of the Toxic Substances Control Act, this CAS number and name should be used for all mixtures of 2,4-TDI and 2,6-TDI.*

1.2 Physicochemical properties

The data reported below concern 2,4- and 2,6-TDI (pure substance), except when otherwise stated.

Table 4: Substance physicochemical properties

Properties				Sources consulted
	2,4 TDI	2,6 TDI	Generic TDI	
Appearance	Colourless to pale yellow liquid, characteristic acrid odour (normal T°C and P) Pungent and penetrating odour detectable at low concentrations (~1 to 2 ppm)			HSDB ; INRS, 2017
Molecular weight ($g.mol^{-1}$):	174,16			INRS, HSDB
Melting point (°C):	21 20.5	18.3	9.511-14	INRS HSDB
Boiling point (°C):	251 °C	250 °C	252-254°C 125°C	INRS HSDB
Vapour density (air=1)	6			HSDB, INRS
Relative density (water=1):	1.22 at 25 °C			HSDB, INRS
Vapour pressure:	2.1 Pa at 21 °C	2.78 Pa at 25°C	1.5 Pa at 20 °C	INRS
	$8.0x10^{-3}$ mm Hg at 20°C => 1,07 Pa	$2.09x10^{-2}$ mm Hg at 25 °C => 2,79 Pa	$2.30X10^{-2}$ mm Hg at 25°C => 3,1 Pa	HSDB
Solubility:	Practically insoluble in water, concentration at saturation 14 $mg.L^{-1}$. soluble in many organic solvents: acetone, benzene and chlorinated hydrocarbons			INRS, HSDB
Octanol/water partition coefficient: log Pow	3.43 (estimated, no information about T°C) 3.74 (estimated)	3.74 (estimated, no information about T°C)	3.43 (estimated, no information about T°C)	INRS, HSDB
Conversion factor at 20°C and 1023 hPa:	1 ppm = 7.24 $mg.m^{-3}$			-
Major impurities	Toluenediamine TDA TDI-tar (name that includes all the distillation residues of TDI that may constitute impurities)			Chematur engineering

HSDB: https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~mn90PT:2, https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~mn90PT:3, https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~mn90PT:4, accessed June 2018)

Chematur engineering : https://chematur.se/process-areas/polyurethane-chemicals/tditoluene-diisocyanate/, accessed June 2018

1.3 European Classification

Under the CLP regulation, TDI has a harmonised classification as described in the table below (table 5).

Table 5: Harmonized classification for Human Health for TDI (CAS 26471-62-5)

Hazard statement Code	Hazard class Code
H315	Skin Irrit. 2
H319	Eye Irrit. 2
H317	Skin Sens. 1
H330	Acute Tox. 2
H335	Stot SE 3
H334	Resp. Sens. 1
H351	Carc. 2

1.4 Majors uses and sources

TDI is used principally to make flexible polyurethane foam products, but is also used in adhesives, sealants, coatings, and elastomers (ECHA 2016).

This substance is manufactured and/or imported in the European Economic Area in 100 000 - 1 000 000 tonnes per year.

This substance is used in articles, by professional workers (widespread uses), in formulation or re-packing, at industrial sites and in manufacturing.

The predominant use of isocyanates (>90 %) is in the direct manufacture of polyurethane plastic materials, where diisocyanates react with polyols and/or other nucleophiles like polyamines.

Typical isocyanate based products include:

- Flexible polyurethanes
- Rigid polyurethanes
- Polyurethane foams (rigid and flexible foam systems)
- Polyurethane fibres
- Assembly foams (e.g. insulation panels)
- Foundry cores (casting)
- Coating materials (paints, lacquers, varnishes)
- Adhesives and glues
- Elastomers
- Sealants
- Pre-polymers in chemical synthesis
- Engineering plastics
- Polyurethane fibres

Occupational exposure to TDI mainly occurs *via* the respiratory route, in gaseous form and/or associated with particles. Although the quantity of the 2,4 TDI isomer predominates in the mixtures on the market, studies in the workplace (Saunders and Frisch 1962 quoted by Prueitt, et al. 2013) or studies in volunteers (Skarping et al. 1991; Tinnerberg et al. 1997), show that the levels of atmospheric exposure to 2,6 TDI are higher than those for 2,4 TDI.

2 Overview of recent recommended occupational limit values

There are no scientific recommendations for TDI from the Scientific Committee on Occupational Exposure Limits (SCOEL) available to date.

The American Conference of Governmental Industrial Hygienists (ACGIH) has adopted a 8-hour threshold value – time weighed average (TLV-TWA) of 1 ppb and a 15-min short-term exposure limit (STEL) of 5 ppb for Toluene-2,4 or 2,6-diisocyanate mixture (TDI) (ACGIH 2016).

The Office of Environmental Health Hazard Assessment (OEHHA) calculated an 8-hour reference exposure level (8-hour REL) of 0.002 ppb based on an accelerated decline in FEV_1 (forced expiratory volume in 1 second) in the absence of TDI-induced asthma based on Diem et al. (1982) study (OEHHA 2016).

Recently, Daniels et al.[12] (affiliate to the National Institute for Occupational Safety and Health, NIOSH), calculated an OEL of 0.3 ppb (corresponding to a working lifetime extra risk of 1/1000 of occupational asthma) based on data on eight TDI-exposed populations considered as suitable for the analysis by the authors, (these studies and their limitations are presented in annex 1, nine studies were selected, one was removed (Daftarian et al. 2000). This value was determined from dose response modelling between occupational asthma and exposure described in the eight TDI-exposed populations (Daniels et al. 2018).

In November 2018, the Dutch expert Committee on Occupational Safety (DECOS) of the Health Council of the Netherlands published a report, which recommended a health-based occupational reference value of 0.1 µg NCO/m^3 (which corresponds to 0.04 ppb for TDI), as an 8 hour TWA for di and triisocyanates. It concluded that, at this concentration, workers have an additional risk of 1% for developing bronchial hyperreactivity (BHR) compared to the background risk in the general population. Indeed, for inhaled allergens, the DECOS considered that it is generally not possible to derive a concentration below which no sensitisation occurs. Moreover, the report deals with isocyanates with 2 or 3 NCO-groups, so the isocyanate concentration in air is expressed as NCO per m^3. Based on two studies (Pronk et al. 2007 and Collins et al. 2017), two exposure-response relationships were derived, for both studies separately. In Pronk et al. (2007) study, workers are exposed to HDI and the DECOS calculated an exposure concentration of 0.1 µg NCO/m^3 corresponding to a 1% extra risk of BHR. In Collins et al. 2017 study workers are exposed to TDI, from these data the committee calculated a concentration of 0.14 µg NCO/m^3 for an extra risk of adverse effect i.e. complaints consistent with occupational asthma. This value is based on short term exposures, but will also limit potential effects due to chronic exposure (Health Council of the Netherlands 2018).

[12] Although the findings and conclusions in this report are those of the author and do not necessarily represent the views of the National Institute for Occupational Safety and Health

3 Toxicokinetics and metabolism

3.1 Introduction

All data in the literature suggest that regardless of the route of administration, TDI in unchanged form is not absorbed or only poorly absorbed at the systemic level. Three mechanisms may limit the quantity of TDI absorbed and distributed in the body:

- rapid reaction in atmosphere and with the compounds of endogenous fluids or tissue with which it is initially in contact in situ polymerisation,
- after oral administration of TDI in animal studies, physicochemical properties of the substance led to the hydrolysis to TDA (toluene diamine, a metabolite of TDI) or formation of polyurea in the stomach.

Several arguments support these assertions (DFG 2003, Bonnard et al. 2006):

- the high reactivity of the isocyanate functional groups, able to react with all compounds with a free hydrogen atom, including water molecules. The reactivity of the isocyanate functional groups is, in decreasing order, amine ≥ thiols > urea > water > alcohols > carboxylic acid;
- the absence of unchanged TDI in the blood, tissues or excreta, in humans as well as in laboratory animals, regardless of the route of exposure. Only metabolites of TDI (TDA and its acetyl derivatives) or adducts of TDI or TDA with endogenous macromolecules such as albumin or haemoglobin, have been identified;
- the presence of TDI polymers in the stomach and TDA (in its free form or mono and diacetyl derivatives) in the urine of rats after gavage with TDI.
- The decrease of the quantity of the 2,4 TDI isomer from the liquid mixtures on the market (containing generally 80 % of 2,4-TDI and 20 % of 2,6-TDI) to the mixture found in the workplace atmospheres.

The nature of the metabolites or adducts of TDI, their rate of absorption and their elimination, depend on:

- the form in which the TDI is administered (liquid, aerosol, gas) and the isomer (2,4- or 2,6-TDI);
- the composition of the medium with which TDI is placed in contact (gastric fluid by the oral route, bronchoalveolar mucus by inhalation, stratum corneum and the dermis by the dermal route).

TDI or TDA can form adducts by binding covalently to endogenous molecules. Severe acid hydrolysis (6M HCl or 3M H2SO4, 100°C for one night) releases all of the adducts formed with TDA and TDI in the form of TDA (TDA_h[13]). In contrast, mild alkaline hydrolysis releases only the adducts of TDA (Bonnard et al. 2006).

[13] TDA after acid hydrolysis

3.2 Absorption

3.2.1 By inhalation

In humans:

Occupational exposure to TDI mainly occurs *via* the respiratory route, in gaseous form and/or associated with particles. Human studies (volunteers and workers) showed that absorption of TDI after inhalation exposure was evident, given the occurrence of metabolites in the urine. In a study with 5 volunteers exposed to 40 µg/m^3 of TDI (30:70 mixture of the 2,4- and 2,6-isomer) for 7.5 hours in a test chamber, inhaled doses of approximately 120 µg were estimated, which resulted in TDI plasma levels of 2.2 µg/L at 8 and 2.4 µg/L at 24 hours (Saunders and Frisch 1962 quoted by Prueitt et al. 2013, Skarping et al. 1991; Tinnerberg et al. 1997).

In TDI-based polyurethane foam production (80:20 mixture of the 2,4- and 2,6-isomer), nine workers who did not wear personal protective equipment were exposed to levels ranged from 9.5 to 94 µg/m^3 (proportion of 2,6-TDI isomer ranged between 44 and 87%). The urinary TDA concentrations varied from 6.5 to 31.7 µg/g creatinine and they were linearly related to the air concentration. Approximatively, 20% of TDI is metabolized to urinary diamines (Maître et al. 1993).

In animals:

Timchalk et al. performed animal experiments with [14C] radiolabelled molecules to determine the intensity of lung retention, and the distribution and elimination of TDI and its metabolites and adducts: male F344 rats exposed to 14C 2,4-TDI vapor (2 ppm) via inhalation for 4 hours excreted most of the radioactivity in the feces (47%); approximately 15% was excreted in urine, while no radioactivity was detected in exhaled air (Timchalk et al. 1994).

3.2.2 By dermal route

In humans:

There are few data in humans, but systemic absorption cannot be ruled out.

To assess dermal absorption's contribution to the total exposure of workers to TDI, Austin (2007) measured the quantity of urinary toluene diamine (uTDA, creatinine-adjusted) in workers (n = 13) and in a control group (n = 13) (Austin 2007). The 13 workers had physical contact with non-polymerized polyurethane foam during their work shift (handlers). The control group was made up of workers from a similar factory block environment to the handlers, but without any physical contact with the non-polymerized foam on the day of sampling (non-handlers). The company used an 80:20 mixture of 2,4 and 2,6 TDI.

The results of this study show higher quantities of uTDA in the skin-exposed group than in the control group. In 10 of the exposed workers, the uTDA values in the end-of-shift urine were above the limits of detection. The results in Table 6 show that the two groups were exposed to

similar ambient concentrations of TDI, suggesting the possibility of dermal absorption among the "handler" workers to explain the difference in uTDA values between the two groups (provided it can be assumed that the breathing rates of both groups were similar). However, no clear relationship was observed between the levels of exposure to TDI in the ambient air and the quantities of uTDA in the end-of-shift urine (Austin 2007).

Table 6: TDI and TDA levels in Handlers and non-handlers (Austin 2007)

	Handlers	**Non-handlers**
N	13	13
Mean TDI	2.7 μg/m^3 NCO	2.6 μg/m^3 NCO
Range	< 3.5-8.4 μg/m^3 NCO	< 3.5-8.4 μg/m^3 NCO
uTDA detected before shift	4/13	0/13
uTDA detected after shift	10/13	2/13
Mean uTDA after shift	2.21 μmol/mol creatinine	0.11 μmol/mol creatinine

In post- shift urine sample, 10 handlers were found to have urinary TDA above detection limits with a mean level of 2.21 µmol/mol creatinine, compared to only 2 non-handlers (0.11 µmol/mol creatinine, calculated mean by the author from only two values mean).

Maitre et al. studied the biological monitoring of occupational exposure on 9 male workers exposed to TDI (80:20 2,4/2,6-TDI). The authors confirmed that 2,4-TDI is predominant in the air at the start of the polymerization process and 2,6-TDI is the major isomer at the end of the process (due to the greater reactivity of 2,4-TDI in the polymerization reaction). They reported the relative proportion of 2,6-TDI in air and 2,6-TDA in urine. They concluded that the comparison between the isomeric ratio in air (TDI) and in urine (TDA) could be a relevant indicator of the cutaneous contact (Maître et al. 1993).

<u>In animals:</u>

Four *in vivo* studies on the percutaneous passage of TDI are reported (Rosenberg and Savolainen 1985; Yeh, Lin et al. 2008; Hoffmann, Leibold et al. 2010; Nayak, Hettick et al. 2014).

Table 7: Summary of the available data on percutaneous passage in animals

Method	Dose	Duration of exposure	Penetrated	Absorbed	Comments	Reference
In vivo **rats**	2,4 TDI at 40% in n butyl ether	3h/day for 4 days			Only 18h collected urine No unchanged or free TDA in urine TDA_h 1.5 µg/mL	Rosenberg et Savolainen1985
In vivo **rats**	2,4/2,6 TDI (80:20) 1.5 mL at 0.2%; 1%; 5% in Olive oil	6 days		0.2% of the dose of TDA_h in urine	Urinary T1/2 20-23 h TDA_h the excretion of hydrolysed TDA in urine amounted to 0.2% of the applied dose. it does not reflect the absorbed dose but an estimation	Yeh et al. 2008
In vivo **rats**	[^{14}C] 2,4 TDI 12 mg/cm^2 pure	30 min - 8h	21% 35%	0.27% > 0.9% (13 µg/cm^2/h)	1/3 of the absorbed dose was retrieved in urine	Hoffman et al. 2010
In vivo **mice**	2,4 TDI at 0.1 and 4% in acetone	3h to 15 days	Location of TDI adducts with proteins in the: Stratum corneum hair follicles, sebaceous glands and Co-location with dendritic and macrophage markers			Nayak et al. 2014

3.2.3 By oral route

No studies seem to have reported on the oral absorption of TDI in humans, and only three animal studies are available in the literature (IPCS 1987, Stoltz et al. 1988 and Timchalk et al. 1994).

These animal studies show that TDI, in the form of 2,4-TDI or 2,6-TDI, is not well absorbed into the systemic circulation in rats. The higher the concentration is, the more TDI is polymerized in the stomach and the more TDI tends to remain in the gastrointestinal tract (GI), the less it is absorbed into the systemic circulation. Its absorption rate has been estimated to be between 12 and 20% when ingested by rats.

3.3 Distribution

3.3.1 By inhalation

The only data available relate to the distribution of radioactivity in some tissues of rats exposed to radiolabelled TDI. After 4 hours of exposure to 0.026 ppm, 0.143 ppm or 0.821 ppm of 2,4 [^{14}C] TDI, all rats tissues examined showed detectable quantities of radioactivity, with the airways, gastrointestinal system and blood having the highest levels which increased with exposure concentration. The concentration of radioactivity in the bloodstream after exposure was linear with respect to the dose (Kennedy et al.1994). However, the concentrations of radioactivity in these tissues did not increase proportionally with the atmospheric concentration of TDI. Similarly, the estimated fraction of the inhaled dose present in the blood of rats exposed to 0.6 ppm (1% of the dose) was twice as high as for rats exposed to 2 ppm for 4 h (0.5% of the dose) (Stoltz et al. 1987) cited by (ECB 2000). In rats exposed, via a head-only chamber, to 2 ppm of 2,4 [^{14}C] TDI for 4 h, nearly 10% of the total radioactivity was present in the skin, and only 0.02% in fats (Timchalket al. 1994).

3.3.2 By dermal route

Few information are available on the kinetics of TDI absorbed by the dermal route. The study by Hoffmann et al. in rats demonstrated low dermal absorption (<1% applied dose) and relatively slow, with a significant amount of the applied dose remaining at or near the application site (Hoffmann et al. 2010)

3.3.3 By oral route

There are no data available in humans.

In the rat, exposed by gavage to 6 or 60 mg/kg, radioactivity was detectable in the blood rapidly, 1% and 0.5% were recovered at 1-2 hours respectively for the low dose and the high dose (Stoltz et al. 1988). Another study also showed that the bioavailability of TDI increased when the administered dose was reduced from 700 to 70 and 7 mg / kg body weight. The authors explained this reduction in bioavailability by partial polymerization of TDI in the stomach at high doses (Timchalk et al. 1994).

3.4 Metabolism

There are no data available in humans.

TDI is characterized by the -N=C=O group, which contains two double bonds and exhibits strong chemical reactivity (Raulf-Heimsoth and Baur 1998). Based on experiments in rats exposed to 2,4-TDI by inhalation, oral or iv routes, a metabolic scheme is proposed in Figure 4 (OEHHA 2016).

As with other isocyanates, TDI can readily react with hydroxyl, sulfhydryl and amine groups on macromolecules found in airway epithelial cells, serum and skin, including hemoglobin, glutathione, laminin, albumin, keratin and tubulin (Brown and Burkert 2002; Bello et al. 2004). In the gut, hydrolysis of TDI generates TDA, which is classified as carcinogenic 1B in the CLP[14] regulation. Free TDA may be absorbed and be further metabolized, or may react with TDI to form polyurea polymers that are poorly absorbed and thus eliminated in the feces.

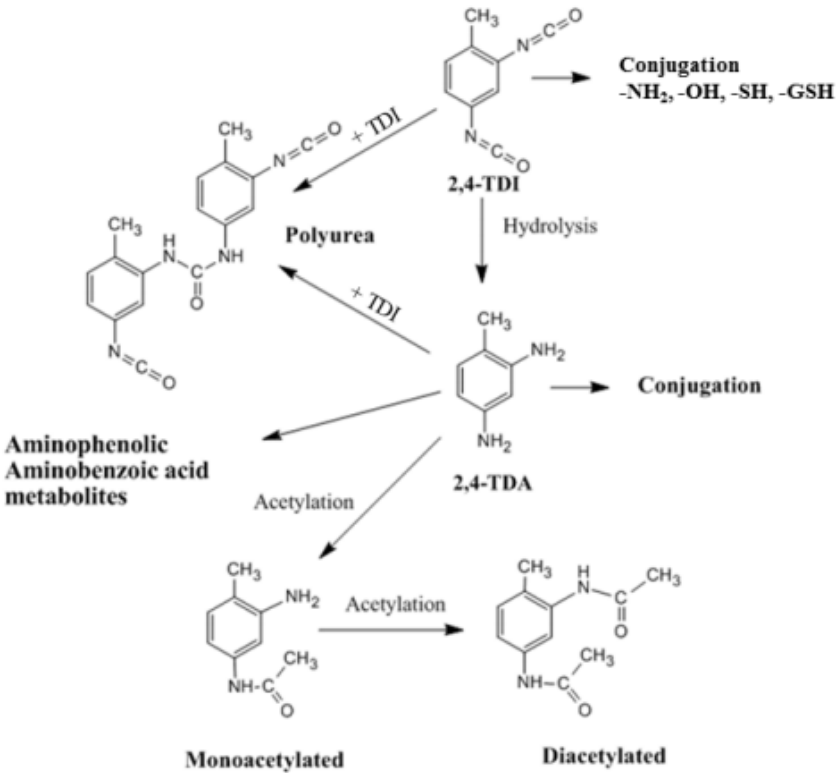


Figure 4: Metabolic Scheme for 2,4-TDI in rat (OEHHA 2016)

3.4.1 By inhalation

The study by Timchalk et al. in rats shows that few (10%) 2,4-TDA is formed after inhalation of 2,4-[^{14}C]TDI as vapors. The majority (90%) of the quantified metabolites were in the form of

[14] Classification, Labelling and Packaging - Regulation (EU) No 1297/2014

acid-labile TDI / TDA conjugates and only 10% were in the form of acetylated TDAs Timchalk et al. (1994).

3.4.2 By dermal route

After application of a solution containing 40% 2,4-TDI in dibutyl ether on rat skin 3 hours / day for 4 consecutive days, no free 2,4-TDA or free 2,4-TDI were detected in the urine 18 h after the last exposure. The average concentration of 2,4-TDA in hydrolysed urine was 1.5 µg/ml (Rosenberg and Savolainen 1985). These results suggest that only a conjugated form is present in the urine. As shown by Hoffmann et al., the authors found radioactivity in the urine but they cannot identify the metabolites. Thus, 0.5 h after the application of a 330 mg/ kg dose of [^{14}C]-TDI in rats, 0.01% of the applied dose was found in the urine, 0.02% after 1 hour of exposure and 0.33% after 8 hours of exposure. No radioactivity was detected in the faeces for the 3 exposure periods (Hoffmann et al. 2010).

3.4.3 By oral route

The formation of metabolites of TDI once absorbed in the body appears highly dependent on physiological conditions, and therefore the route of administration. Thus, at pH 7, TDI will bind very easily with the proteins while at acidic pH, TDI will bind poorly with a protein but will hydrolyze to form TDA or derived polymers urea (Doe et Hoffmann 1995).

3.5 Excretion

3.5.1 By inhalation

In rats, the elimination of body radioactivity is slow. Forty-eight hours after exposure to 2 ppm of 2,4 [^{14}C] TDI, 34% of the total radioactivity is still present in the tissues and carcass of the exposed rats. During the 48h following the end of exposure, the fraction of residual radioactivity decreases at a variable rate, depending on the tissue with, in decreasing order: lung (-86%) > liver (-78%) > kidney (-64%) > skin (-43%) (ECB 2000).

Table 8 summarises the plasma and urine elimination of radioactivity or TDAh (TDAh = TDA after acid hydrolysis) following inhalation exposure of animals or volunteers/workers

Table 8: Plasma and urine elimination of [^{14}C] or TDA_h (h, TDA after acid hydrolysis) following exposure by inhalation

Species	Product	Dose /duration	Urine Percentage of [^{14}C]//TDA_h Retrieved after inhalation	Urine $T_{1/2}$ [^{14}C]/TDA_h after hydrolysis	Plasma $T_{1/2}$ [^{14}C]/TDA_h after hydrolysis	Reference
Rat	^{14}C 2,4/2,6 TDI	0.6 ppm /4h/96h	24% 20%			Stoltz et al.1988
Guinea pig	^{14}C 2,4 TDI	0.13 ppm 1h/15 days			About 11d	Kennedy et al.1989
Rat	^{14}C 2,4/2,6 TDI	2 ppm 4h/48h	15%	20h		Timchalk et al.1994
Volunteers N=2	2,4/2,6 TDI (30:70)	10 ppb 4h/3 weeks	13-23%		$T_{1/2}$ fast 2-3 h $T_{1/2\ slow}$ = 6d	Brorson et al.1991
Volunteers N=5	2,4/2,6 TDI (48:52)	5.6 ppb 7.5h/28h	8-18 %	$T_{1/2}$ fast <2h $T_{1/2\ slow}$ =5h		Skarping et al.1991
Volunteers N=2	Workshop	0.14-1.4 ppb 1d/3-8d		5-8h		Lind et al.1996,1997
Workers N=6	Workshop	0.14-1.4 ppb			$T_{1/2}$ fast	Lind et al.1997

		1d/3-4 weeks			=6-12 days $T_{1/2\ slow}$ 19 days	
Workers N=10	Workshop	0.01-17 ppb 1d/4-5 weeks			21 days	Lind et al.1996,1997

3.5.2 By dermal route

No information is available in the literature for this route of exposure.

3.5.3 By oral route

Following ingestion of radiolabelled TDI in rats, there is mainly the formation of TDI-polyureas, which are then excreted by the feces (about 80% of the ingested dose, but percentage depending on the concentration), and a low formation of free TDA, acetylated or conjugated which are excreted in the urine.

3.6 Conclusion on toxicokinetics

TDI reacts intensely with compounds present in the atmosphere and with the structures of the uptake routes (digestive tract, lung, skin). After oral administration of TDI, physicochemical properties of the substance led to the hydrolysis to TDA or formation of polyurea in the stomach. It is the TDA which is subsequently absorbed and metabolized. This does not happen by inhalation. The comparison between the isomeric ratio in air (TDI) and in urine (TDA) could be a relevant indicator of the cutaneous contact.

It forms polymers or conjugates with all molecules presenting labile hydrogen atoms.

In the blood, TDI is present mainly in the form of adducts with albumin and, to a lesser extent, globin.

After acid hydrolysis, the adducts present in biological media are released in the form of TDAh.

- The plasma concentration in TDAh
    - is proportional to the amount inhaled
    - decreases slowly with a half-life of 10 to 20 days
    - reflects long-term exposure
- The urinary concentration in TDAh
    - decreases rapidly (in a few hours)
    - reflects recent exposure

4 Toxicity data

Comprehensive information about the toxicity of TDI is available in various reviews or agency documents (DFG 2003, US EPA 2012, ATSDR 2015, US EPA, ECHA 2016, OEHHA 2016, Health Council of the Netherlands 2018). Based on these reviews, in this report, only studies that were considered useful to be used for the derivation of OEL have been included. Only toxicity endpoints relevant for the derivation of an OEL for TDI (asthma, irritation, respiratory and skin sensitisation, lung irritation and carcinogenicity) are considered.

Only the studies considered adequate by the experts (e.g. exploitable for the derivation of an OEL) were described in this part. Some of these studies are listed in annex 1.

Animal data were not described when it was judged that human data were sufficient to describe the effects.

According to the harmonised classification and labelling (CLP) approved by the European Union, TDI is fatal if inhaled, causes serious eye irritation, is suspected of causing cancer, is harmful to aquatic life with long lasting effects, causes skin irritation, may cause an allergic skin reaction, may cause allergy or asthma symptoms or breathing difficulties if inhaled and may cause respiratory irritation, and is suspected of causing cancer.

The toxicity of TDI in humans is mainly based on relatively old exposure studies in human volunteers, case studies, and epidemiological studies. As reported by OEHHA (2016), exposure to TDI in the workplace is mainly by aerosols, given its high boiling point and its low volatility at room temperature. Aerosols are mainly generated during the use and / or handling of commercial products. They are particularly present when the products are sprayed during industrial processes. TDI is one of the main agents responsible for occupational asthma (5 to 15% of occupational chemical asthmas) (OEHHA 2016).

In a study on isocyanate worker exposures (Bello et al. 2004), the authors summarize current knowledge of diisocyanate exposure and its effects on health. They report sensitisation and asthma, but also mention that the influence of exposure characteristics on consequences of health, such as duration, peaks or mean exposures, chemical composition and route of exposure, are not yet clearly defined. Based on experimental animal data, it appears that dermal exposure is the cause of isocyanate sensitisation in animals. These findings may explain the role of dermal exposure in triggering sensitisation in humans.

4.1 Acute toxicity

Although many experimental data are available on the acute toxicity of TDI, the studies are old. Most of them predate the application of good laboratory practices, and the protocols and methodologies used do not meet current standards (Collins 2002). However, the human data relating in particular to the irritant and asthmogenic properties of TDI are unequivocal.

The inhalation of low concentrations of isocyanates may cause headaches and digestive disorders such as nausea and vomiting, as well as irritation to the eyes, nose and throat (DFG 2003).

At high concentrations, short-term exposure can cause broncho-pulmonary symptoms, including coughing, a feeling of suffocation, dyspnoea as well as asthma. For some isocyanates such as TDI,

the respiratory symptoms can be delayed until 4 to 8 hours after exposure and may persist for 3 to 7 days. The inhalation of high concentrations of diisocyanates causes immediate lesions of the respiratory tract and lungs, mainly by inducing inflammatory reactions of the mucous membranes *via* the activation of macrophagic cells and eosinophils (Nakashima et al. 2002). In annex 2, studies on respiratory tract effects (human volunteers) are detailed.

Early studies exposing healthy volunteers to TDI were carried out during the 1950s/60s. They mainly revealed irritation of the respiratory tract (DFG 2003[15]).

There are also many reports of poisoning, relating in particular to cases of bronchial asthma following occupational exposure with TDI. In 1960, counting only those cases reported in the American literature, a total of 222 cases of TDI poisoning were identified, 54 of which were considered to be severe (Elkins et al. 1962). After 1960, the number of cases reported fall down considering the prevention measures put in place where workers were exposed or suspected to be exposed to TDI (Lareng et al. 1972). The reported cases are accidents in the workplace, and one case of collective poisoning of firefighters responding without respiratory protection to a fire affecting a polyurethane factory in which two tanks of TDI were damaged (Axford et al. 1976). Many symptoms are described, and may relate to exposure by different routes, especially dermal and respiratory routes. The causal link with TDI exposure is sometimes difficult to establish, because of co-exposures in the workplace (e.g. co-exposure to chemical product in the firefighters). Accidental exposure to TDI can cause acute respiratory distress syndrome or even induce a chronic obstructive bronchial disease such as asthma, liable to persist even at a great distance from any exposure (Moller et al. 1986). No dose-effect relationship is currently available (DFG 2003).

4.2 Irritation

4.2.1 Respiratory tract irritation

Studies by Henschler et al. in human volunteers reported that acute exposures to TDI resulted in mild respiratory tract irritation at 50 ppb TDI, but not at 20 ppb (Henschler et al. 1962). Baur (1985) reported mild irritation in healthy subjects and more severe respiratory symptoms, cough and chest tightness, in 4 of 15 asthmatics exposed to 10 ppb during one hour followed by a 45 min break, then a one hour exposure to 20 ppb (Baur 1985 described by OEHHA 2016). Other studies detected no evidence of irritation, inflammatory response, or changes in forced expiratory volume (FEV_1) in subjects, BHR, in non-isocyanate asthmatics, or in healthy controls exposed to TDI (18 or 20 ppb for respectively 30 min and 15 or 20 min, respectively) (Chester et al. 1979; Fabbri et al. 1987; Moller et al. 1986).

Vandenplas et al. exposed 17 subjects (eight smokers and nine non-smokers) without occupational exposure to isocyanates and without respiratory symptoms suggestive of asthma and chronic bronchitis, once to ambient air and once to TDI (5 ppb for 6 hrs followed by 20 ppb TDI for 20 min,

[15] Several studies are mentioned: Baader and Holstein 1955, Dodson 1966, Elkins et al. 1962, Fuchs and Valade 1951, Ganz and Mager 1954, Hama 1957, Jennings and Gower 1963, Johnstone 1957, Kessler 1960, Lane cited by Munn (1965), Munn 1965, Reinl 1953, Sands *et al.* 1957, Schur 1959, Schürmann 1955, Silver 1963, Skonieczny 1963, Swensson et al. 1955, Trenchard and Harris 1963, Walworth and Virchow 1959, Woodbury 1957. The references of these articles are available in the DFG report (2003).

four weeks later) (Vandenplas et al. 1999). Exposure to TDI did not result in respiratory symptoms or in decrease of FEV_1. However, slight, but statistically significant, decreases in specific airway conductance (sGAW), parameter which reflects the conductance of the respiratory tract independently of the respiratory volumes. This finding therefore suggests an association between TDI exposure and an effect on the airways. A decrease of MEF25% (Maximal expiratory flow at 25%) of FVC (forced vital capacity) was observed. This last parameter would reflect the flow of the most distal airways but its reliability is questionable. These observations suggest an association between TDI and the development of an effect on both small and large airways. The authors did not observe an increase in the concentrations of potential indicators of epithelial cell dysfunction (secretory component and CC16[16]) and pro-inflammatory cytokines (TNFα[17], IL-4, IL-5, IL-6, and IL-8) in BAL, however a slight increase in BAL (bronchoalveolar lavage) albumin level measured in exposed subjects (26.4+/-12.5 mg/L after TDI exposure compared to non-TDI exposure, 21.8+/-8.6 mg/L, p=0.044). This suggests a loss of the integrity of the alveolo-capillary barrier.

In conclusion, short-term exposure to TDI levels near the French regulatory value (i.e. 20 ppb for 5 min) can cause detectable, although minimal, changes in airway calibre and epithelial permeability (assessment of methodology for measuring atmospheric levels for the Vandenplas study is presented in annex 3).

Persons diagnosed with non-TDI related asthma or BHR have been found to be more sensitive to inhalation exposures to TDI, experiencing symptoms and changes in specific airway resistance at TDI concentrations of 10–20 ppb. It has been noted that persons diagnosed with TDI-induced asthma can be more sensitive, reacting to lower concentrations of TDI (O'Brien et al. 1979 a, b). Another study (Chester et al. 1979) did not find alterations in specific airway resistance (sRAW) in healthy or asthmatic (not TDI-induced) subjects exposed to 20 ppb TDI for 20 minutes.

4.2.2 Skin and eye irritation

After skin contact (with variable duration), isocyanates can cause severe skin irritation, second-degree burns and dermatitis. Similarly, severe irritation of the eyes (conjunctivitis) with possible secretions may occur in the event of contact with the eyes.

Cases of dermatitis caused by skin contact with TDI have been reported (Rothe, 1976). An atypical keratopathy was observed among the workers of a polyurethane foam factory. It was not attributed to TDI but to the curing agents derived from aliphatic amines, which can produce these types of lesions (Potts et al. 1986).

Two studies have reported skin effects associated with occupational exposures to TDI (Daftarian et al. 2002 and Huang et al. 1991).

[16] Clara Cell specific protein
[17] Tumor Necrosis Factor

Table 9: Summary of Controlled Acute TDI Exposure Studies in Naïve Subjects from OEHHA (2016)

Study	TDI exposure conditions	Pulmonary/sensory findings
Henschler et al. 1962	6 subjects, 1 exposure/day 30 min exposure to: 10, 20, 50, 75, 100, 500 and 10 min exposure to 1300 ppb	No symptoms at 20 or 20 ppb; increasing sensory irritation with increasing TDI concentration starting at 50 ppb and above.
Vogelmeier et al. 1991 ; Baur et al. 1994	10 normal subjects, 20 ppb for 2 h 15 asthmatics, 10 ppb for 1h, 45 min break, then 20 ppb for 1h	Normals: No significant pulmonary decrement; 3 complained of eye irritation and/or cough Asthmatics: 1/15 had ≥100% increase in Raw at 10 ppb; 1/13 had ≥100% increase in Raw at 20 ppb; overall, 5 complained of chest tightness, rhinitis, cough, dyspnea, throat irritation, and/or headache
Fruhmann et al. 1997	15 normal subjects, 20 ppb for 2h 15 asthmatics, 10 ppb for 1h, 45 min break, then 20 ppb for 1h	Normals: No significant inscrease in Raw Asthmatics: 3/15 had ≥100% increase in Raw; one-third of subjects experienced significant, but unspecified, changes or complaints
Chester et al. 1979	10 normal subjects and 10 asthmatics 20 ppb for 1h	No increase in SRaw greater than 50% in any subject
Fabbri et al. 1987	10 normal subjects 18 ppb for 30 min	No change in FEV_1 or airway responsiveness to methacholine
Moller et al. 1986	10 subjects with positive methacholine challenge test, up to 20 ppb for 15 min	No change in FEV_1 observed with methacholine challenge after TDI exposure
Mapp et al. 1986	8 asthmatics subjects 18 ppb for 30 min	No decrease in FEV_1 ≥20% observed; No decrease in the PD20 FEV_1 greather than 2-fold with methacholine challenge
Vandenplas et al. 1999	17 normal subjects 5 ppb for 6h followed by 20 ppb for 20 min, with pulmonary function test every h	Decreased sGaw(p=0.053) and $MEF_{25\%}$ (p=0.015) measured by regression analysis of repeated measures; increased BAL albumin level (p=0.044) and BL macroglobulin (p=0.044[18]) concentration
Raulf-Heimsoth et al. 2013	10 non-exposed subjects with bronchial hyperresponsiveness 5, 10, 20 and 30 ppb for 30 min each	No FEV_1 decrease ≥20% observed; No increase in eosinophils and soluble inflammatory biomarkers in nasal lavage and induced sputum

[18] In the Vandenplas study, p = 0.021

Concerning eye irritation, studies on human volunteers are detailed in annex 2.

4.3 Sensitisation

4.3.1 Respiratory sensitisation

TDI can cause a specific hypersensitivity of the tracheobronchial tree, also known as "isocyanate asthma". Specific IgE-dependent mediation mechanisms as well as non-specific mechanisms could be responsible for these reactions. In some cases, the presence of circulating antibodies against TDI in sensitised individuals confirms the allergic origin of the bronchial asthma (Taylor 1970). The positivity of the specific tests is not, however, the rule. There is thus a weak correlation between the clinical symptoms and the results of IgE assays specific to the conjugates of TDI by RAST (radioallergosorbent test) (Baur et al. 1994, Diller et al. 1980). The weak correlation could also be explained by technical difficulties in measuring TDI-specific IgE. In addition, IgE dependent immunological mechanisms may not appear to be involved in all cases of isocyanate asthma.

TDI asthma is usually accompanied by a specific and non-specific bronchial hyperreactivity detected in particular by the methacholine test. Isolated cases of hypersensitivity have been described after a single exposure to high doses of TDI, constituting a reactive airway dysfunction syndrome (RADS). Similarly, cases of extrinsic allergic alveolitis, a rare form of interstitial pneumonitis consecutive to respiratory sensitisation, have been reported following exposure to diisocyanates (DFG 2003).

The triggering of bronchial sensitisation after skin contact has also been mentioned (DFG 2003). In volunteers sensitised to TDI, the main factor determining the triggering of an asthmatic reaction was not the concentration (C) or duration of exposure (T), but the total dose administered (C*T).

The induction of respiratory sensitisation was evaluated by exposing Guinea pigs to TDI vapor at concentrations of 20 ppb for 70 days or 120–7600 ppb for a week, followed by an inhalation challenge with TDI–guinea pig serum albumin conjugates (TDI–GSA) (Karol 1983). Respiratory responses were evaluated by measuring increases in respiratory rate and antibody production after challenge with 1% TDI–GSA. Based on antibody production, the authors reported no effects at 120 ppb but shown pulmonary sensitivity at 360 ppb or greater. Using a mouse model, Matheson et al. reported respiratory tract responses following the daily inhalation of 20 ppb TDI for 6 weeks. After a two week non-exposure period, animals were challenged *via* inhalation to 20 ppb TDI for 1 h. TDI-treated mice demonstrated enhanced airway inflammation and some hyperreactivity to methacholine (PENH), elevated IgE and IgG antibody levels, and increased Th1/Th2 cytokine expression in lung tissue. These responses support a LOEL for induction of 20 ppb in this mouse model (Matheson et al. 2005).

Pauluhn developed a respiratory sensitisation /elicitation protocol in Brown Norway rats to determine a threshold dose of TDI for elicitation of asthma-like responses in sensitised and re-challenged rats. The data presented as total dose (ppm × min) suggests that both the priming response and the elicitation response are linked to irritation/inflammation of the lung airway tissue Pauluhn 2014).

4.3.2 Skin sensitisation

Among the population of patients suffering from dermatitis as a result of exposure to isocyanates, the rate of positive results from the TDI patch test is low. In relation to the extent of professional use of TDI, the probability of developing an allergic skin reaction as a result of repeated contact with TDI therefore seems relatively low. According Daftarian et al., it is unclear if the skin effects are attributable to primary irritation or sensitisation, the authors suggest that irritant dermatitis is more common than allergic contact dermatitis (Daftarian et al. 2002).

4.4 Chronic toxicity on respiratory function

This chapter contains a review of toxicological information for TDI relevant to workplace exposures. Existing reviews from national or international authorities who adequately reviewed vast amounts of literature, were consulted for the bulk of the health information. However critical original papers and reports identified in the reviews have been sourced and consulted. An updated literature (including the key word OEL, limit value, exposure levels, occupational, chemicals, health effects, metrology, measurement methods, workplace, toluene diisocyanate, TDI) search was also conducted to identify recently published relevant information (Pubmed, Scopus were consulted until December 2017).

In annex 1, pertinent epidemiological studies selected by international authorities are listed with their limitations according to the Anses's experts.

4.4.1 Deutsche Forschungsgemeinschaft19 (DFG) report (2003)

Several studies have shown that repeated exposure to TDI can cause a deterioration of lung function (DFG 2003). However, the data relating to the conditions of exposure are not sufficiently precise or are too heterogeneous in most of these studies to enable an assessment of the respective influence of the duration and levels of exposure (these vary or are not specified). The most recent publications include more detailed occupational exposure data. On the basis of these data, no significant deterioration of lung function (with an observation period between 1.5 and 25 years) was suspected after an 8-hour time-weighted exposure to 0.005 mL/m^3 (ppm or 0.036 mg/m^3) and exposure peaks of 0.02 mL/m^3 (ppm or 0.142 mg/m^3) (DFG 2003[20]; Ott et al. 2003), whereas at these levels irritation of the respiratory tract and eyes was frequently reported in comparison with the control group of workers not exposed to TDI.

DFG identified as key study the Jones et al. (1992). In this study, the mean individual atmospheric concentration was 0.0045 mL/m^3 (ppm or 0.032 mg/m^3), and thus similar to the concentrations in studies reporting negative results. A certain portion of the exposure peaks was higher than 0.02 mL/m^3 (ppm or 0.142 mg/m^3).

Jones et al. (1992) in this five-year longitudinal study of workers (n=435) in polyurethane foam factories, found no link between exposure to TDI (5 ppb) and the annual decrease in the FEV1 or

[19] The DFG is the self-governing organisation for science and research in Germany. It serves all branches of science and the humanities. In organisational terms, the DFG is an association under private law. Its membership consists of German research universities, non-university research institutions, scientific associations and the Academies of Science and the Humanities.
[20] See the references shown in Table 4 of the DFG report (2003) (pp. 302-304).

other lung function parameters. However, a significant correlation was observed between the prevalence of chronic bronchitis and cumulative exposure to TDI, after adjustment for age, tobacco consumption and gender. The working environment was characterised by the presence of other chemical compounds (e.g. catalysts, surfactants, foaming agents, etc.) used in the manufacture of foam . According to Clark et al., the symptoms described in the study by Jones et al. (1992) could also be caused by exposure to the glues used in the foam manufacturing processes (Clark et al. 2003).

4.4.2 US EPA report (2012)

The US EPA conducted a literature review and compared the negative studies with those finding an effect of exposure to TDI (US EPA 2012). Wegman et al. demonstrated a dose-response relationship between atmospheric concentrations of TDI and annual variations in the FEV_1 (Wegman et al. 1974, 1977, 1982). Peters (1974) and Peters et al. (1969, 1968, 1975) reported a correlation between changes in the FEV_1 during shifts and the annual decrease in the FEV_1.

US EPA identified as key study Diem et al. (1982). In this study, a total of 2093 samples from 143 workers representing all the work shifts were collected. The workers were divided into two cumulative exposure groups: below 68.2 ppb x month (0.0682 ppm or 0.486 mg/m^3), or above this value. These two groups were then subdivided into six further groups depending on the smoking status (non-smoker vs former or active smoker) then the level of tobacco consumption. In addition, the work stations were classified on the basis of the mean exposure levels on the one hand, and the time spent above certain exposure levels on the other (20, 40, 60 and 80 ppb).

A questionnaire was administered to workers to assess their smoking habits and respiratory symptoms. The pulmonary function tests included a spirometry test, a measurement of the diffusion capacity of the lungs and an assessment of the residual volume (from the volume of expired nitrogen). The critical effect chosen was the chronic reduction in ventilatory parameters, in particular the forced expiratory volume in 1 second (FEV_1), assessed by the paper ribbon method. The baseline was calculated from 168 individuals without prior exposure to TDI in the 6 months preceding the beginning of TDI production. Atopy was assessed by the skin-prick test for 16 common aeroallergens and was defined as a positive result in two or more tests.

According US EPA, the results of this study revealed:

- a strong correlation between the reduction in the FEV_1 and cumulative exposure to TDI, after adjustment for cumulative smoking;
- a significantly higher mean annual decrease in the FEV_1 (38 mL/year) in the group of non smokers exposed to more than 68.2 ppb than in the low-exposure group. This difference was not found in the active or former smokers;
- a correlation between the time spent above 20 ppb and the mean annual decrease in the FEV1. In non-smokers, the excess annual decline in the FEV_1 was 24 mL/year in the group exposed most often to more than 20 ppb. This difference was also observed in smokers (excess decline of 18 mL/year in the FEV_1).

In this study, the data on individuals who had never smoked suggest that the excess annual fall in the FEV_1 and the forced expiratory flow at 25-75% (FEF25-75) are caused by long-term exposure above a mean TDI concentration of 1.9 ppb (arithmetic mean). For US EPA, the

LOAEL was therefore set at 1.9 ppb (0.0019 ppm or 0.014 mg/m^3) and the NOAEL at 0.9 ppb (0.0009 ppm or 0.006 mg/m^3).

This study was preferred by US EPA to others (Kaufman and Overcash 1993; Omae et al. 1992a), because:

- confounding factors such as multiple environmental exposures, an atopic disposition or smoking were better taken into account;
- it has good statistical power: it includes a large number of workers (277 men), monitored longitudinally over a period of 5 years. Measurements of lung parameters were taken from 3 to 9 times over a period of 5 years in 147 of these workers;
- the authors took intra- and inter-individual variations into account in the ventilatory parameters tested.

For the experts from Anses, the Diem et al. (1982) study does not provide sufficient evidence of an accelerated rate of decline in FEV_1 among TDI-exposed employees at this level of exposure. Methodological limitations of this study do not allow the determination of a threshold (see annex 4).

4.4.3 OEHHA report (2016)

A comprehensive summary about the chronic TDI exposure is available in OEHHA 2016. Diem et al. (1982) was used as the basis of the 8-hour reference exposure level. The critical effect of accelerated decline in FEV_1 in the absence of TDI-induced asthma strongly indicates a chronic inflammatory response in the lung airways of the workers resulting in the loss of pulmonary function.

As US EPA, OEHHA consider a LOAEL of 1.9 ppb (0.0019 ppm or 0.014 mg/m^3) and a NOAEL at 0.9 ppb (0.0009 ppm or 0.006 mg/m^3).

For the experts from Anses, the Diem et al. (1982) study does not provide sufficient evidence of an accelerated rate of decline in FEV_1 among TDI-exposed employees at this level of exposure. Methodological limitations of this study do not allow the determination of a threshold (see annex 4).

4.4.4 Dutch expert Committee on Occupational Safety (DECOS) of the Health Council of the Netherlands

After analyzing all available epidemiological studies, the DECOS selected two workplace studies, the studies of Pronk et al. (2007, 2009) on BHR and asthma symptoms and Collins et al. (2017) on 'TDI-consistent' cases of occupational asthma.

In two publications, Pronk et al. (2007, 2009) reported an exposure-response relationship of respiratory symptoms and sensitisation in a large cohort occupationally exposed to isocyanate oligomers during spray painting.

In the first study (Pronk et al. 2007), 581 car spray painters exposed to hexamethylene diisocyanate or HDI were followed and specific IgE and IgG to HDI were assessed in serum using various assays and an evaluation of respiratory symptoms by questionnaire was performed. In the second study

(Pronk et al. 2009), a subsample of 229 subjects were assessed based on BHR, lung function parameters, serology and exhaled nitric oxide. Mean exposure was 4,530 µg NCO/m^3*h*months (range 15.4-66,464). The authors observed that workers with higher isocyanate exposure were more often hyperresponsive. They reported a statically significant exposure-related decreased FEV1, FEV1/FVC and flow –volume parameters independent of BHR.

Based on this study, the DECOS applying a logistic regression analysis on the individual's data calculated a value of 0.10 µg NCO/m^3 for a 1% increase of BHR prevalence.

In Collins (2017) study, 197 workers in facilities producing TDI were monitored from 2007-2012 and the authors report new cases of asthma by application of a standardized annual medical assessment including spirometry and questionnaires on symptoms and exposure (Cassidy et al. 2017 and Middendorf et al. 2017). Increased risk of cases consistent with TDI asthma was observed for cumulative exposure with OR =2.08 (CI 1.07-4.05) per unit increase in log ppb-years and for peak TDI exposures with OR = 1.18 (CI 1.06-1.32) per unit increase in parts per billion.

Based on this study, the DECOS calculated the exposure level corresponding to a 1% extra risk of being a case 'consistent with TDI-induced asthma' from the exposure-response relationship reported by the authors (with data on cumulative exposure). The DECOS derived a value of 0.14 µg NCO/m^3 corresponding to an extra risk of 1%.

According to the DECOS, this value supported the risk calculation based on the study of Pronk, so for di and triisocyanate, the committee derives risk based advisory value of 0.1 µg NCO/m^3 (which corresponds to an extra risk of BHR of 1%).

Before the public consultation of the DECOS report, and as part of the collaboration, Anses's experts decided to analyze the epidemiological studies on respiratory effects and isocyanate exposure summarized by DECOS. Most studies, however, have addressed the effect of isocyanate exposure on lung function (usually measured as FEV1 and FVC). Finally, DECOS reported 42 studies with critical concentrations associated with lung function effect.

These studies were analyzed by Anses via an approach adapted from OHAT[21] method (Annex 5).

In this approach, steps 1 to 3 lead to the identification of relevant studies following criteria as the studied Population, type of Exposure, Comparator tools and selected Outcome (PECO statement). Out of the 42 studies analysed, only 23 consider professional TDI exposure only, 12 include more than 100 subjects and 10 measure the decrease of FEV1 (see annex 5). Therefore, only 6 studies comply these 3 criteria and passed stage 3: Diem et al. 1982, Bodner et al. 2001, Littorin et al. 2007, Ott et al. 2000, Wegman et al. 1977 and 1982.

Despite a large number of available studies, none of the epidemiological studies were considered adequate for deriving a quantitative value. The cause of this lies in limitations of the studies, but is also inherent to the mechanism of the disease. No study overcomes the problem that sensitive predictive markers for diisocyanate sensitisation are missing, and that dermal exposure as well as inhalation peak exposure likely contribute to the induction of sensitisation, but cannot be assessed appropriately to date.

[21] Office of Health Assessment and Translation

4.5 Mechanism of action- sensitisation/irritation

TDI has been shown to cause respiratory irritation in animals and humans and, depending on the respective study setup, the delineation of sensitisation from irritation is difficult.

TDI has sensitising properties, particularly after respiratory exposure. Studies on the inhalation of TDI vapors show that the majority of the inhaled TDI is rapidly found in the blood and tissue, in particular in the form of conjugates with the body's biomolecules from reaction with nucleophilic sites such as the NH_2 of proteins. This conjugation competes with the hydrolysis reactions degrading TDI into TDA that occur in acidic conditions (Holdren et al. 1984; Prueitt et al., 2013).

Nakashima et al., in their review on occupational exposure to TDI, argued that the formation of TDI adducts with epithelial proteins could cause epithelial lesions and induce changes in the permeability of the respiratory tract and in cell signaling, and increase the sensitivity of the respiratory tract receptors (Nakashima et al. 2002).

Clinical data

TDI asthma observed clinically appears as a classic asthma with inflammation, bronchoconstriction and mucus hypersecretion. As with any occupational asthma, the only effective treatment remains cessation of exposure. However, while some people become asymptomatic after this removal, others may present persistent asthma symptoms. Thus, it was reported that 6 months after the cessation of exposure to TDI, asthmatic subjects showed persistent inflammatory infiltrate, despite the decrease in the thickening of their basal membrane (Saetta et al. 1992). TDI asthma develops with a variable latency period that can range from a few months to several years after exposure (Mapp et al. 1985). The asthma-like reaction can be immediate (< 1 hour), delayed (2 to 4 hours later) or both immediate and delayed; it usually occurs following exposure to low concentrations of isocyanates (Wegman et al. 1982), and is complicated to recognize and diagnose.

Exceptionally, asthma can occur after exposure to high concentrations and may be linked to the irritating effects of the isocyanates. TDI thus presents irritant effects occurring without a latency period, from 0.5 ppm for acute exposure (Bonnard et al. 2006) in the form of non-specific bronchial hyperreactivity due to direct toxicity (alteration of the bronchial epithelium + inflammatory infiltrate) (Shin et al. 2013). Some individuals may retain sensitisation as a result of a single exposure of this type (Moller et al. 1986; Leroyer et al. 1998).

Pathophysiological mechanisms

A number of arguments suggest an immunological mechanism for respiratory sensitisation to TDI:

- the latency period between the first contact and the onset of the asthmatic response,
- the low incidence in relation to the large number of subjects exposed (5 to 10% of workers),
- the similarity of the symptoms observed in TDI asthma to those triggered by inhaled allergens,
- the presence of IgE (even low) in the serum of subjects with lung sensitisation (Karol and Jin, 1991).

However, there are differences between the classic IgE-mediated immunological lung sensitisation and sensitisation to TDI, such as the delayed response (Finotto et al. 1991), the atypical reactions to the bronchial challenge tests (Perrin et al. 1991) and the low rate of detection of specific IgE in subjects diagnosed with TDI asthma (Son et al. 1998). The limited success in detecting IgE in the

cases of sensitisation to TDI could be explained by a lack of information on the conjugate complexes and therefore on the antibodies raised against these conjugated forms (Karol and Jin, 1991). Inappropriate diagnosis of sensitisation to TDI has also been mentioned: out of 75 subjects diagnosed positive by questionnaire, less than half responded to the challenge with high molecular-weight allergens (Karol and Jin, 1991).

Pulmonary sensitisation could occur with repeated exposure to TDI (from 0.05 ppm for chronic exposure, Bonnard et al. 2006), stimulating the immune mechanisms of exposed individuals who may then react to lower doses of TDI by triggering the immune response.

The mechanistic assumption made to explain pulmonary sensitisation to TDI is as follows: TDI may behave as a hapten, i.e. a low molecular-weight substance, non-immunogenic in itself, which, once captured by the lung epithelial cells, binds to proteins to form adducts. The structure of the conjugates formed is not clearly identified (possible bond with albumin, laminin or cell membrane proteins) but they may be able to initiate an immunological response after being captured by the immature dendritic cells of the airways. After maturation, the dendritic cells prepare the TDI conjugates and migrate in the lymph nodes to present them to naive T lymphocytes and polarise the T lymphocytes by directing them towards the differentiation path most suited to aggression (Raulf-Heimsoth and Baur, 1998). The adaptive immunity depends on the activation, clonal expansion and differentiation of B and T lymphocytes, which are specific to a given antigen. It is orchestrated by the helper T lymphocytes (Th), which are able to differentiate into distinct sub-populations: Th1, Th2, Th17 and T regulators (Treg). The Th1 lymphocytes promote a cellular immune response by activating the different cytotoxic cells. The Th2 lymphocytes guide the response towards the humoral mediated immunity involving the B lineage and the production of high-affinity antibodies. The Th17 cells, discovered recently, are characterized by their ability to produce a strongly pro-inflammatory cytokine, IL-17. Conversely, the Treg are immunosuppressive cells that control and limit the immune response. In the case of lung sensitisation to TDI, a predominance of Th2 responses has been reported, with secretion of IL-4, IL-5 and IL-13 supporting a humoral-type immune response leading to the production of specific IgE. During subsequent exposure, these IgE attach themselves to the mast cells, causing their degranulation and the release of histamine responsible for bronchoconstriction (Maestrelli et al. 1997; Ban et al. 2006). This is a Type I hypersensitivity reaction generally associated with classic respiratory hypersensitivity.

However, the development of delayed reactions and chronic symptoms associated with TDI asthma, as well as the fact that atopy (predisposition to develop an IgE-mediated immune hypersensitivity) is not an acknowledged risk factor for the occurrence of TDI asthma, seem to imply a Type IV hypersensitivity reaction. A cellular-type immune response involving the $CD8^+$ T lymphocytes has also been described, explaining the delayed manifestations observed in the occurrence of TDI asthma. In a mouse model exposed by inhalation to TDI, not only was a predominant role of $CD4^+$ T lymphocytes secreting Th2 cytokines reported, but also interactive cooperation with $CD8^+$ T lymphocytes secreting Th1 cytokines such as IFNγ (Matheson et al. 2005). The presence of $CD8^+$ T lymphocytes producing IL-5 and IFNγ has also been identified in the bronchial mucosa of subjects sensitised to TDI (Maestrelli et al. 1994). These cytokines are then able to recruit and activate inflammatory cells such as neutrophils and eosinophils (Bentley et al. 1992; Sun et al. 2006; Świerczyńska-Machura et al. 2012; 2014) that in turn secrete mediators of inflammation responsible for the asthma-like response.

Moreover, cross-sensitisation between isocyanates has been reported (Malo et al. 1983). For example, it is known that all the residues of the albumin targeted by isocyanates to which 4,4-methylene diphenyldiisocyanate (MDI) binds are analogous to those of TDI (Hettick and Siegel, 2011). However, a very recent study report the absence of cross-reactivity between MDI and TDI in a mouse model of isocyanate-induced asthma (Pollaris et al. 2015).

Pharmacological mechanisms of action of isocyanates directly on the bronchi have also been reported as participating in the respiratory response to TDI. Indeed, an inhibitory action of TDI on the acetylcholinesterases in the bronchial walls could play a role in the development of bronchoconstriction (Brown et al. 1982; Dewair et al. 1983; Brondeau et al. 1990), as could the effect reported on the beta-adrenergic system (Borm et al., 1989).

Lastly, **genetic factors** such as genotypes on the proteins of the major histocompatibility complex, glutathione transferase or N-acetyltransferase, might predispose the individual to development of an occupational isocyanate asthma according to the resulting phenotype (Mapp et al., 2005).

In conclusion, lung sensitisation to TDI leading to the development of an occupational asthma is the result of mechanisms that are more complex than those observed in classic immunological lung sensitisation.

Links between skin and respiratory sensitisation

In workers exposed to products containing TDI, rare cases of skin sensitisation in the form of contact dermatitis or allergic urticaria have been reported (Goossens et al. 2002), despite the fact that absorption of TDI by the dermal route is controversial.

A study of dermal penetration of TDI in rats reported absorption of less than 1% in the form of protein adducts (Pauluhn 2014). In experimental animal models, it has been shown that topical application of TDI was responsible for an increase in the Th2 response with the production of IgE in mice (Dearman et al. 1996), but a Th1 response with an increase in IFNγ secretion was also revealed in another model of dermal exposure (Vanderbriel et al. 2000). It has been shown that dermal exposure to TDI followed by inhalation exposure influenced the allergic sensitisation to TDI (Vanoirbeek et al. 2004) and induced a local and systemic Th2 response (Ban et al. 2006) in mice. In this mouse model, an important role of B-lymphocytes without major involvement of IgE and without help from T-lymphocytes has also been recently described (De Vooght et al. 2013; Haenen et al. 2015).

In humans, the link between skin sensitisation and the occurrence of TDI-induced respiratory sensitisation remains unresolved. Because of the dermal exposure through skin contact in workplaces that produce or use isocyanates (Bello et al. 2007, 2008), many concerns have been raised about skin exposure as a potential route of allergic sensitisation that could possibly lead to asthma (Redlich 2010). Bello et al. (2007) concluded that there was some inconclusive evidence that skin exposure to isocyanates in humans could contribute to the development of isocyanate asthma (Bello et al. 2007).

In this way, Pauluhn has proposed that TDI-induced respiratory allergy could involve two sequential mechanisms: a dermal exposure able to cause systemic sensitisation which followed by inhalation exposure, would initiate and amplify allergic inflammation and progression to asthma (Pauluhn, 2014). Another recent study in mice suggests that hair follicles and their associated sebaceous

glands could be a reservoir for TDI conjugated to self-proteins and its uptake immune cells such as local dendritic cells capable of producing allergic sensitisation after presentation to T-cells in the lymph nodes (Nayak et al. 2014).

In their review on animal data, Schupp and Collins (2012) reported NOAECs for respiratory sensitisation in the range of 0.005 to 0.03 ppm (LOAECs about 0.02 to 0.4 ppm). They concluded that the current animal database is not in contradiction with the current workplace exposure limits for TDI. The animal data and human experience for respiratory sensitisation are in concordance. The authors added that in this case, application of additional factors is not required for the derivation of occupational workplace exposure limits.

Genetic factors

Genetic factors have been implicated in the susceptibility to occupational asthma by TDI and other diisocyanates. OEHHA (2016) summarizes pharmacogenetic and provide a review of the literature on the influence of genotype on the health effects of TDI. A number of gene variants have been reported to be associated with increased sensitivity to the disease (asthma) in workers. These studies provide information on genetic association that could lead to explain the interindividual variability observed in asthma. However, these studies are not useful to the OEL derivation based on TDI-induced asthma or other effects.

4.6 Genotoxicity

4.6.1 In Vitro data

4.6.1.1 DNA damage

An *in vitro* study showed that TDI induces double-strand breaks in the DNA of sheep white blood cells and a degradation of mitochondrial DNA and large DNA fragments in rabbit white blood cells, which may result from apoptosis, into small DNA fragments (Marczynski et al. 1993).

4.6.1.2 Mutagenesis

TDI induced mutations in *Salmonella* Typhimurium TA98, TA100 and TA1538 in the presence of a metabolic activation system (IARC 1999). All of the studies used dimethylsulfoxide (DMSO) as a solvent for the test compounds, suggested that TDI was mutagenic with metabolic activation in at least one strain of *S. typhimurium* (NTP 1986; Zeiger et al. 1987). Mutagenicity tests on bacteria carried out in the presence of DMSO or EGDE (ethylene glycol dimethylether, used as a solvent have) have been performed (Herbold et al., 1998; Nakashima et al. 2002). When DMSO is replaced by EGDE (ethylene glycol dimethylether, used as a solvent), TDI is mutagenic in TA1537 and TA98 bacteria (Seel et al. 1999).

TDI induced mutations in the $Tk^{+/-}$ locus in L5178Y mouse lymphoma cells (*in vitro*) in the presence of a metabolic activation system (McGregor et al. 1991).

TDI induced sex-related recessive lethal mutations in *Drosophila* (Foureman et al. 1994).

The complete analysis of TDI genotoxicity tests based on the Prueitt et al. 2013 study is presented in annex 6.

4.6.1.3 Damage at chromosomal level

Sister chromatid exchanges but not chromosomal aberrations occurred in hamster ovary cells treated with TDI (Gulati et al. 1989).

4.6.1.4 Genotoxicity of TDA

Prueitt et al. stated that the positive results of some in vitro tests with TDI were due to the use of solvents that resulted in the formation of TDA. TDA showed mutagenic activity after metabolic activation in *S. typhimurium* strains (TA98, TA100, and TA1538), and 2,6-TDA was mutagenic only in *S. typhimurium* strains (TA98 and TA1538) in the presence of a metabolic activator (Prueitt et al. 2013, 2017).

4.6.2 Animal data

4.6.2.1 Protein adducts

Although adducts of TDI with proteins are not an expression of genotoxicity, they are often considered in parallel with genotoxicity as biomarkers of exposure and toxicity. Studies in animals following inhalation of radioactive ^{14}C-TDI showed the presence of radioactivity in the epithelia of the upper respiratory tract and the formation of protein adducts, mainly with albumin. Adducts also are formed in the blood with albumin.

4.6.2.2 DNA adducts

Diisocyanates can form adducts not only with proteins but also with DNA. Their metabolites can also form adducts with DNA. The formation of DNA adducts with isocyanates has been little studied. The only article published up to now shows that the formation of adducts with 4,4'-methylenediphenyl diisocyanate (MDI) from its diamine derivative, 4,4'-methyllenedianiline (MDA), in the olfactory epithelium of rats exposed to MDI (Vock et al. 1996). Although there are no studies published to date on DNA adducts with TDI, it can be assumed that, as with MDI, adducts may form by covalent binding to DNA via two pathways:

- reaction of the electrophilic group -N=C=O with nucleophilic atoms of DNA to give isocyanate adducts,
- formation of arylamines (aromatic amines) after hydrolysis of TDI into carbamic acid and decarboxylation into arylamine TDA. The arylamine requires an enzyme activation to give a reactive electrophilic metabolite (nitrenium/carbenium ions) which reacts with DNA and forms a DNA-arylamine adduct.

4.6.2.3 DNA damage

One study conducted on micronuclei *in vivo* were negative: 2,4-toluene diisocyanate did not induce micronuclei in rats and mice exposed *in vivo* to concentrations of 1.1 mg/m^3, 6 hours per day, 5 days/week for 4 weeks. A more recent study on mice exposed to TDI by inhalation for 24 hours

demonstrated the formation of specific haemoglobin adducts (with TDI and not its amine TDA) in peripheral blood but no micronuclei in the bone marrow (Lindberg et al. 2011).

4.6.3 Human data

TDI induced double-strand breaks in the DNA of human white blood cells, *in vitro* (Marczynski et al. 1992a). In a cell-free system, DNA treated with TDI did not undergo fragmentation. This means that a biotransformation probably took place and it was the metabolites of TDI that interacted with the DNA.

TDI induced chromosomal aberrations and sister chromatid exchanges in human lymphocytes in culture after 24 hours of exposure in the absence of a metabolic activation system (Maki-Paakkanen et al. 1987 cited by IARC 1999).

A study on workers (n = 26) exposed to TDI (the two isomers) for approximately 12 years in a plastic manufacturing company showed an increase in the frequency of structural chromosomal aberrations and sister chromatid exchanges in their lymphocytes (for a TDI concentration between 0.007 mg/m^3 and 0.016 mg/m^3). Smoking was taken into account in this study (18 in the group in contact with TDI (smoking index of 272), 10 in the control group (smoking index of 140)) but the number of subjects was small (Bilban 2004). In a previous study with MDI and not with TDI, Marczynski et al. (1992b) had shown DNA fragmentation in the white blood cells of a worker who had been exposed to MDI by inhalation (from 5 to 20 ppb).

The alkaline version of the Comet assay was used to analyse DNA strand breaks in lymphocytes of workers having respiratory symptoms and with a history of exposure to diisocyanates (Marczynski et al. 2005). In a controlled atmosphere chamber, 5 subjects were exposed to industrial-grade TDI (80:20 mixture of 2,4-and 2,6-TDI) in the following sequence: 30 minutes at 5 ppb, 30 minutes at 10 ppb, 90-minute break, 30 minutes at 20 ppb, 90-minute break, and ending with 30 minutes at 30 ppb. Blood was sampled for use in the comet assay before as well as 30 minutes and 19 hours after the end of exposure. Mean values of the olive tail moment before and after exposure did not differ significantly, nor between the groups.

Protein adducts as biomarkers of exposure have been describing by several authors and report:

Blood protein adducts are good markers of exposure in the workplace: the adducts found in the plasma of workers exposed to TDI are essentially covalent adducts with albumin. Other adducts are formed with haemoglobin: TDI-haemoglobin formed by carbamoylation (IARC 1999).

Mhike et al. 2016 performed a study to compare TDI and HDI covalent adducts with human blood proteins. They found that TDI was more reactive to both albumin and haemoglobin than HDI at pH 7.4.

Säkkinen *et al* found that TDI-derived adducts were found in 77% of plasma and in 59% of globin samples from exposed workers manufacturing flexible polyurethane foam (Säkkinen.et al. 2011).

4.6.4 Conclusion on TDI genotoxicity

In conclusion, inconsistent results have been observed in *in vitro* genotoxicity assay. Negative as well as positive findings have been reported for TDI, for which the positive findings may be attributed to the formation of mutagenic TDA when using DMSO as vehicle. Therefore, on the basis of the available studies, the data are equivocal and it is not possible to conclude as to the mutagenicity and genotoxicity of TDI.

4.7 Carcinogenicity

In 1999, IARC classified TDI as possibly carcinogenic to humans (Group 2B) as there were inadequate evidence for the carcinogenicity of TDI in humans and sufficient evidence for the carcinogenicity of TDI in experimental animals (IARC 1999 –Table 11).

NTP considers that TDI is reasonably anticipated to be human carcinogen based on sufficient evidence of carcinogenicity from studies in experimental animals (NTP 2016).

In the gut, hydrolysis of TDI generates toluene diamine (TDA), a carcinogen which explains that by the oral route TDI is carcinogenic in animal. Inhalation exposure leads preferentially to the formation of TDI conjugates and little or no measurable TDA (Timchalk et al. 1994; Lindberg et al. 2011). OEHHA (2016) stated that route-dependent differences may explain the observed carcinogenicity of TDI by the oral (with conversion to TDA) but not the inhalation route in experimental animals (Collins 2002).

4.7.1 Human data

Three large cohort studies on occupational cancer have been carried out in Sweden, England and the United States and examined by IARC (IARC 1999).

In the Swedish study (Hagmar et al. 1993), the standardised mortality ratios (SMRs) were very low and were attributed to the healthy worker effect. In addition, the workers included in the study had a relatively short duration of exposure to isocyanates.

This study was updated with 11 more years of follow up (Mikoczy et al. 2004), and confirmed the conclusion of the first study: the data did not provide a link between isocyanate exposed employment and lung cancer risk.

For England and Wales, 20% of the individuals in the study were deceased. Although the cohort was made up of relatively older individuals, this study found no link between the workers exposed to isocyanates inhalation and the risk of lung cancer or non-malignant diseases of the respiratory system. The significant increase in lung cancer among women was attributed by the authors to smoking (International Isocyanate Institute 1992b quoted by DFG 2003, Sorahan and Pope 1993, Sorahan et Nichols 2002).

This study was updated through 2011 (Pinkerton et al. 2016). The authors found that lung cancer mortality was not related to exposure duration or cumulative TDI exposure, but was associated with employment duration in finishing jobs.

Concerning the American study, it showed a possible relationship between the time that elapsed between the first job in the polyurethane factory and the appearance of non-Hodgkin lymphomas and Hodgkin's disease (Schnorr et al. 1996). TDI worker exposure was assessed using atmospheric concentrations, without taking into account any other possible exposure noted at the workplace.

In the end, on the basis of these three cohorts from the polyurethane industry, and the absence of cases of cancer in the workers after occupational exposure to diisocyanates, TDI does not seem to induce an increased overall risk of cancer in humans by inhalation (Table 10). Regarding the carcinogenicity of TDA, no data are available on case reports or epidemiologic studies of TDA carcinogenicity to humans.

Table 10: Description of cohorts studies on occupational cancer from ATSDR 2015

	US Cohort		UK Cohort		Swedish Cohort	
Reference	Schnorr et al. 1996		Sorahan and Nichols 2002		Mikoczy et al. 2004	
Cohort size (plants)	4,611 (4)		8,288 (11)		4,175 (9)	
Follow-up period	1958–1993		1958–1998		1959–1998	
Person-years at risk	90,393		200,262		83,023	
Cancer site	**Number of cases**	**SMR (95% CI)**	**Number of cases**	**SMR (95% CI)**	**Number of cases**	**SMR (95% CI)**
Females						
Lung	8	173	35	181[a] (126–251)	10	352[a] (169–648)
Rectum	0	NA	2	53 (6–192)	–	–
Non-Hodgkin's lymphoma	–	–	3	110 (23, 321)	–	–
Hodgkin's disease	–	–	0	NA	–	–
Males						
Lung	12	79	134	107 (90–127)	7	49 (20–101)

Rectum	3	390	10	65 (31–120)	–	–
Non-Hodgkin's lymphoma	–	–	6	65 (24–142)	–	–
Hodgkin's disease	–	–	1	44 (1–243)	–	–
Females + Males combined						
Lung	20[b]	101 (62–156)	–	–	17	99 (58–159)
Rectum	3	278 (57–813)	–	–	–	–
Non-Hodgkin's lymphoma	4	154 (42–395)	–	–	–	–
Hodgkin's disease	2	232 (28–838)	–	–	–	–

Notes:

[a] Significantly different from null hypothesis at $p<0.05$.

[b] Includes tumors of the lung, trachea, and bronchus.

– = not reported; NA = not applicable; SMR = standardized mortality ratio; CI = confidence interval.

4.7.2 Animal data

TDI

The carcinogenicity of TDI has been investigated in studies in rats and mice exposed by oral and respiratory routes. The results tend to show a risk after oral ingestion but not after inhalation exposure. This could be explained by a different metabolic pathway between the two routes of exposure, including the formation of TDA after ingestion of TDI but not after inhalation of TDI (IARC 1999; OEHHA 2016).

Oral exposure to TDI (gavage) in rats and mice can result in tumors in different tissues. TDI administration (85% 2,4-TDI and 15% 2,6-TDI) has been shown to cause liver tumors (hepatocellular adenomas) in rats and female mice, benign mammary gland tumors (fibroadenomas) in female rats, and benign pancreatic tumors in male rats. It also increases the combined incidence of benign and malignant subcutaneous tissue tumors (fibroids and fibrosarcomas) in male rats and blood vessels tumors (hemangiomas and hemangiosarcomas) in female mice (Dieter *et al.* 1990, quoted by IARC 1999). TDI was administered in corn oil by gavage 5 days / week for 105 weeks in mice and 106 weeks in rats. The estimated doses were 23 or 49 mg / kg in male rats, 49 or 108 mg / kg in female rats and female mice, 108 or 202 mg / kg in male mice. It seems that TDA, a product of hydrolysis of TDI, especially in the stomach, induces the same types of tumors.

In inhalation exposure, no carcinogenic effect was observed in a study exposing rats and mice at concentrations of 0.05 and 0.15 ppm TDI (2.4 TDI/2.6 TDI: 80/20) 6 hours / day, 5 days / week for 2 years (Bonnard et al. 2006, Löser 1983). In rats, there were no differences between the exposed and control groups; histopathological examinations did not reveal chronic effects on the lungs or tumor incidence. In mice, there was no difference in exposure to clinical or hematological chemical indices, and pathological examinations did not show oncogenic effects (Collins, 2002, Löser, 1983).

TDA

NCI[22] performed a carcinogenic study in rat and mice:

Groups of 50 male and 50 female B6C3F1 mice received 2,4-TDA in the diet ad libitum at concentrations of 100 or 200 ppm for 101 weeks. A statistically significant incidence of hepatocellular carcinomas occurred in low-dose ($p=0.007$) and high-dose ($p=0.008$) female mice; a statistically significant increase also occurred in the incidence of lymphomas ($p<0.001$) in the low-dose female mice. No significant increase in tumors occurred in male mice treated with 2,4-TDA (NCI 1979).

Groups of 50 male and 50 female F344/N rats were given 2,4-TDA in the diet ad libitum at of 125 or 250 ppm for 40 weeks. When incidences of hepatocellular carcinomas and neoplastic nodules were combined, dose-related linear trends occurred in males ($p=0.014$) and females ($p=0.008$). In addition, the incidence of mammary gland fibroademonas was dose-related in treated female rats and statistically significant for those on the high dose ($p<0.001$).

[22] National Cancer Institute

Conclusion of carcinogenicity

NTP reported TDA as a substance which may be a carcinogen because there is sufficient evidence of carcinogenicity in experimental animals even though no evidence exists for humans (NTP 1985).

The transformation of TDI into TDA, a known animal carcinogen with genotoxic properties, is the most plausible explanation for the observed tumors following oral administration of TDI. The differential formation of TDA *via* the two routes of exposure may contribute to the mechanism by which TDI was carcinogenic in mice and rats by oral exposure but not by inhalation.

In conclusion, the epidemiology data are not sufficiently robust to support TDI as a human carcinogen (TDI is classified as a Group 2B carcinogen, possibly carcinogenic to humans by IARC); there is no specific concern for genotoxic carcinogenicity from the available diisocyanate dataset in animals or from human case reports, after inhalation. Therefore the carcinogenicity will not be considered for the derivation of the OEL.

5 Construction of the OELs

The available human studies (pulmonary chronic toxicity in the workplace) show evidence that TDI exposure is associated with respiratory effects (such as asthma, BHR, decrements in lung function and sensitisation). Nevertheless, the human studies reporting these effects present limitations (see annexes 1 and 3 for pertinent epidemiological studies selected by National, international authorities and their limitations according the experts) for the establishment of a dose-response relationship:

- Dermal and peak exposures are not quantifiable

- Real exposure is difficult to establish (e.g. use of personal protective equipment, previous exposures and dermal exposure and co-exposure with chemicals like irritants (phosgene) were not taken into account,

- The size of the test populations is limited

- Lack of more objective outcome measurements. The key indicators of pulmonary function employed in these studies were the forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) (FEV1 alone is not seen as a sensitive predictive marker of asthma). The followed period (<5 years, e.g in the Diem et al. (1982), Omae et al. (1992a) studies…) was insufficient to provide evidence of an accelerated rate of decline in FEV1 among TDI-exposed employees.

The available animal studies indicate that the induction of respiratory sensitisation is a threshold phenomenon:

In a review from Schupp and Collins (2012), the authors concluded that the NOAELs and LOAELs for both irritation and sensitisation are in the same order of magnitude across animal species. They also reported that literature data available allow the conclusion that both respiratory irritation and sensitisation may be interdependent, and both irritation and sensitisation by TDI is a threshold phenomenon. Pauluhn developed an animal model indicating that the respiratory effect produced by TDI are threshold events and that OEL based on irritation adequately protect against both irritation and sensitisation (Pauluhn 2014).

OEHHA and Pauluhn concluded that staying below the irritation/sensitisation threshold dose should also be sufficient to avoid this adverse health effect (OEHHA 2016, Pauluhn 2014).

Predicting a safe exposure level for sensitised individuals is difficult due to high variability in individual susceptibility. For this reason, prevention of sensitisation in naïve individuals, rather than elicitation in already sensitised persons, appears to be a more suitable and health protective health endpoint to serve as the basis of an OEL (Dotson et al. 2015). For sensitised individuals, elicitation of clinical symptoms may occur at or below an OEL derived to prevent induction of sensitisation in naïve individuals. Banks et al. demonstrated that a 15-min exposure at the 8-hr TWA-OEL (at that time the OEL was 20 ppb) to TDI was sufficient to elicit an asthmatic attack in a sensitised worker (Banks et al. 1989). O'Brien et al. reported challenge elicitation of TDI-induced asthma in a subgroup of highly sensitised workers at ≤ 1 ppb (O'Brien et al. 1979).

In the study by Lemière et al., the authors showed that exposure of subjects confirmed with occupational asthma caused by isocyanates to a concentration of 1 ppb and to a dose as low as 10.7% (47 ± 37.3 ppb · min) of the total dose of isocyanates (371.3 ± 361.1 ppb · min) that

had induced an asthmatic reaction at the time of diagnosis caused an asthmatic reaction (Lemière et al. 2002)

Based on all this evidence, pulmonary irritation was selected by the experts as the critical end point to derive an OEL.

The experts consider that protection against irritation will avoid sensitisation, but not allergic reactions in "sensitised" individuals (see chapter mechanism of action).

5.1 Construction of a short-term exposure level (STEL)

To determine the point of departure to establish a STEL for respiratory irritation, the experts retained the study of Vandenplas et al. (1999) as the key study.

Vandenplas et al. exposed 17 subjects (eight smokers and nine non-smokers) without occupational exposure to isocyanates and without respiratory symptoms suggestive of asthma and chronic bronchitis, once to ambient air and once to TDI (5 ppb for 6 hrs followed by 20 ppb TDI for 20 min, 4 weeks later). Vandenplas et al. examined sub clinical endpoints and estimated the risk of respiratory irritation induced by the inhalation of TDI. Briefly, TDI exposure resulted in a slight increase in BAL albumin level (TDI: 26.4+12.5 µg/ml vs. air: 21.8+8.6 µg/ml, p=0.044) and in BL (bronchial lavage), α2-macroglobulin concentration (TDI: 0.07+0.061 µg/ml vs. air: 0.05+0.04 µg/ml, p= 0.021). A slight decrease in specific airway conductance (sG_{AW}) (p=0.053) and MEF at 25% ($MEF_{25\%}$) (p=0.015) were also observed.

The observed increase in BAL albumin content after TDI exposure is likely to represent indirect evidence of changes in permeability of the epithelial barrier and slight leakage of blood plasma components into the alveolar compartment.

- Choice of the critical concentration:

As reported by Vandenplas et al. 1999, an exposure of 20 ppb for 20 min TDI with a continuous exposure at 5 ppb during 6 hours resulted in healthy subjects in changes in permeability of the pulmonary epithelial barrier characterized by a slight increase in BAL albumin level and α2-macroglobulin.

The experts consider therefore this concentration of 20 ppb as a LOAEC.

- Assessment factor :

Since the critical study is a human study, no interspecies adjustments is required.

Since the starting point for the STEL calculation is a LOAEC, the experts use an assessment factor of 3 (for the transition from the LOAEC to the NOAEC).

The experts do not recommend for local effects (irritation) applying an assessment factor for the intra-species variability. Nevertheless TDI is able to induce more severe effect at the same range of exposure, as described by Vogelmeier et al. 1991 and Baur et al. 1994, where TDI showed sensory irritation in 3 healthy subjects with 2 hr exposure to 20 ppb and a severe pulmonary response to 10 ppb during 1 hr in a asthmatics (1/15).

The experts use an assessment factor for intra-species variability of 5 in order to take account higher sensitivity among worker and to prevent chronic effect.

- STEL calculation:

A final assessment factor of 15 is applied to the LOAEC of 20 ppb leading to a value of 1.3 ppb.

Anses's experts recommend this value of 1.3 ppb as a 15-min short-term limit value.

As described in the choice of the critical effect, a 15 min-STEL based on pulmonary irritation has been derived, this STEL should offer protection against sensitisation, but not against allergic reaction in "sensitised" individuals.

5.2 Construction of an 8 hours occupational exposure level (8-hour OEL)

Because of the potential severity of the effects of TDI and since the control of a STEL values involves a measurement during peaks of exposure that can sometimes be difficult to implement over the duration of an 8-hour workshift, the experts estimate that it is important to recommend, in addition to the STEL, an exposure limit not to be exceeded over an 8-hour period.

In the absence of adequate data, the experts propose to establish a **pragmatic 8h-OEL**, which does not aim to set a value below which there is no health risk, but rather to provide OSH experts with a risk management tool for limiting occupational exposure (Anses 2017). In order to minimize the risk of exceeding the 15 min-STEL over the duration of an 8-hour workshift (i.e. 32 times 15 minutes) the atmospheric concentration of TDI should not exceed the 15 min-STEL / 32 on a working day of 8 hours.

i.e.

$$8h - OEL = \frac{15\ min{-}STEL}{32} = \frac{1{,}3}{32} = 0.04 \text{ ppb}$$

experts recommend a pragmatic 8h-OEL of 0.04 ppb, which is in agreement with the DECOS value (0,1 µg.m^{-3} of NCO corresponding to 0.04 ppb of TDI). This pragmatic 8h-OEL does not protect against allergic reaction in "sensitised" workers.

5.3 "Skin" notation

Although skin sensitisation is not a criterion for assigning a "skin" notation but as far as the penetration of TDI by the dermal route may lead to a possible systemic effect which can result in general immuno-allergic pathologies of particular concern (respiratory sensitisation), the experts recommend assigning the "skin" notation for TDI.

5.4 “Noise” notation

In the absence of scientific data on the ototoxic effect of TDI, no "noise" notation has been assigned for this substance.

6 Conclusions of the collective expert appraisal

15 min-STEL: 1.3 ppb

Pragmatic 8h-OEL: 0.04 ppb

“Skin” notation: yes

“Noise” notation: none

The experts further recommend to avoid any skin contact as dermal uptake is likely to contribute to respiratory sensitisation as well being associated with skin sensitisation.

The experts want to underline that the values hereby recommended **are not intended to protect already sensitised workers**

The experts point out that the ALARA (As Low As Reasonably Achievable) principle should be applied with sensitisers.

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Partie B – Report on the assessment of methods for measurement of exposure levels in workplace atmospheres

1 Presentation and discussion of methods for measuring TDI 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 5).

The general principle is as follows:

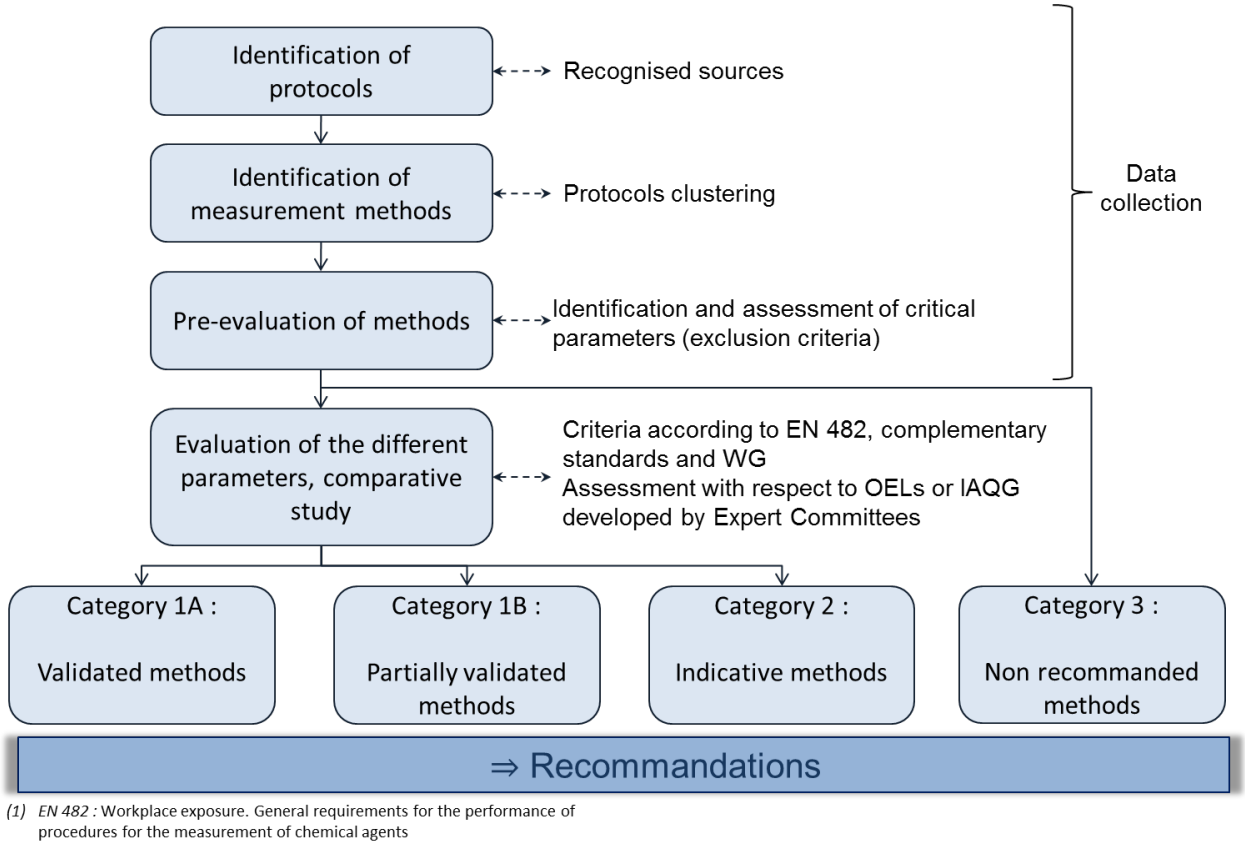

Figure 5 : General principle for the classification of analytical methods (Anses, 2017)[23]

It should be noted that only the evaluation of the measurement methods was carried out with regard to the STEL for the public consultation. The assessment with regard to the 8h-OEL has been supplemented after the public consultation.

[23] 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

Respiratory occupational exposure to TDI occurs in gaseous and/or particulate form. Exposure is related to the vapour pressure: at ambient temperature TDI do not tend to be volatilized but when heated or aerosolised, the volatility rapidly increases. At 75°C, the TDI generic mixed isomers vapour pressure is close to 100 Pa and rises sharply (Perry and Green, 2008). Therefore, the first critical issue for the measurement of TDI is the choice of sampling devices. The working group has decided to only retain the sampling devices able to collect simultaneously both the gaseous and particulate phases of TDI in various workplace atmospheres. The working group has considered that the low vapour pressure of TDI induces a high probability that part of the TDI generated in gaseous form is condensed in very fine droplets or adsorbed on the surface of particles. As a result, the protocols based on gas diffusion sampling badges have not been investigated, as they are not able to collect the particle phase.

Moreover, the working group has not retained the protocols exclusively developed to measure ambient exposure levels in a workplace area and not adapted to personal exposure sampling.

Instruments that monitor real time isocyanates exposure level are produced and commercially available. They are based on spectrometric detection and measurement of either the ionic mobility or the reaction products that result from the reaction of the isocyanate with a reagent impregnated paper. But these instruments only take the vapour phase into account.

To evaluate the personal vapours and aerosols exposure to TDI in workplace atmospheres, numerous indirect measurement methods (active sampling methods) are available. They included the same steps: A TDI sampler able to collect both vapour phase and aerosol of widely varying particles sizes with a dissolution into a solvent contained in an impinger and/or an adsorption onto a filter; a reaction with a derivatising reagent to create a non-volatile derivative which stabilises the species of interest to analyse them by liquid chromatography coupled with spectrometric detection.

Active sampling methods

Depending on the different conditions of exposure, some pump sampling methods must be preferred to collect vapours and aerosols.

Impingers are usually efficient to sample aerosols but particles less than 2 µm in diameter can pass through them. Impregnated glass fibre filters are efficient to collect particles of widely varying sizes and vapours.

NIOSH 5525 (2003), p. 14, shows some examples of exposure scenarios and the corresponding sampler to choose:

- The fibre filters impregnated with derivatising reagent can be used to sample gas phase isocyanates, aliphatic isocyanates aerosols, aromatic isocyanates aerosols with particles diameter < 2 µm ;
- The impingers can be used to sample aromatic isocyanates aerosols with particles diameter > 2 µm ;
- The combination of an impinger with a filter can be used to sample vapours and aerosols of aliphatic or aromatic isocyanates (particles greater or less than 2 µm in diameter).

Impingers are not samplers of the inhalable fraction. These devices, when combined with a downstream filter to collect particles < 2µm, overestimate the fraction collected or may have a similar collection efficiency to a filter impregnated in an IOM sampler (study in autobody shop - NIOSH 5525).
Therefore, methods using an impinger followed by a filter will not be excluded according to this criterion but will be considered as indicative at best.
The choice of sampler – filter, impinger, or impinger and filter in series - is dictated by the exposure scenario and depends on the environment where the samples are taken.

Derivatisation

All methods are based on the derivatisation of the reactive isocyanate groups to lead to products that can be analysed by liquid chromatography. These methods have several objectives: 1) trap gas phase isocyanate in a non-volatile form, 2) block the isocyanate function to avoid side reactions of polymerization or degradation and to graft a chromophore group to promote the UV detection. A lot of derivatising agents have been studied. According to the method used different derivatising agent may be preferred (ISO/TR17737, Henneken et al. 2007, NIOSH NMAM Chapter K)

Derivatising agents and abbreviations:

- 1-(2-methoxyphenyl)piperazine) : 1,2-MPP ;
- 1-(2-pyridyl)piperazine) : 1,2-PP ;
- dibutylamine : DBA ;
- 9-(methylaminomethyl)-anthracene : MAMA ;
- 1-(9-anthracenylmethyl)piperazine : MAP ;
- N-[(4-nitrophenyl) methyl] propylamine: Nitro reagent.

1,2-MPP and 1,2-PP reagents react quantitatively with aromatic isocyanates and are readily soluble in many solvents. They are also both UV and time resistant in solution.

MAMA and MAP reagents give derivatising products highly sensitive to UV detection, i.e. around 2 or 3 times more sensitive than 1,2-MPP or 1,2-PP. On the contrary, the derivatising compounds formed are UV sensible and their solubility is low in many common organic eluents used in liquid chromatography.

DBA reagent is highly reactive in solution, which is often used in impinger sampler.

Analysis by liquid chromatography

After sampling, filters are extracted with a solvent that may contain, or not, a derivatising reagent. For filter and impinger solution, the excess of reagent is generally destroyed with acetic anhydride, the solution filtered and concentrated before injection into the chromatographic system. Liquid chromatographic separation with ultraviolet, fluorimeter, electrochemical, nitrogen or mass detection systems, are the common detection techniques for all methods.

Table 11 presents the measurement methods that were identified and evaluated in the present report.

The details of the seven methods mentioned (sampling support, flow rate, sampling treatment, analysis conditions and validation data) are given in Annex 7.

Table 11: Information on methods identified as relevant for measuring workplace exposure to TDI in light of the 15 min-STEL recommendation

	Methods				
N°	Similar protocols	Sampling	Supports	Desorption/extraction	Analysis
1	ISO 16702 (2008) ; HSE-MDHS 25/4 (2011)	Active	Impinger (1,2 mpp in toluene) + 1,2-MPP impregnated filter	acetic anhydride + ACN or CAN/sodium acetate buffer	HPLC-UV, ECD (DAD, MS/MS)
2	ISO 17734-1 (2013)	Active	Impinger (DBA in toluene) + non impregnated glass fibre filter	ACN	LC-MS (LC-MS/MS, -UV, -ND)
3	ISO 17735 (2009) ; NIOSH 5525 (2003)	Active	Impinger (MAP in butyl benzoate) + MAP impregnated glass fibre filter	Solid-phase extraction : Methylene chloride, acetonitrile/methanol, methanol	HPLC-UV, Fluo
4	ISO 17736 (2010), , IRSST 376	Active	PTFE filter associated with MAMA impregnated glass fibre filter in three-piece cassette 37 mm	PTFE filter : 1,2 mpp in toluene Glass filter : DMF/triethylamine buffer/ACN	HPLC-UV, Fluo
	ISO 17735 (2009), NIOSH 5525 (2003)		MAP impregnated glass fibre filter in closed cassette or IOM	MAP-ACN solution and acetic anhydride	
5	IFA 7120 (2010) ; IFA 7670 (2004) ; MAK Diisocyanate (2006) ; INRS MétroPol 245, 246, 249, 250 (2003) ; ISO 14382 ; OSHA 42 (1983)	Active	1 or 2 glass fibre filters impregnated with 1,2-MPP or 1,2-PP	ACN,THF or ACN/DMSO	HPLC, UV, Fluo
6	NIOSH 5521 (1994)	Active	Impinger with derivatising agent : 1,2-MPP in toluene	Acetylation : Anhydride acetic, evaporation and redissolution in MeOH	HPLC, UV, Fuo, ECD
	NIOSH 5525 (2003)		Impinger with derivatising agent : MAP in butylbenzoate	Solid-phase extraction : Methylene chloride, acetonitrile/methanol, methanol	
	OSHA 18 (1981)		Impinger with derivatising agent : Nitro reagent in toluene	Evaporation, redissolution in methylene chloride	
7	ISO 17734-1 (2013)	Active	Denuder tube impregnated with DBA terminated by a glass fibre filter impregnated with DBA	Methanol/H2SO4/toluene before evaporation, dissolution in toluene, evaporation and final dissolution in ACN	LC, MS, MS/MS

Three other protocols describing a method similar to the method n°6 were also identified, but they involve 2 or 3 impingers in series (MAK HDI TDI (1985), INSHT MTA/MA-034/A95 (1995)) or the use of tryptamine in DMSO (NIOSH 5522). They are therefore dedicated to an ambient measurement of the atmosphere and not evaluated in this report.

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

1.2 Detailed assessment of the methods

For comparison to the 8h-OEL and 15 min-STEL values:

Requirements: Considering the 15 min-STEL recommended by the experts, methods should be validated in the following concentration range:

- 0.1 to 2 *8h-OEL: 0.029 – 0.58 µg.m^{-3} (for the technical regulatory control)
- 0.1 to 2 *15min-STEL: 0.94 – 18.8 µg.m^{-3} (for the technical regulatory control)
- 0.5 to 2 *15-minSTEL: 4.7 – 18.8 µg.m^{-3} (for the monitoring of short exposure)

The following table presents the rating of identified methods relevant to measure worker's TDI exposure (Table 12). The evaluation is described in the following paragraphs.

Remarks:

The assessment methodology was updated during this expertise (Anses, 2020), and concerning substances in mixed phase, a differentiated classification of the sampling method and the analysis method must be carried out.

The evaluation of the TDI measurement methods does not take this update into account. However, this has no impact on the overall assessment of the measurement methods. Indeed, the use of an impinger followed by a filter overestimates the inhalable fraction, and consequently this device is not excluded according to this criterion but is considered as indicative at best.

Table 12 : Rating of monitoring methods for workplace TDI assessment

TDI Monitoring					
Methods		Protocols	8h-OEL	15min-STEL	
			Technical regulatory control	Technical regulatory control	Exposure monitoring
1	Impinger (1,2-MPP in toluene) followed by 1,2-MPP impregnated glass fibre filter	ISO 16702 (2008) ; HSE-MDHS 25/4 (2011)	**3**	**3**	**3**
2	Impinger (DBA in toluene) followed by non-impregnated glass fibre filter	ISO 17734-1 (2013)	**3**(*)	**3**(*)	**3**(*)
3	Impinger (MAP in butyl benzoate) followed by MAP impregnated glass fibre filter	ISO 17735 (2009) ; NIOSH 5525 (2003)	**3**	**2**	**2**
4	PTFE filter associated with glass fibre filter impregnated with MAMA or MAP in closed cassette or IOM	ISO 17736 (2010), ISO 17735 (2009), NIOSH 5525 (2003), IRSST 376	**3**	**3**	**3**
5	1 or 2 glass fibre filters impregnated with 1,2-MPP or 1,2-PP	IFA 7120 (2010) ; IFA 7670 (2004) ; MAK Diisocyanate (2006) ; INRS MétroPol 245, 246, 249, 250 (2003) ; ISO 14382 ; OSHA 42 (1983)	**3**	**3**	**2**
6	Impinger with derivatising agent (1,2 mpp, MAP or nitro reagent)	NIOSH 5521 (1994) ; 5525 (2003) ; INSHT MTA/MA-034/A95 (1995) ; MAK HDI TDI (1985); OSHA 18 (1981)	**3**	**3**	**3**
7	Denuder tube impregnated with DBA terminated by a glass fibre filter impregnated with DBA	ISO 17734-1 (2013)	**3**	**3**	**3**
(*) method classified in category 3 due to a lack of validation data					

The following figure presents the ranges for which the various methods were tested and their limit of quantification for the 8h-OEL and 15 min-STEL value recommended by the experts (Figure 6 and Figure 7). The methods included in category 3, based on exclusion criteria, appear for indicative purposes and therefore are written in italics and not underlined.

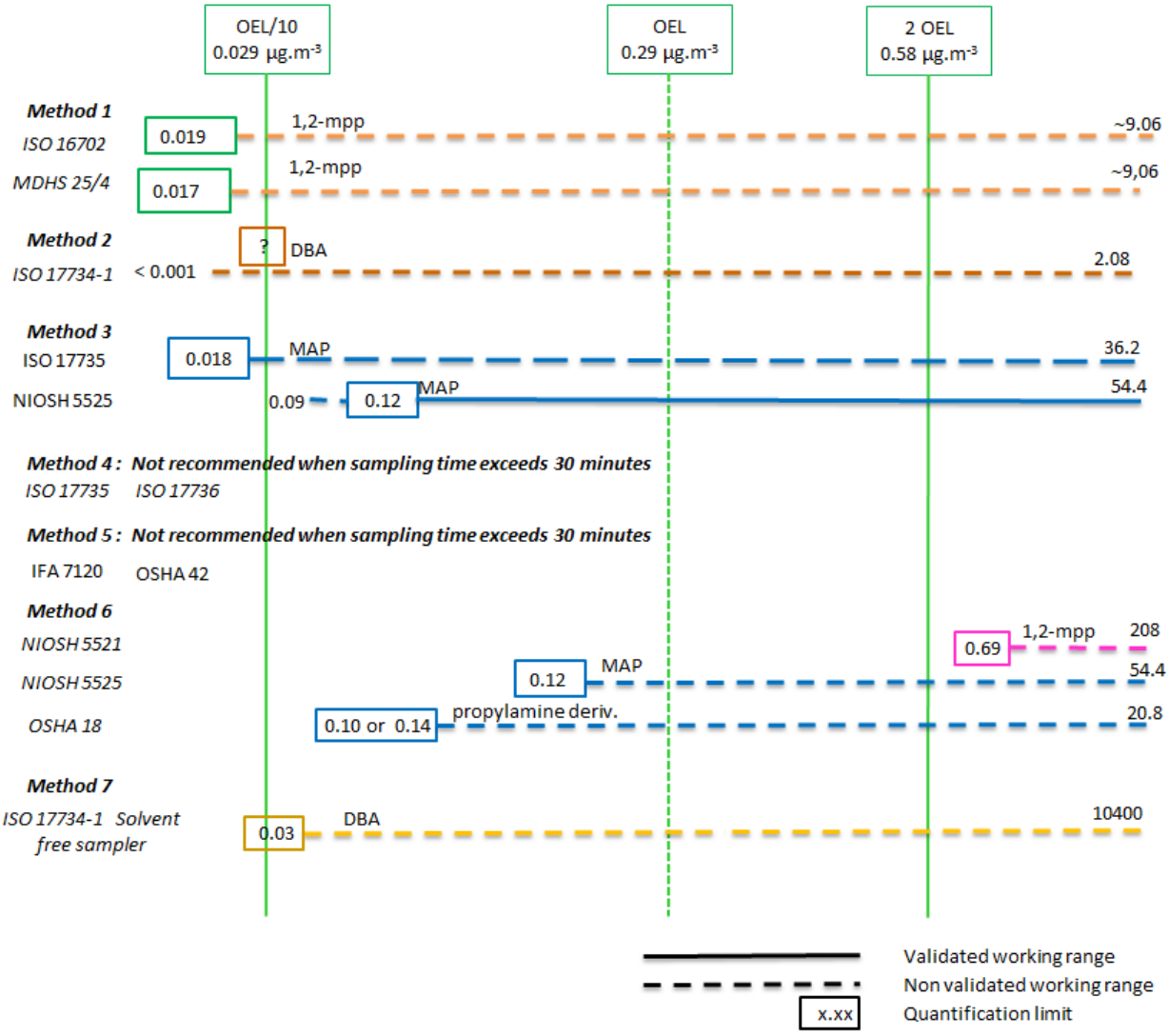

Figure 6 : Working range and quantification limit of the different methods compared to the 8h-OEL

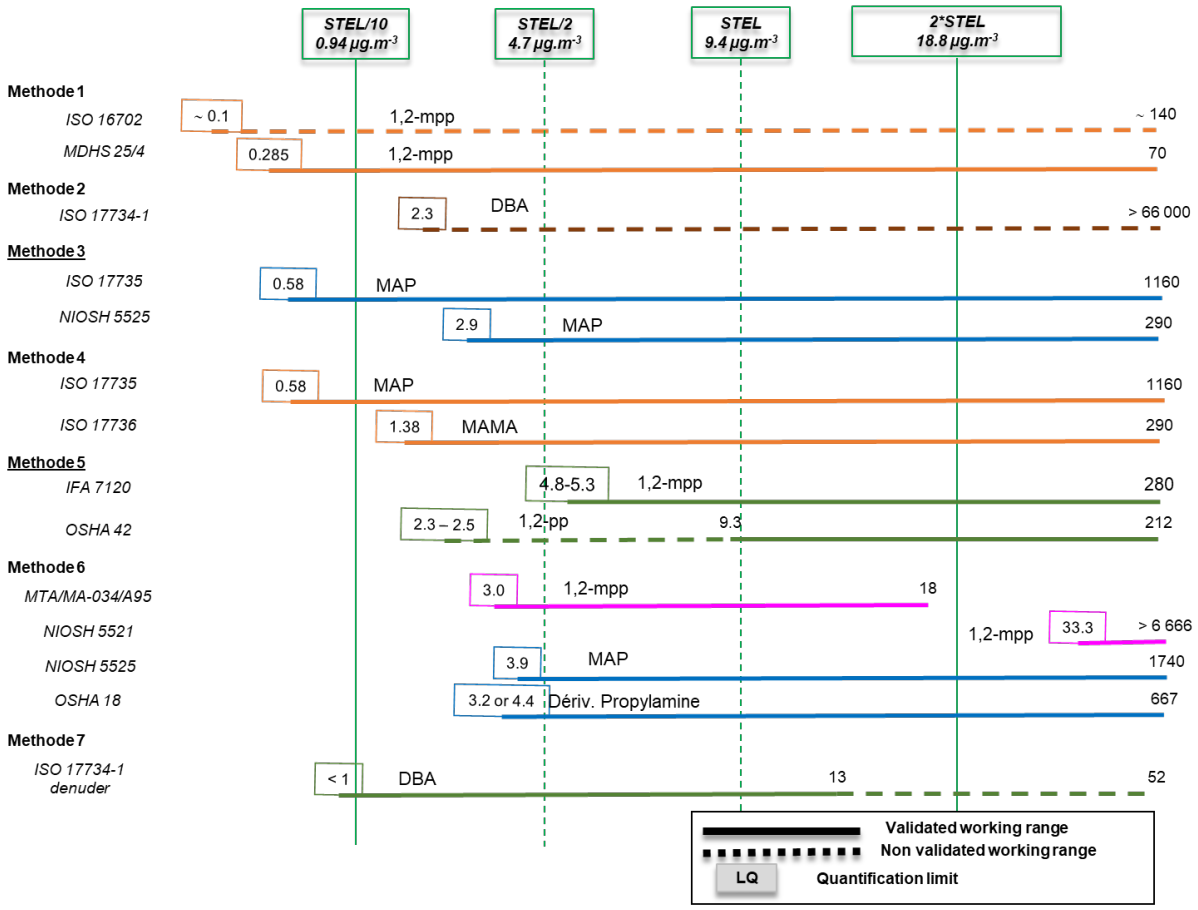

Figure 7 : Working range and quantification limit of the different methods compared to the 15min-STEL

1.2.1 Method 1

Description: Active sampling through an impinger containing 1,2-MPP in toluene followed by a glass fibre filter impregnated with 1,2-MPP, liquid chromatography analysis with ultra-violet, electrochemical or mass detection. The method is described in the ISO 16702 Standard (2011) and MDHS 25/4 protocol (2008).

As the objective of the method is the determination of the total isocyanates concentration in air (µg NCO.m^{-3}), few validation data for TDI are available. The working range and limit of quantification are mentioned, but not specifically for TDI. The sampling efficiency is not mentioned. The expanded uncertainty of the method is estimated between 47 and 50% for TDI (upon the range 0.1 to 2 µg NCO), which is high, close to the maximum allowed by the NF EN 482 Standard requirements for both short and long term reference period.

For these reasons, method 1 is ranked in category 3 and is not recommended for the regulatory control of the 8h-OEL or the 15min-STEL nor for the short term exposure monitoring.

In addition, taking into account the potential health effects of toluene, individual sampling with a individual sampling with an impinger filled with a toluene absorbing solution should be avoided in order to avoid a risk of exposure of workers.

1.2.2 Method 2

Description: Active sampling through an impinger containing DBA in toluene followed by a glass fibre filter non impregnated, liquid chromatography analysis with ultra-violet, nitrogen or mass detection. The method is described in the ISO 17734-1 Standard (2013).

The data about sampling and recovery efficiencies are missing and rank the method in class 3* for the regulatory control of the 8h-OEL and the 15min-STEL, as well as for the short term exposure monitoring.

Like method 1, individual sampling with an impinger filled with a toluene absorbing solution should be avoided in order to avoid a risk of exposure of workers.

1.2.3 Method 3

Description: Active sampling through an impinger containing MAP in butyl benzoate followed by a glass fibre filter impregnated with MAP, liquid chromatography analysis with ultra-violet or fluorimetric detection. The method is described in the ISO 17735 Standard (2009) and NIOSH 5525 protocol (2003).

Sampler: Impinger, containing 15 mL of a solution of MAP 10 µM in butyl benzoate, followed by a glass fibre filter impregnated with MAP. Flow rate 1 $L.min^{-1}$ for impinger sampling.

Sample preparation: Impinger solution is subjected to a solid-phase extraction conditioned first with butyl benzoate then eluted respectively with methylene chloride, acetonitrile (ACN)-methanol mixture 9:1 and pure methanol. The eluate volume is reduced to an exact volume of one mL by using a nitrogen stream. Filter is extracted in field with 5 mL of 1.10^{-4} M MAP in ACN, and acetylated with 5 µL of acetic anhydride after overnight extraction.

Analysis: HPLC UV/Fluorescence detection. Reverse phase Inertsil C8 column 150x4.6 mm, 5 µm, or suitable equivalent column. Flow rate 1.5 $mL.min^{-1}$.mobile phase 65/35 (V/V) ACN-triethylammonium phosphate/formiate (100 mM in both), pH gradient from 6.0 to 1.6. Injection volume of 15 µL. UV detection at 253 nm, fluorescence detection (xenon lamp - ex: 368 nm, em: 409 nm - or deuterium lamp – ex: 254 nm, em: 409 nm -). Calibration with the MAP derivatives of 2,4- and 2,6- TDI.

Linearity of detector response: UV and fluorescence responses are linear in the range 1.5 x 10^{-5} to $1x10^{-8}$ $M.L^{-1}$. UV and fluorescence give similar response for monomer measurement.

Storage stability: Samples are refrigerated immediately upon receipt. Samples containing TDI-MAP gave results 16 to 24% lower than the original values when reanalysed after storage of nine months in freezer (-10°C) (NIOSH 5525).

Maximum capacity: For an impinger followed by a glass fiber impregnated sample, at 1 $L.min^{-1}$ flow rate, the maximum concentration reached is 250 ppb giving 1780 $µg.m^{-3}$ for TDI for a 15 L air sample and 7.8 ppb equivalent to 56.6 $µg.m^{-3}$ for a 480 L air sample (NIOSH 5525). The ISO 17735 standard indicates that a volume of 960 L should be achievable with an IOM sampling cassette at a 2 $L.min^{-1}$ flow rate.

Working range tested: 0.58 to 1160 $µg.m^{-3}$ for a 15 L air sample, equivalent to 0.018 to 36.25 $µg.m^{-3}$ for a 480 L air sample - ISO 17735- ; 2.9 to 1740 $µg.m^{-3}$ for a 15 L air sample equivalent to 0.09 to 54.4 $µg.m^{-3}$ for a 480 L air sample (NIOSH 5525)

Quantification limit: 0.2x3.33 nM NCO giving 3.9 µg.m^{-3} for a 15 L air sample or 0.12 µg.m^{-3} for a 480 L air sample, but the lowest borderline concentration tested for the working range is closed to 2.9 µg.m^{-3} for a 15 L air sample or 0.09 µg.m^{-3} for a 480 L air sample - NIOSH 5525 -. 0.05 nM NCO giving 0.58 µg.m^{-3} for a 15 L air sample or 0.018 µg.m^{-3} for a 480 L air sample (ISO 17735 Standard).

Sampling with recovery efficiencies: No test with a standard vapour atmosphere generator was performed; only solutions of 2,4-TDI-MAP at nominal levels of 42, 127, 425, 1275 and 4249 ng into quartz and glass fibre filters were used with mean recoveries of 91% and no correlation between recovery and spiking levels (NIOSH 5525).

Specificity: The liquid chromatographic system calibrated with derivatised standard matching solutions gives a great specificity to the method.

Interferences: Any compound that reacts with MAP can be separated by altering the pH gradient - NIOSH 5525, ISO 17735 Standard -.

Uncertainty, accuracy: Bias, overall precision and accuracy were not determined in NIOSH 5525. In the ISO 17735 Standard, the expanded uncertainty is 36 % (with a coverage factor of 2) without considering the uncertainty contribution from the collection efficiency.

The protocols associated with this method provide validated data compliant with the metrology requirements guide used by the working group, in particular about working range, maximum capacity, storage stability, information on interfering compounds and expanded uncertainty of the method.

Despite this, the method is not validated for a long sampling time of 480 minutes and a 480 L air sample due to:

- ***An expanded uncertainty of the method estimated greater than the maximum allowed by the NF EN 482 Standard requirements***
- ***A non-conventional evaluation of the sampling and recovery (with spiked liquid derivative TDI solutions on filter);***
- ***The lack of environmental studies.***

The method 3 is ranked in category 3 and is not recommended for 8h-OEL technical regulatory control.

The method is validated for a sampling time of 15 minutes and a 15 L air sample. But because of non-conventional evaluation of the sampling and recovery (with spiked liquid derivatised TDI solutions on filter), the lack of environmental studies, the method is classified as category 2 with regard to the STEL-15 min for the regulatory control and the short term exposure monitoring.

1.2.4 Method 4

Description: Active sampling through a PTFE filter followed by a glass fibre filter impregnated or extracted with MAMA or MAP; PTFE filter extracted with 1,2-MPP; analysis with liquid chromatography, ultra-violet or fluorimetric detection. The method is described in the ISO 17736 Standard (2010), ISO 17735 Standard (2009), NIOSH 5525 (2003) and IRSST 376 protocols.

The filter method is designed to determine short-term (15 min) exposure concentrations of TDI in vapour and aerosols forms. Sampling time can be extended to 8 hours if the exposure is expected to be in vapour form only.

Data about overall uncertainty is available in ISO 17735 and 17736 Standards. Expanded uncertainty values are high, close to or above the NF EN 482 Standard requirements, 36% without taking in account the uncertainty contribution from the collection efficiency – ISO 17735 Standard -, 50% for the measurement of the TDI vapour form and 90% of the TDI aerosol form – ISO 17736 Standard -. For this reason, method 4 is ranked in class 3 for the regulatory control of the 8h-OEL or the 15min-STEL and for the short term monitoring.

1.2.5 Method 5

Description: Active sampling through one or two glass fibre filters impregnated with 1,2-MPP or 1,2-PP, liquid chromatography analysis with ultra-violet or fluorimetric detection. The method is described in several similar protocols and standards : IFA 7120 (2010), IFA 7670 (2004), MAK diisocyanate (2006), INRS MetroPol 245 – 246 – 249 – 250 (2003), OSHA 42 (1983) and ISO 14382 Standard (2012). The different protocols use different sampling devices: open face cassette sampler, closed face cassette sampler or GSP sampler. However this does not modify the validation data attached to this method since the collection efficiency is not studied. Sampling should be performed with a device able to collect the inhalable fraction. The filter method is designed to determine short-term (15 min) exposure concentrations of TDI. If the exposure is not expected to be in vapour form only, then sampling time can not be extended to 8 hours. IFA 7120, IFA 7670 provide data for sampling times of one and two hours respectively, but for TDI taken in vapour form. In presence of aerosols both protocols advise to use method 3, i.e. sampling through an impinger followed by a filter.

The MAK diisocyanate protocol does not present any validation data related to TDI.

Sampler: Glass fibre filter 37 mm impregnated with 1,2-MPP. Flow rate 3.5 $L.min^{-1}$ (GSP sampler) - IFA 7120 & 7670 -. Glass fibre filter 25 mm impregnated with 1,2-MPP. Flow rate 0.2 to 2 $L.min^{-1}$ (closed face cassette sampler) - MAK diisocyanates - 2 quartz fibre filters 37 mm impregnated with 1,2-MPP. Flow rate 0.2 to 2 $L.min^{-1}$ (closed face cassette sampler) (INRS MetroPol 245 – 246 – 249 – 250). Glass fibre filter 37 mm impregnated with 1,2-PP. Flow rate 1 $L.min^{-1}$ (open face cassette sampler) (OSHA 42 & ISO 14382 Standard).

Sample preparation: Filters are extracted with 3 to 5 mL of solvent, and then acetylated to destroy the excess reagent with 5 µL of acetic anhydride.

Extraction solvent: ACN + 1,2-MPP for IFA protocols, ACN for MAK, MetroPol, ACN + dimethylsulfoxide for OSHA and ISO Standard protocols. The extraction solvent volume is evaporated and the residue is recovered in ACN or tetrahydrofuran (MAK and MetroPol).

Analysis: HPLC UV/Fluorescence detection. Reverse or normal HPLC, ODS, nucleosil, C8, 250x4.6 mm, 5 µm, or suitable equivalent column. Flow rate 1 to 1.2 $mL.min^{-1}$. Mobile phase buffer ammonium solution/ACN or methanol/water. Injection volume 10-25 µL. UV detection at 245-250 or 254 nm, fluorescence detection with deuterium lamp (excitation: 240 nm, emission: 380-390 nm). Calibration with the 1,2-MPP derivatives of 2,4- and 2,6- TDI, 1,2-PP derivatives for OSHA 42 and ISO 14382 Standard protocols.

Linearity of detector response: UV and fluorescence responses are linear in the range 0.3 to 3 µg.mL^{-1} (IFA 7120) and 0.05 to 5 µg.mL^{-1} (INRS MetroPol)

Storage stability: No significant variation at room temperature for 15 days (IFA 7120), at 4 °C for 14 days (MAK diisocyanate), 3 weeks if the excess of reagent is not destroyed (INRS MetroPol).

Maximum Capacity: > 280 µg.m^{-3} for a 52.5 L air sample (IFA 7120); maximum of 5.6 mg.m^{-3} for a 15 L air sample (ISO 1438); maximum of 560 µg.m^{-3} for a 15 L air sample (OSHA 42).

Working range tested: 4.8 to 280 µg.m^{-3} for a 52.5 L air sample (IFA 7120); 9.3 to 212 µg.m^{-3} for a 15 L air sample (OSHA 42).

Quantification limit:

	Volume - 15 min sampling (L)	LOQ (µg.m^{-3})
IFA 7120	52.5	4.8 for 2,4 TDI 5.2 for 2,6 TDI
OSHA 42 ISO 14382	15	2.5 for 2,4 TDI 2.3 for 2,6 TDI

LOQ are sufficient to achieve the STEL/2 value but not enough for the STEL/10 value.

Sampling with recovery efficiencies: 96 – 97 % in the working range 4.8 to 280 µg.m^{-3} for a 52.5 L air sample, no precision about the technique used to measure these efficiencies (IFA 7120). By vapour spiking, 78 %RH, at TDI concentration levels of 180 and 210 µg.m^{-3}, respectively 80 and 89.7% of recovery 2,4- and 2,6-TDI. Liquid spiking, 80 %RH, same concentration with glass fibre filters pre-moistened, 100 and 81.5% of recovery (OSHA 42).

Specificity: The liquid chromatographic system calibrated with derivatised standard matching solution gives a great specificity to the method.

Interferences: Any compound that react with 1,2-MPP or 1,2-PP can be separated by modifying the pH gradient. Benzaldehyde identified as 2,4-TDI interfering (OSHA 42).

Uncertainty, accuracy: In the IFA 7120 protocol, relative standard deviation between 5 to 100 µg.m^{-3}, 2,4-TDI: 2.9 to 3.3 %; 2,6-TDI: 2.2 to 3.4%. Overall precision, 2,4-TDI: 16.5 to 16.9%; 2,6-TDI: 15.8 to 16.6%. OSHA 42, overall precision at 95% confidence level, 2,4-TDI 13.5% and 2,6-TDI 14.9%. The ISO 14382 Standard provides an expanded uncertainty, by using a coverage factor of 2, of 20 %, without taking into account the uncertainty contribution from the collection efficiency.

Note : For TDI, and more generally for aromatic isocyanates, literature reports that filter sampling is not as efficient as impinger sampling for the following reasons:

- *The isocyanate adsorbed on particulate matter reacts on the filter surface with the reagent at the impact point. For large size particles, the filter is locally reagent depleted and isocyanate will be only partially derivatised. The same phenomenon applies when the isocyanate covers the whole surface of the particle (Streicher et al. 2000).*
- *In presence of polyalcohol and/or curing agent in the atmosphere, the polymerization reaction with isocyanate competes directly with the derivatising reaction. Several*

articles highlight the concentration difference measured with filter sampling in laboratory using an isocyanate atmosphere generator versus real atmosphere in production workshops of polyurethane foam (Mattsson et al. 2008, Streicher et al. 2000, Guglya 2000). The filter sampling systematically underestimates the TDI level. A model developed by Sennbro et al. (2004) gives a 2.5-fold underestimation of the true exposure levels for 7h30 sampling. They determined an underestimation factor of 1.7 for the 2,4-TDI and 1.5 for the 2,6-TDI for 4h sampling whereas these values were calculated to be 1.4 for the 2,4-TDI and 1.3 for the 2,6-TDI by Mattsson et al. (2008). The hypothesis most formulated is that the kinetic of the derivatising reaction is too low compared to polymerization reaction kinetic for vapour phase and there is also a chemical degradation of the derivatised compounds over time.

But a sampling period under 20 minutes and a filter desorption in a solution containing the reagent, in the field just after the sampling, allows this bias to be greatly reduced and minimized (White et al. 2012, Tinneberg et al. 1996, Streicher et al. 1994, and ISO 17736; ISO 17737; MA 25/4; NIOSH 5525).

The method is not validated for a long sampling beyond to 20 minutes because of the underestimation due to the chemical degradation of the derivative compounds over time. The method 5 is not recommended for 8h-OEL technical regulatory control and is ranked in category 3.

For a sampling time of 15 minutes and a maximum of a 15 L air sample, the numerous protocols that composed this method provide validated data compliant with the metrology requirements guide used by the working group, in particular about working range, maximum capacity, storage stability, sampling and recovery efficiencies, influence of environmental conditions and expanded uncertainty of the method. But, considering the important elements set out in the note and despite the limited information on interference the working group considers that the method is classified as category 2 with regard to the 15-min-STEL for the short term exposure monitoring and is classified as category 3 for the regulatory control, as the quantification limit is not enough to achieve the STEL/10 value.

1.2.6 Method 6

Description: Active sampling through single or multiple impingers in series containing derivatising reagent, 1,2-MPP; 1,2-PP or MAP; liquid chromatography analysis with ultra-violet, fluorimetric or electrochemical detection. The method is described in the NIOSH 5521 (1994), NIOSH 5525 (2003) and OSHA 18 (1981) protocols.

This method is ranked in class 3 for 8h-OEL and 15min-STEL measurements for the following reasons:

- ***when a single impinger is used, the aerosol fraction sampled is not compliant with the conventional inhalable fraction: the particles less than 2 µm diameter can pass through it ;***
- ***only considering the regulatory control, the quantification limit is not enough to achieve the STEL/10 value.***

Furthermore the exposure of the worker through personal sampling with an impinger filled with a toluene or xylene absorbing solution should be avoided as they are dangerous chemical agents.

1.2.7 Method 7

Description: Denuder tube impregnated with DBA terminated by a 13 mm glass fibre filter impregnated with DBA sampled at the flow rate of 0.2 L.min-1. Prolonged sampling (>8 h) can be performed without loss of sampling performance. Filters are extracted consecutively with H_2SO_4, methanol and toluene; the toluene phase is centrifuged three times before evaporation and, at last, taken up with ACN. Analysis conducted by liquid chromatography coupled with mass or mass/mass detection. This method is described in the ISO 17734-1 standard.

In addition to the fact that the collected fraction is unknown (inlet hole with 8 mm diameter and a flow rate of 0.2 L.min^{-1}) numerous validation data are lacking such as capacity limit, sampling and recovery efficiencies, and uncertainty data of the method. For all these reasons, this method is ranked in class 3 for the regulatory control of the 8h-OEL and the 15min-STEL as well as for the short term exposure monitoring.

2 Conclusions and recommendations

Seven methods of 2,4- and 2,6-TDI measurement in workplace atmosphere have been assessed according to the criteria set out in particular in NF EN 482 Standard.

The methods have been selected and grouped according to the sampling device, impinger, filter or denuder tube, and for some of them, to the derivatising reagent used. :

- Method 1: Impinger containing 1,2-MPP in toluene followed by a glass fibre filter impregnated with 1,2-MPP ;
- Method 2: Impinger containing DBA in solution in toluene followed by a glass fibre filter non impregnated ;
- Method 3: Impinger containing MAP in solution in butyl benzoate followed by a glass fibre filter impregnated with MAP ;
- Method 4: PTFE filter associated with glass fibre filter impregnated with MAMA in inhalable sampler;
- Method 5: 1 or 2 glass fibre filters impregnated with 1,2-MPP or 1,2-PP in inhalable sampler
- Method 6: Impinger alone containing derivatising reagent (1,2-MPP; 1,2-PP or MAP)
- Method 7: Denuder tube impregnated with DBA terminated by a glass fibre filter impregnated with DBA.

For all of the methods, the analysis is similar: liquid chromatography with normal or reverse phase, UV, fluorimetric, electrochemical, nitrogen or mass detection.

Sampling should be performed with a device able to collect the inhalable fraction.

Nevertheless, even if impingers are not samplers of the inhalable fraction, methods using these devices combined with a downstream filter to collect particles < 2 µm weren't excluded because they may have a similar collection efficiency (NIOSH 5525) or they overestimate the fraction collected.

The choice of sampler – filter, impinger, or impinger and filter in series - is dictated by the exposure scenario and depends on the environment where the samples are taken.

The assessment methodology was updated during this expertise (Anses, 2020), and concerning substances in mixed phase, a differentiated classification of the sampling method and the analysis method must be carried out.

The evaluation of the TDI measurement methods does not take this update into account. However, this has no impact on the overall assessment of the measurement methods. Indeed, the use of an impinger followed by a filter overestimates the inhalable fraction, and consequently this device is not excluded according to this criterion but is considered as indicative at best.

Considering the validation data provided in the various protocols:

- Method 1 has been classified in category 3 for the regulatory control of the 8h-OEL and of the 15-min STEL, as well as for the short term exposure monitoring because essential criteria for validation are lacking or inappropriate and the expanded uncertainty is close to the maximum allowed by the NF EN 482 Standard requirements.
- Method 2 has been classified in category 3* for the regulatory control of the 8h-OEL and of the 15-min STEL, as well as for the short term exposure monitoring because essential criteria for validation are lacking.
- Method 3 has been classified in:

  - category 3 for the regulatory control of the 8h-OEL because the expanded uncertainty is greater than the maximum allowed by the NF EN 482 Standard requirements, because of a non-conventional evaluation of certain criteria and of the lack of environmental studies.
  - category 2 for the regulatory control of the STEL- 15min and for the short term exposure monitoring, as result of non-conventional evaluation of the sampling and recovery and of the lack of interfering and environmental studies. The method covers the concentration range from 1/10 to 2* STEL-15 min value, 0.94 to 18.8 µg.m^{-3}.

- Method 4 has been classified in :
  - category 3 for the regulatory control of the 8h-OEL because the filter method is only designed to determine short-term (15 min) exposure concentrations of TDI in vapour and aerosols forms.
  - category 3 for the regulatory control of the 15min-STEL and for the short term exposure monitoring because the expanded uncertainty is close or greater than the maximum allowed by the NF EN 482 Standard requirements.
- Method 5 has been classified in:
  - category 3 for the regulatory control of the 8h-OEL because the filter method is only designed to determine short-term (15 min) exposure concentrations of TDI in vapour and aerosols forms. A chemical degradation of the derivatives compounds over time may lead to an underestimation.
  - category 2 for the short term exposure monitoring and in category 3 for the regulatory control of the 15min-STEL. The method covers the concentration range from 1/2 to 2* STEL-15 min value, 4.7 to 18.8 µg.m^{-3}, but does not cover the concentration range from 1/10 to 2* STEL-15 min value.

Method 5, filter sampling without the use of an impinger, offers the benefit to be the most practical method but it requires to put the filter(s) in a jar with the desorption solvent immediately after sampling in the field. The TDI derivatives generated from 1,2-MPP and 1,2-PP are stable over time and light insensitive. Standards 2,4- and 2,6-TDI derivatives in solution are commercially available to calibrate the detectors. They produce a suitable response with UV-detector, fluorimeter, electrochemical detector or mass spectrometer. Some protocols, ISO 17736 and ISO 17737 Standard, HSE MDHS 25/4 and NIOSH 5525, indicate that, for a 15 minutes period sampling, this method is the most appropriate.

- Method 6 has been classified in category 3 for the regulatory control of the 8h-OEL and the 15-min STEL, as well as for the short term exposure monitoring because the aerosol fraction sampled is not compliant with the conventional inhalable fraction and the quantification limit not sufficient.
- Method 7 has been classified in category 3 for the regulatory control of the 8h-OEL and the 15-min STEL, as well as for the short term exposure monitoring because the aerosol fraction sampled is unknown and essential criteria for validation are lacking.

In conclusion, the experts recommend method 3 as indicative for the regulatory control of the 15min-STEL, and methods 3 and 5 as indicative for the monitoring of short-term exposures (Table 13).

The implementation of method 3, involving the individual wearing of glass impingers, may be problematic in the field due to the risk of inhalation exposure of the worker, the risk of breakage of the impinger and the discomfort caused by wearing it. The use of a micro-impinger for personal sampling reduces these risks.

The group recommends the development of a method validated according all its metrological requirements for the technical regulatory control of the 8h-OEL and 15min-STEL.

Table 13: Recommended methods for TDI assessment in workplace atmosphere in light of the 8h-OEL and 15min-STEL

TDI Monitoring					
Methods		Protocols	8h-OEL	15min-STEL	
			Technical regulatory control	Technical regulatory control	Short Exposure monitoring
3	Impinger : MAP in butyl benzoate followed by glass fibre filter impregnated with MAP Analysis by HPLC/UV/Fluorescence	ISO 17735 (2009) ; NIOSH 5525 (2003)	**3** **(not recommended)**	**2**	**2**
5	1 or 2 glass fibre filters impregnated with 1,2-MPP or 1,2-PP, in inhalable sampler Analysis by HPLC/UV/Fluorescence	IFA 7120 (2010) ; IFA 7670 (2004) ; MAK Diisocyanate (2006) ; INRS MétroPol 245, 246, 249, 250 (2003) ; ISO 14382 ; OSHA 42 (1983)	**3** **(not recommended)**	**3** **(not recommended)**	**2**

3 Bibliography

3.1 Protocols and Standards

Date of inventory: May 2018

FD ISO/TR 17737 Juin 2013 - Atmosphères des lieux de travail - Lignes directrices pour la sélection des méthodes analytiques d'échantillonnage et d'analyse des isocyanates dans l'air (Workplace atmospheres - Guidelines for selecting analytical methods for sampling and analysing isocyanates in air)

NF EN 482 + A1 Novembre 2015 : Exposition sur les lieux de travail - Exigences générales concernant les performances des procédures de mesure des agents chimiques (Workplace exposure. General requirements for the performance of procedures for the measurement of chemical agents)

NF ISO 16702 Octobre 2008 - Qualité de l'air des lieux de travail - Dosage des groupements isocyanates organiques totaux dans l'air par dérivatisation avec la 1-(2-méthoxyphényl)pipérazine et par chromatographie en phase liquide (Workplace air quality - Determination of total organic isocyanate groups in air using 1-(2-methoxyphenyl)piperazine and liquid chromatography)

HSE MDHS 25/4 : HSE - Methods for the Determination of Hazardous Substances (MDHS) guidance – MDHS 25/4 - Organic isocyanates in air - Laboratory method with derivatisation in situ either on treated glass fibre filters or in solution using impingers with a treated back-up filter in series, followed by high-performance liquid chromatography analysis – December 2014 (http://www.hse.gov.uk/pUbns/mdhs/pdfs/mdhs25-4.pdf, accessed May 2018)

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INSHT - MTA/MA-034/A95 - Determinación de isocianatos orgánicos (2,6 y 2,4-toluen-diisocianato, hexametilendiisocianato, 4,4'-difenilmetano-diisocianato) en aire - Método de

derivación y doble detección ultravioleta y electroquímica / Cromatografía líquida de alta resolución

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IRSST 376 - Workplace Air Contaminants Sampling Guide - IRSST Method 376 : Toluene diisocyanate (TDI) (isomers mixture)

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ISO 17734-2:2013 December 2013 - Determination of organonitrogen compounds in air using liquid chromatography and mass spectrometry - Part 2: Amines and aminoisocyanates using dibutylamine and ethyl chloroformate derivatives

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(http://www.cdc.gov/niosh/docs/2003-154/pdfs/5525.pdf, accessed May 2018)

OSHA 18 – OSHA Sampling and analytical methods – Diisocyanates 2,4-TDI and MDI – method 18 : February 1980.

(https://www.osha.gov/dts/sltc/methods/organic/org018/org018.html, accessed May 2018)

OSHA 42 – OSHA Sampling and analytical methods – Diisocyanates : 1,6-Hexamethylene Diisocyanate (HDI), Toluene-2,6-Diisocyanate (2,6-TDI), Toluene-2,4-Diisocyanate (2,4-TDI) - method 42 : February 1983 - March 1989 (Revised)

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3.2 Publications

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Anses (2020). RAPPORT de l'Anses relatif à la méthodologie d'évaluation des méthodes de mesure dans l'air des lieux de travail et l'air intérieur, Maisons-Alfort. 52 p.

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Date de validation du rapport d'expertise collective par le CES « Valeurs sanitaires de référence » : 10 décembre 2020

Signature :

Maisons-Alfort, le ,

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

F Michiels
Président du CES

ANNEXES

Annex 1: Relevant epidemiological studies selected by OEHHA, US EPA and their limitations according to Anses's experts

Study	Limitations
Daftarian et al. 2000:	the results of this study cannot determine conclusively whether there is an increased prevalence of asthma in this workforce and whether TDI exposure is responsible for the apparently increased prevalence identified
Omae et al. 1992 (a,b)	the followed follow-up period (<5 years,) was insufficient to provide evidence of an accelerated rate of decline in FEV1 among TDI-exposed employees.
Belin et al. 1983	Co-exposition with volatile amines
Clark et al. 1998, 2003	the annual declines in FEV_1 and FVC were not related to exposure to TDI, and were typical of those measured in other populations not exposed to TDI.
Jones et al. 1992	the annual declines in FEV_1 and FVC were not related to exposure to TDI, and were typical of those measured in other populations not exposed to TDI.
Diem et al. 1983	the followed follow-up period (<5 years,) was insufficient to provide evidence of an accelerated rate of decline in FEV1 among TDI-exposed employees.
Ott et al. 2003	Annual FEV_1 loss not associated with exposure
Bodner 2001	the followed follow-up period (<5 years,) was insufficient to provide evidence of an accelerated rate of decline in FEV1 among TDI-exposed employees.
Karol (1981)	the followed follow-up period (<5 years,) was insufficient to provide evidence of an accelerated rate of decline in FEV1 among TDI-exposed employees.

Annex 2: Effects on the respiratory tract and eyes as a result of exposure to constant and determined concentrations of TDI in human volunteers (translated and summarised from the DFG (2003)).

Increase in irritation of the respiratory

Short-term exposure (30 min)	**Concentrations tested (ppm)**									
	0.01	**0.02**	**0.05**	**0.08**	**0.1**	**0.2**	**0.3**	**0.5**	**1.3**	
6 healthy volunteers	No odour No irritation	Odour threshold	Irritation threshold	Slight conjunctival irritation Tingling in the nose	Slight irritation of the eyes, nose and throat	Pronounced irritation of the eyes	Burning sensation in the eyes and nose	Severe and immediate irritation of the conjunctival and nasal mucous membranes, as well as the throat, persists for 24 h after exposure	Pronounced lacrimation, conjunctival hyperaemia, coughing and catarrhal symptoms, persist for several hours after exposure /	Henschler et al. (1962) Bayer 1970 Brugsch and Elkins 1963 Woolrich 1982
15 healthy and 15 asthmatic subjects	Asthma in 1/15 people	Increase in the resistance of the respiratory tract in 5/15 people								Baur et al. 1994 Fruhmann et al. 1987

Annex 3: Assessment of methodology for measuring atmospheric levels

Studies	Quality of metrological analyses TDI	Co-exposition
Littorin et al. 2007	**++**	MDI, IPDI
Clark et al. 2003	**++**	
Diem et al. 1982	**++**	Phosgene
Ott et al. 2000	**+ (++)**	Phosgene
Bodner et al. 2001	**+**	Phosgene
Wegman et al. 1982	**+**	
Venables et al. 1985	**-**	

Assessment of methodology for measuring atmospheric levels for the Vandenplas et al 1999 study:

In this study, the atmospheric levels of TDI were underestimated for at least 2 reasons:

1- The reliability of the MDA model used because it is a colorimetric reaction on impregnated paper tape. For HDI, Dharmarajan et al. indicated that the under-estimation is around 50% of the true concentration (when it is measured by a method by derivation and HPLC) (Dharmarajan et al. 1980).

2- Vandenplas et al. indicated that the tip of the sampling tube was located at a distance of ~50 cm from the subjects' mouth. The vapors of TDI sampled will be adsorbed on the tube so it is probable that the device will measure only 2,6-TDI less reactive that 2,4-TDI (Vandenplas et al. 1999).

In conclusion, the exposure measure is underestimated. However, the level of error is lower than that related to the use of assessment factors.

Annex 4: Analysis of Diem's study

In the Diem et al. (1982) study, workers were divided into two groups, those with low and high cumulative exposures to TDI (> (limit between groups = 68.2 ppb-・month<). When comparing the two groups, the authors reported a significant decline in lung function among never smokers in the high exposure group, but not among previous or current smokers. The 68.2 ppb ppb・months corresponds to the division point (68.2 =1.1 ppb x 62 months) and was chosen because it corresponds with the calculated cumulative exposure of a worker who spent all 62 months from the time of initial TDI production to the end of the study in the lowest time weighted average exposure category (1.1 ppb x 62 months).

Moreover, the decline in FEV_1 was observed in several studies and in follow-up studies of workers who continued to work after their diagnosis of occupational asthma.

Accelerated lung function decline is not seen as a sensitive predictive marker of asthma, as asthma is characterised by variable airflow limitation, and lung function may not be decreased permanently. The time of day at spirometry may therefore have a large impact on lung function.

Annex 5: OHAT approach

The OHAT approach for Systematic Review and Evidence Integration[24] provides an approach for assessing the study quality or “risk of bias.” The tool applies a parallel approach to the evaluation of the risk of bias for human and experimental animal studies.

OHAT (Office of Health Assessment and Translation) approach has been developed by the National Toxicology Program (NTP, 2015). The aim is to improve transparency and consistency in the analysis of studies. It includes a 7-step framework for systematic review and evidence integration (annex 1a).

Epidemiological studies on respiratory effects and isocyanate exposure were summarised by DECOS in their draft opinion “Di- and triisocyanates”. Most studies, however, have addressed the effect of isocyanate exposure on lung function (usually measured as FEV1 and FVC). Finally, DECOS reported 42 studies with critical concentrations associate with lung function effect.

These 42 studies were analysed *via* an approach adapted from OHAT.

Step 1 to 3 lead to the identification of relevant studies following criteria as the studied Population, type of Exposure, Comparator tools and selected Outcome (PECO statement).

- **P**opulation: Professional (only studies with more than 100 subjects were included)
- **E**xposure: only TDI exposure
- **C**omparator: exposure based on workstation
- **O**utcome: FEV1

On 42 analysed studies, only 23 concern professional TDI exposure only, 12 include more than 100 subjects and 10 mesure the decrease of FEV1. Therefore, only 6 studies comply these 3 criteria and passed stage 3.

[24] https://ntp.niehs.nih.gov/pubhealth/hat/noms/index-2.html

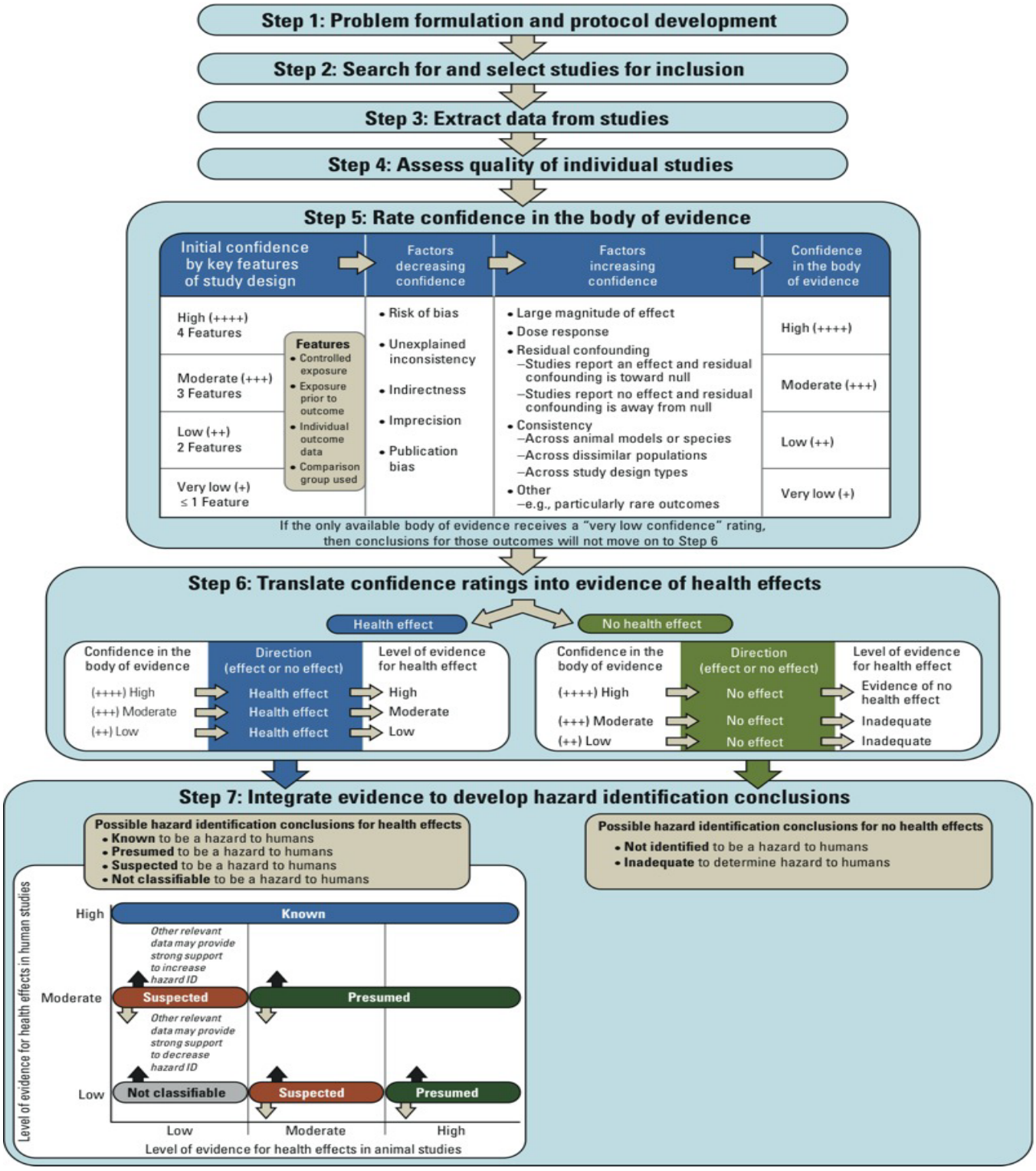

Figure 8 Summary of OHAT approach (handbook, 2015)

Step 4:

Selection Bias	Diem et al. 1982	Bodner et al. 2001	Littorin et al. 2007	Ott et al. 2000	Wegman et al. 1982	Wegman et al. 1977
Did the study design or analysis account for important confounding and modifying variables ?	(++)	(+)	(+)	(+)	(-)	(-)
Can we be confident in the exposure characterization ?	(++)	(++)	(+)	(-)	(++)	(+)
Can we be confident in the outcome assessment ?	(+)	(+)	(-)	(+)	(+)	(-)

++	***Definitely Low** risk of bias*: There is direct evidence of low risk of bias practices (May include specific examples of relevant low risk of bias practices)
+	***Probably Low** risk of bias*: There is indirect evidence of low risk of bias practices OR it is deemed that deviations from low risk of bias practices for these criteria during the study would not appreciably bias results, including consideration of direction and magnitude of bias
– NR	***Probably High** risk of bias*: There is indirect evidence of high risk of bias practices OR there is insufficient information (e.g., not reported or "NR") provided about relevant risk of bias practices
—	***Definitely High** risk of bias*: There is direct evidence of high risk of bias practices (May include specific examples of relevant high risk of bias practices)

Step 5:

Features for initial confidence rating

Table 8. Study Design Features for Initial Confidence Rating					
Study Design	**Controlled Exposure**	**Exposure Prior to Outcome**	**Individual Outcome Data**	**Comparison Group Used**	**Initial Confidence Rating**
Human controlled trial[a]	likely	likely	likely	likely	high
Experimental animal	likely	likely	likely	likely	high
Cohort	unlikely	may or may not	likely	likely	low to moderate
Case-control	unlikely	may or may not	likely	likely	low to moderate
Cross-sectional[b]	unlikely	unlikely	likely	likely	low
Ecologic[b]	unlikely	may or may not	may or may not	likely	very low to moderate
Case series/report	unlikely	may or may not	likely	unlikely	very low to low

[a]Human controlled trial study design as used here refers to studies in humans with a controlled exposure, including randomized controlled trials and non-randomized experimental studies.

[b]Cross-sectional study design as used here refers to population surveys with individual data (e.g., NHANES), as distinct from population surveys with aggregate data on participants (i.e., ecologic studies).

	Diem et al. 1982	Bodner et al. 2001	Littorin et al. 2007	Ott et al. 2000	Wegman et al. 1977	Wegman et al. 1982
Number of key features of studies	2	3	3	3	2	2
Initial confidence rating	Low (++)	Moderate (+++)	Moderate (+++)	Moderate (+++)	Low (++)	Low (++)

Factors increasing/decreasing confidence

Downgrade Confidence Rating	Diem et al. 1982	Bodner et al. 2001	Littorin et al. 2007	Ott et al. 2000	Wegman et al. 1982	Wegman et al. 1977
1- Risk of bias of the body of evidence based on step 5	0	0	-1	-1	-1	-1
2- Unexplained inconsistency	0	0	0	0	0	0
3- Indirectness	0	0	0	0	0	-1
4- Imprecision	-1	-1	-1	0	-1	-1
5- Publication bias	-1	-1	-1	-1	-1	-1
Total 1	**-2**	**-2**	**-3**	**-2**	**-3**	**-4**
Upgrade confidence rating	Diem et al. 1982	Bodner et al. 2001	Littorin et al. 2007	Ott et al. 2000	Wegman et al. 1982	Wegman et al. 1977
a- Large magnitude of effect	1	1	0	0	1	0
b- Dose response	1	0	0	0	1	1
c- Residual confounding increases confidence	0	0	1	0	0	0
d- Cross-species/population/study consistency	0	0	0	1	0	0
Total 2	**2**	**1**	**1**	**1**	**2**	**1**
Total	**0**	**-1**	**-2**	**-1**	**-1**	**-3**

Confidence	Moderate (+++)	Low (++)	Very low (+++)	Low (++)	Low (++)	Very low (+)

Annex 6: Review of genotoxicity city test based on Prueitt *et al.* 2013

Table I

in vitro studies											
Ames test											
Ref.	Substance	Solvent	Concentration (µg/plate)	Metabolic activation	*Salmonella typhimurium*						*E.coli*
					TA 97	TA 98	TA 100	TA 1535	TA 1537	TA 1538	WP2uvrA
Anderson et Styles 1978	2,4 TDI	DMSO [25]	4-2500	Yes		-	-	-		-	
Andersen et al. 1980	80/20 TDI	DMSO	125-1000	No		-	-		-	-	
				Yes		+ (125)	+		-	+	
JETOC 1996	2,4 TDI	DMSO	20-5000	No		-	-	-	-		-
				Yes		-	+ (200)	-	-		-
	2,6 TDI	DMSO	20-5000	No		-	-	-	-		-
				Yes		+ (200)	+ (200)	-	-		-
JMHLW 2001	80/20 TDI	DMSO	78.1-5000	No		-	-	-	-		-
			9.77-5000	Yes		-	+	-	-		-
	2,4-TDI	EGDE	50-1000	No		-	-	-	-		

[25] DMSO : dimethylsulfoxide

See et al. 1999				Yes		+ (100)		-	+ (200)		
	2,6-TDI	EGDE	150-4800	No		-	-	-	-		
				Yes		+ (300)		-	-		
	80/20 TDI	EGDE	125-2000	No		-	-	-	-		
				Yes		+ (125)	+ (250)	-	+ (1000)		
Zeiger et al. 1987 (NTP 1986)	2,4-TDI	DMSO	33-3333	No	-	-	-	-			
				Yes	-	+ (100)	+ (333)	-			
	2,6-TDI	DMSO	3-10 000	No		-	-	-	-		
				Yes (rat)		+ (33)(x2)	-	-	-		
				Yes (hamster)		+ (10)(x2)	+ (333) (x2)	-	-		
	80/20 TDI	DMSO	3-10 000	No		-	-	-	-		
				Yes (rat)		+ (100)(x2.5)	-	-	-		
				Yes (hamster)		+ (100)(x2)	-	-	-		

Table II

In vitro studies											
Ames test											
Ref.	Substance	Solvent	Concentration (µg/plate)	Metabolic activation	*Salmonella typhimurium*						*E.coli*
					TA 97	TA 98	TA 100	TA 1535	TA 1537	TA 1538	WP2uvrA
NTP 1980	2,4-DAT	DMSO	100-10000	No		-	-	-	-		
				Yes (rat)		+ (100)(x3)	+ (3333)(x2)	-	+ (333)(x2)		
				Yes (hamster)		+ (100)(x7)	+ (333)(x2)	-	+ (100)(x2)		

Table III

In vitro studies							
Test sur cellules de mammifères							
Ref.	Substance	Solvent	Substrate	Assay	Dose (µg/ml)	Activation métabolique	Résultats
Gulati et al. 1989	2,4-TDI	DMSO	Chinese hamster ovary (CHO) cells	Chromosome aberration	300-1000	No	-
					300-750	Yes	-
	2,6-TDI				300-1000	No	+(600)
					160-350	Yes	-
	2,4-TDI			Sister Chromatide exchange (SCE)	5-500	No	E (300)
					16-500	Yes	-
	2,6-TDI				5-300	No	+(50)
					16-600	Yes	-
JETOC 1996	2,4-TDI	DMSO	Chinese hamster lung (CHL) cells	Chromosome aberration	50-800	Yes	-
					50-600	No	-
	2,6-TDI				50-600	Yes	+(300)
					50-600	No	+(400)
JMHLW 2001	80/20 TDI	CMC sodium 1%	CHC cells	Chromosome aberration	78.1-625	No	+(313)
					625-5000	Yes	-

Maki-Paakkanen et Norppa 1987	80/20 TDI	Acetone	Human ymphocytes	Chromosome aberration	23-183	No	+(92)
					11-92	Yes	+(46)
				SCE	1.8-180	No	-
					1.8-180	Yes	-
Marczynski et al. 1992	80/20 TDI	Not reported	Human ymphocytes	DNA damage	2440-5490	No	+(2440)
				DNA damage	30 ppb	No	+
Marczynski et al. 1993	80/20 TDI		Sheep lymphocytes	DNA damage	488-1952	No	+(488)
			Rabbit lymphocytes de lapin	DNA damage	3050	No	+
MacGregor et al. 1991	2,4-TDI	DMSO	L5178Y	Mutagenicity (foward mutation assay)	6.25-150	No	+(150)
					50-150	Yes	+(75)
	2,6-TDI				15.6-250	No	+(50)
					10-200	Yes	+(25)
Shaddock et al. 1990	80/20 TDI	DMSO	Rat Hepatocytes de rat	DNA repair	0.5-50	No	-
						Yes	-

Table IV

Etudes *in vivo*									
Test sur cellules de mammifères									
Ref.	Compound	Species	Assays	Substrate	Sexe	Exposure route	Exposure duration	Dose	Results
Benford et Riley 1988	80/20 TDI	Rat	Unscheduled DNA synthesis UDS	hepatocytes ; lung	Male	Inhalation	4 hrs	0.08-1.5ppm	-
Benford & Riley 1989	80/20 TDI	Rat	UDS	hepatocytes ; lung	Male	Inhalation	4h/d, 5d	0.1-0.92 ppm	-
Lindberg et al. 2011	80/20 TDI	Mouse	Micronucleus	Bone marrow Peripheral blood	Male	Inhalation	1h/d-5d	0.15-0.34 ppm	-
Loeser 1983	80/20 TDI	Rat	Micronucleus	Bone marrow	Male /Female	Inhalation	6h/d-5d/week.-4 weeks.	0.05-0.15 ppm	-
		Mouse	Micronucleus	Bone marrow	Male /Female	Inhalation	6h/d-5d/week.-4 weeks	0.05-0.15 ppm	-
MacKay 1992	80/20 TDI	Mouse	Micronucleus	Bone marrow	Male	Inhalation	6 h	5.9-19ppm	-
					Female			3.6-11.9ppm	-

NTP 1989	80/20 TDI	Mouse	SCE	Bone marrow	Male	Intraperitoneal injection	Once	25-100 mg/kg	-
NTP 1989	80/20 TDI	Mouse	SCE	Bone marrow	Male	Intraperitoneal injection	Once	12.5-100 mg/kg	-
Whong *et al.* 1991	80/20 TDI	Rat	Micronucleus	Lung	Male	Intratracheale	3 times in 24hrs	100-400 mg/kg	-
			SCE	Lung	Male	Intratracheale	3times in 24hrs	100-400 mg/kg	-

Annex 7: Annex part B - Technical support - Details of analytical methods for workplace assessment

Annex 7B.1: Method 1 - Impinger containing toluene solution of 1,2-MPP followed by a glass fibre filter impregnated with 1,2-MPP

Table 7: Descriptive parameters of the method 1

Method 1 – Sampling – Sampling treatment – analysis		ISO 16702 Standard	MDHS 25/4
Gas /vapour - Aerosol - Combined		Combined	
Sampling	Active / passive	Active	
	Sampler	Impinger containing 10 mL mixture of 1,2-MPP in toluene followed by a glass fibre 25 mm filter impregnated with 1,2-MPP. After sampling the filter is extracted in field with 2 mL of 1,2-MPP in toluene	
	Flow rate	1 $L.min^{-1}$	
	Duration	0.5 to 480 min ISO 16702, 15 to 480 min MDHS 25/4	
	Volume	0.5 to 480 L ISO 16702, 15 to 480 L MDHS 25/4 (with level monitoring of toluene in impinger)	
Analysis	Sampling treatment	24h after the sampling, add 100 µL of acetic anhydride, evaporate and take up with 2 mL of ACN or mobile phase then filter the solution	
	Analytical technique	HPLC UV or ECD, external calibration	
	Analytical parameters	C8 column, 100 mm x 4.6 mm ID, eluent : ACN/ acetate buffer, UV 242 nm, ECD +0.8 V	

Table 8: Validation data of the method 1

Method 1 – Sampling – Sampling treatment – analysis	ISO 16702 Standard	MDHS 25/4
Working range	Approx. 0.1 to 140 $\mu g.m^{-3}$ [NCO]@ 15L i.e. 2.1 to 290 $\mu g.m^{-3}$ [TDI] @ 15 L or0.0065 to 9.06 $\mu g.m^{-3}$ [TDI] @ 480 L	Non available, (data of ISO and MDHS 25/4 have the same origin, §15 MDHS 25/3)
Sampling and recovery efficiencies	Non available	Not measured but taken to be 100% for both monomers for most practical purposes
Capacity limit	≥ 140 $\mu g.m^{-3}$ [NCO] i.e. ≥ 290 $\mu g.m^{-3}$ TDI @ 15L or ≥ 9.056 $\mu g.m^{-3}$ [TDI] @ 480 L	≥ 140 $\mu g.m^{-3}$ [NCO] i.e. ≥ 290 $\mu g.m^{-3}$ TDI @ 15L or ≥ 9.056 $\mu g.m^{-3}$ [TDI] @ 480 L (MDHS 25/3)
Detector response linearity	Non available	
Storage stability	Non available	TDI on filters and in toluene have been found to be stable up to 90 days (73% and 81% recovery respectively).
Environmental condition	Non available	
Selectivity / Interfering	Studied, aromatic amines identified	
Speciation	Given by derivatisation and HPLC separation	

Method 1 – Sampling – Sampling treatment – analysis		ISO 16702 Standard	MDHS 25/4
Conditions for the 8h-OEL or 15min-STEL determination	Estimated expanded uncertainty	47 – 50 %	
	Detection limit	0.07 µg.m^{-3} [NCO] i.e. 0.145 µg.m^{-3} [TDI] @ 15L or 0.0045 µg.m^{-3} [TDI] @ 480 L	
	Quantification limit	0.3 µg.m^{-3} [NCO] i.e. 0.62 µg.m^{-3} [TDI] @ 15L or 0.019 µg.m^{-3} [TDI] @ 480 L	0.27 µg.m^{-3} [NCO] i.e. 0.55 µg.m^{-3} [TDI] @ 15L, 0.017 µg.m^{-3} [TDI] @ 480 L
Additional details		-	

Annex 7B.2: Method 2 -Impinger containing toluene solution of DBA followed by a glass fibre filter non impregnated

Table 9: Descriptive parameters of the method 2

Method 2 – Sampling – Sampling treatment – analysis		ISO 17734-1 Standard
Gas / vapour- Aerosol - Combined		Combined
Sampling	Active / passive	Active
	Sampler	Impinger containing 10 mL mixture of DBA (10 mM) in toluene followed by a glass fibre 13 mm non-impregnated filter. After sampling the filter is extracted in field in the impinger solution or separately in a jar.
	Flow rate	1 $L.min^{-1}$
	Duration	15 -30 min up to 480 min with level monitoring of toluene in impinger every 30 min
	Volume	15 L to 480 L
Analysis	Sampling treatment	Addition of an internal standard, ultra sonication, centrifugation, solvent evaporation and residues dissolution in 0.5 mL ACN and ultra sonication
	Analytical technique	HPLC MS, MS-MS, nitrogen or UV detection, internal or external calibration
	Analytical parameters	LC MS and LC Nitrogen detector : C18 column, 50 mm x 1 mm ID, eluent : MeOH/water 50/50 or Methanol/water/ACN 20/50/30, 0.1 $mL.min^{-1}$

Table 10: Validation data of the method 2

Method 2 – Sampling – Sampling treatment – analysis		ISO 17734-1 Standard
Working range		0.3 $ng.m^{-3}$ to 66.6 $mg.m^{-3}$@ 15L; 0.009 $ng.m^{-3}$ to 2.08 $mg.m^{-3}$ @ 480 L
Sampling and recovery efficiencies		Non available
Capacity limit		≥ 66 $mg.m^{-3}$ @ 15 L; ≥ 2.08 $mg.m^{-3}$ @ 480 L
Detector response linearity		Non available
Storage stability		6 months at 8°C
Environmental condition		Non available
Selectivity / Interfering		Studied, no loss measured
Speciation		Given by derivatisation and HPLC separation
Conditions for the 8h-OEL or 15min-STEL determination	Estimated expanded uncertainty	24 % (Mass or Nitrogen detection)-
	Detection limit	0.2 nmol TDI/sample. The ISO 17334-1 standard mentions that for a 15 L air sample this corresponds to 0,02 ng.m-3 for 15L. But 0.2 nmol corresponds to 0.2*174.2=34.8 ngTDI/sample. And for a 15 L air sample this corresponds to 2.3 $\mu g.m^{-3}$; 0.073 $\mu g.m^{-3}$ @ 480 L Not consistent with the order of magnitude of the working range
	Quantification limit	10 LD/3 : 7.7 $\mu g.m^{-3}$ @ 15L; 0.24 $\mu g.m^{-3}$ @ 480 L

Method 2 – Sampling – Sampling treatment – analysis	ISO 17734-1 Standard
Additional details	-

Annex 7B.3: Method 3 - Impinger containing toluene solution of MAP followed by a glass fibre filter impregnated with MAP

Table 11: Descriptive parameters of the method 3

Method 3 – Sampling – Sampling treatment – analysis		ISO 17735 Standard	NIOSH 5525
Gas / vapour- Aerosol - Combined		Combined	
Sampling	Active / passive	Active	
	Sampler	Impinger containing 15 mL mixture of MAP in butyl benzoate followed by a glass fibre 37 mm filter impregnated with MAP. After sampling the filter is extracted in field in the impinger solution	
	Flow rate	1 L.min^{-1}	
	Duration	15 - 480 min	
	Volume	15 - 480 L	1 – 500 L
Analysis	Sampling treatment	Add 5 µL of acetic anhydride, 2 hours after, extract with a SPE cartridge with methylene chloride then ACN/methanol and finally pure methanol, evaporate and take up with ACN	
	Analytical technique	HPLC UV or fluo., external calibration	
	Analytical parameters	C8 column, 100 mm x 4.6 mm ID, eluent: ACN/triethylammonium-phosphate-formiate buffer, 1.5 mL.min^{-1}, post column ACN/phosphoric acid 0.7 mL.min^{-1}, UV 253 nm, fluo. Xenon lamp ex. 368 nm, em. 403 nm, deuterium lamp ex. 254 nm, em; 409 nm.	

Table 12: Validation data of the method 3

Method 3 – Sampling – Sampling treatment – analysis	ISO 17735 Standard	NIOSH 5525
Working range	10^{-10} à 2.10^{-7} mol NCO/sample or 8,7 ng to 17,4 µg TDI / sample, or 0.58 to 1160 µg TDI.m^{-3} @ 15L; 0.018 to 36.2 µg TDI.m^{-3} @ 480 L	0,5 to 300 nM NCO per sample, or 2.9 to 17400 µg TDI.m^{-3} @ 15L; 0.09 to 54.4 µg TDI. m^{-3} @ 480 L
Sampling and recovery efficiencies	Non available	Liquid spiked with 2,4-TDI-MAP recovery ≥ 97%, MAP filter spiked with 2,4-TDI at 4 levels at 21 to 2100 ng NCO group, 91% recovered without correlation between recovery and spiking level
Capacity limit	≥ 1160 µg TDI.m^{-3} @ 15L ≥ 36.2 µg TDI.m^{-3} @ 480 L	≥ 261 µg per air sample
Detector response linearity	Linear 1.10^{-7} à 2.10^{-4} moles NCO/L or 8.7 pg to 17.4 mg TDI.mL^{-1}	Non-linear fluo. response in the very low concentration
Storage stability	Non available	Samples containing TDI-MAP, losses of 16 to 24% after 9 months in freezer
Environmental condition	MAP sensitive to light	

Method 3 – Sampling – Sampling treatment – analysis		ISO 17735 Standard	NIOSH 5525
Selectivity / Interfering		Studied and identified	Interfering compounds do not fluoresce using 368 nm excitation and 409 emission and interfering UV signal can be separated by altering pH gradient
Speciation		Given by derivatisation and HPLC separation	
Conditions for the 8h-OEL or 15min-STEL determination	**Estimated expanded uncertainty**	36 %	Not determined
	Detection limit	Not determined	0.2 nM NCO = 17.4 ng TDI, 1,16 µg TDI.m^{-3} @ 15 L 0.036 µg TDI.m^{-3} @ 480 L
	Quantification limit	≤ 0.58µg TDI.m^{-3} @ 15 L ≤ 0.018 TDI m^{-3}@480 L	3.87 µg TDI.m^{-3} @ 15 L 0.12 µg TDI.m^{-3} @ 480 L
Additional details		-	-

Annex 7B.4: Method 4 - Glass fibre filter impregnated with MAMA or MAP

Table 13: Descriptive parameters of the method 4

Method 4 – Sampling – Sampling treatment – analysis		ISO 17736 Standard, IRSST 376, GF impregnated MAMA	ISO 17735 Standard NIOSH 5525, GF impregnated MAP
Gas / vapour- Aerosol - Combined		Combined only for STEL monitoring not for extended time above 30 min	
Sampling	Active / passive	Active	
	Sampler	PTFE filter associated with glass fibre filter impregnated with MAMA	Glass fibre filter impregnated with MAP
	Flow rate	1 L.min^{-1}	1 or 2 L.min^{-1} (IOM)
	Duration	15 min	15 min
	Volume	15 L	15 or 30 L (IOM)
Analysis	Sampling treatment	PTFE filter is extracted in field with 5 mL MP toluene solution. The solution is evaporated and the residue dissolved in ACN with acetic anhydride. Glass filter id extracted with 2 mL of mobile phase eluent (mixture of DMF/triethanolamine buffer/ACN)	In field extract with 5 mL or 10 mL (IOM) of MAP-ACN solution. Add 5 µL of acetic anhydride, 2 hours after.
	Analytical technique	HPLC UV or fluo., external calibration	
	Analytical parameters	C18 column, 150 mm x 3.2 mm ID, eluent: ACN/triethylammonium-phosphate-formiate buffer, 0.6 mL.min^{-1}, post column ACN/phosphoric acid 0.7 mL.min^{-1}, UV 242-254 nm, fluo. deuterium lamp ex. 254 nm, em; 412 nm.	C8 column, 100 mm x 4.6 mm ID, eluent: ACN/triethylammonium-phosphate-formiate buffer, 1.5 mL.min^{-1}, post column ACN/phosphoric acid 0.7 mL.min^{-1}, UV 253 nm, fluo. Xenon lamp ex. 368 nm, em. 403 nm, deuterium lamp ex. 254 nm, em; 409 nm.

Table 14: Validation data of the method 4

Method 4 – Sampling – Sampling treatment – analysis	ISO 17736 Standard, IRSST 376, GF impregnated MAMA	ISO 17735 Standard NIOSH 5525, GF impregnated MAP
Working range	1,38 to 290 µg.m^{-3} @ 15L (ISO)	0.58 to 1160 µg.m^{-3} @ 15L (ISO) ; 2.9 to 17400 µg.m^{-3} @ 15L (NIOSH)
Sampling and recovery efficiencies	Not available	Liquid spiked with 2,4-TDI-MAP recovery ≥ 97%, MAP filter spiked with 2,4-TDI at 4 levels at 21 to 2100 ng NCO group, 91% recovered without correlation between recovery and spiking level
Capacity limit	≥ 4.35 µg per sample (ISO)	≥ 17.4 µg per air sample (ISO) ≥ 261 µg per air sample (NIOSH)
Detector response linearity	Linear	Linear 8.7 pg to 17.4 mg.mL^{-1} (ISO); non-linear fluo. response in

Method 4 – Sampling – Sampling treatment – analysis		ISO 17736 Standard, IRSST 376, GF impregnated MAMA	ISO 17735 Standard NIOSH 5525, GF impregnated MAP
			the very low concentration (NIOSH)
Storage stability		Not available	Samples containing TDI-MAP, losses of 16 to 24% after 9 months in freezer
Environmental condition		Not available	MAP sensitive to light
Selectivity / Interfering		Studied, amines identified	Interfering compounds do not fluoresce using 368 nm excitation and 409 emission and interfering UV signal can be separated by altering pH gradient
Speciation		Given by derivatisation and HPLC separation	
Conditions for the 8h-OEL or 15min-STEL determination	**Estimated expanded uncertainty**	50 % vapour, 90 % aerosol (ISO)	36 % (ISO)
	Detection limit		0.2nM NCO = 17.4 ng TDI, 1,16 µg.m^{-3} @ 15L (NIOSH)
	Quantification limit	0.01 µg NCO equivalent per sample, corresponding to approximately 0.67 µg NCO per cubic meter for a 15 Lsample volume This corresponds to : 0.67*174,2/(2*42) = 1,38 µg TDI / m3	≤ 1x10^{-10} mole NCO For a 15 L air sample : 0.5x174.2 10^{-10}/0.015 = 580 ng TDI.m^{-3} (§1 ISO 17735) 3.87 µg.m^{-3} @ 15L (NIOSH)
Additional details		-	-

Annex 7B.5: Method 5 - Glass fibre filter impregnated with 1,2-MPP or 1,2-PP

Table 15: Descriptive parameters of the method 5

Method 5 – Sampling – Sampling treatment – analysis		IFA 7670-7120; MAK diisocyanate; MetroPol 245, 246, 249, 250	OSHA 42 = ISO 14382 Standard
Gas / vapour- Aerosol - Combined		Combined only for STEL monitoring not for extended time above 20 min	
Sampling	Active / passive	Active	
	Sampler	Glass or Quartz fibre filter impregnated with 1,2-MPP	Glass fibre filter impregnated with 1,2-PP
	Flow rate	3.5 L.min^{-1} GSP (IFA) 2 L.min^{-1} (MAK & MetroPol)	1 L.min^{-1}
	Duration	15 - 20 min	
	Volume	52.5 L (IFA); 40 & 30 L (MAK & MetroPol)	15 L
Analysis	Sampling treatment	Extracted with ACN with acetic anhydride, solution evaporated and the residue dissolved in ACN, THF or mobile phase eluent	Extracted with 2 mL ACN/DMSO 90/10
	Analytical technique	HPLC UV or fluo., external calibration	
	Analytical parameters	C18 column, 250 mm x 4.6 mm ID, eluent: ACN/water/buffer acetate or formiate, pH 6.2, 1 mL.min^{-1}, 0.6 or 1 mL.min^{-1}, UV 242-254 nm, fluo. Deuterium lamp ex. 254 or 240 nm, em; 412 or 380 nm.	C8 column, 250 mm x 4.6 mm ID, eluent : ACN/water/buffer acetate, pH 6.2, 1 mL.min^{-1}, post column ACN/phosphoric acid 0.7 mL.min^{-1}, UV 254 and 313 nm, fluo., deuterium lamp ex. 240 nm, em; 370 nm.

Table 16: Validation data of the method 5

Method 5 – Sampling – Sampling treatment – analysis	IFA 7670-7120; MAK diisocyanate; MetroPol 245, 246, 249, 250	OSHA 42 = ISO 14382 Standard
Working range	4.8 to 280 µg.m^{-3} @ 52.5 L (IFA)	2,4-TDI 4.3 to 140 µg.m^{-3}, 2,6-TDI 5.3 to 140 µg.m^{-3} @ 15 L
Sampling and recovery efficiencies	96-97 % 4.8 to 280 µg.m^{-3} (IFA)	Vapour spiking 78 %RH 0.18 and 0.21 µg.m^{-3} 89.7 & 80 % for 2,4- and 2,6-TDI. Liquid spiking 80 %RH, 87.9 & 82.4 %. Test on pre-moistened filter: 100 & 81.5 % recovery.
Capacity limit	≥ 14.7 µg per sample (IFA)	≥ 2.1 µg per air sample
Detector response linearity	Linear	
Storage stability	9 to 96 µg.m^{-3}, no significant variation after 15 days at room T°C (IFA); stable 14 days at 4°C (MAK); stable 3 weeks (MetroPol)	After 18 days at -22°C, 86.3 & 81.3 % recovery for 2,4- & 2,6-TDI; 18 days at 22°C, 86.4 & 80.3% recovery.

Method 5 – Sampling – Sampling treatment – analysis		IFA 7670-7120; MAK diisocyanate; MetroPol 245, 246, 249, 250	OSHA 42 = ISO 14382 Standard
Environmental condition		Not available	Not available
Selectivity / Interfering		Studied, amines identified	Benzaldehyde interfere with 2,4-TDI
Speciation		Given by derivatisation and HPLC separation	
Conditions for the 8h-OEL or 15min-STEL determination	Estimated expanded uncertainty	2,4-TDI : 16.5 to 16.8 % at 5 to 100 µg.m^{-3}; 2,6-TDI : 15.8 to 16.6 % (IFA)	Analytical prec. : 0.009; Overall prec.: 14.9 & 13.5 %; Reproducibility : 101.5 & 105.4 % (OSHA) 20 % (ISO)
	Detection limit		1,3 & 1.6 µg.m^{-3} for 2,4- & 2,6-TDI @ 15L
	Quantification limit	2,4-TDI : 4.8 µg.m^{-3} 2,6-TDI : 5.2 µg.m^{-3} @ 15L (IFA)	≤ 0.58 µg.m^{-3} @ 15L (ISO) 3.87 µg.m^{-3} @ 15L (NIOSH)
Additional details		-	-

Annex 7B.6: Method 6 - Impinger alone containing derivatising agent

Table 17: Descriptive parameters of the method 6

Method 6 – Sampling – Sampling treatment – analysis		NIOSH 5521, 5525 ;; OSHA 18 ;
Gas / vapour- Aerosol - Combined		Combined
Sampling	Active / passive	Active
	Sampler	One impinger containing 1,2-MPP in toluene – NIOSH 5521, ; MAP in butyl benzoate – NIOSH 5525; nitro reagent in toluene or xylene – OSHA 18;
	Flow rate	1 L.min^{-1}
	Duration	15 min recommended, possible 1h without adding toluene, 8h by adjusting the level of toluene in the impinger every hour (OSHA 18)
	Volume	30 L MTA/MA 15 L to 480 L (OSHA 18), 500 L (NIOSH 5521)
Analysis	Sampling treatment	Acetylation of the excess of reagent, evaporation and redissolution in ACN
	Analytical technique	HPLC UV or fluo. Or electrochemical (NIOSH 5521), external calibration
	Analytical parameters	C8 column, 75 mm x 4.6 mm ID, eluent: ACN/methanolic or acetate buffer, 1 mL.min^{-1}, UV 242 nm, fluo. Xenon lamp ex. 368 nm, em. 403 nm, deuterium lamp ex. 254 nm, em; 409 nm, electroch. +0.8 V vs Ag/AgCl

Table 18: Validation data of the method 6

Method 6 – Sampling – Sampling treatment – analysis	NIOSH 5521, 5525 ;; OSHA 18 ; NIOSH 5525
Working range	NIOSH 5521 2,4-TDI : 33.3 to 6666 µg.m^{-3} @ 15L, 1.0 to 208 µg.m^{-3} @ 480L 2,6-TDI : 46.6 to 6666 µg.m^{-3} @ 15L; 1.46 to 208 µg.m^{-3} @ 480L; NIOSH 5525 : 2.9 to 17400 µg.m^{-3} @ 15L; 0.09 to 54.4 µg.m^{-3} @ 480L; OSHA 18 : 3.2 or 4.4 to 667 µg.m^{-3} @ 15L, 0.10 or 0.14 to 20.8 µg.m^{-3} @ 480 L
Sampling and recovery efficiencies	OSHA 18: spiked samplers, closed to 100 % at 96 to 385 µg.m^{-3} @ 15L.
Capacity limit	NIOSH 5521 & 5525 : ≥ 8 µg per air sample OSHA 18 : ≥ 10 µg per air sample
Detector response linearity	Linear
Storage stability	NIOSH 5521 : 88 +- 7%, 7 days at 4°C OSHA 18: > 91% 17 days at room temp. or refrigerated
Environmental condition	OSHA 18 : RH% studied, few influence
Selectivity / Interfering	Studied and identified Compounds interfering UV signal can be separated by altering pH gradient
Speciation	Given by derivatisation and HPLC separation

Method 6 – Sampling – Sampling treatment – analysis		NIOSH 5521, 5525 ;; OSHA 18 ; NIOSH 5525
Conditions for the 8h-OEL or 15min-STEL determination	Estimated expanded uncertainty	OSHA 18 : Standard error : 5..5 %
	Detection limit	OSHA 18 : 1.33 µg.m^{-3} @ 15L MAK HDI TDI : 2.67 µg.m^{-3} @ 15L NIOSH 5521 0.1 µg TDI/sample
	Quantification limit	MTA/MA : 3 µg.m^{-3} @ 15L NIOSH 5521 (electroc.) : 22.2 µg.m^{-3} @ 15L, 0.69 µg.m^{-3} @ 480L; NIOSH 5525 3.87 µg.m^{-3} @ 15L. 0.12 µg.m^{-3} @ 480L NIOSH 5525 : 3.9 µg.m^{-3} @ 15L OSHA 18 : 3.2 to 4.4 µg.m^{-3} @ 15L, 0.10 to 0.14 µg.m^{-3} @ 480 L MAK TDI HDI : 8.89 µg.m^{-3} @ 15L
Additional details		-

Annex 7B.7: Method 7- Denuder tube followed by a filter impregnated with DBA

Table 19: Descriptive parameters of the method 7

Method 7 – Sampling – Sampling treatment – analysis		ISO 17734-1 (Solvent free sampler)
Gas / vapour- Aerosol - Combined		Combined
Sampling	Active / passive	Active
	Sampler	Denuder tube followed by 13 mm glass fibre filter. The walls of the denuder tube are cover with glass fibre. Glass fibre in the tube and glass fibre filter are impregnated with DBA
	Flow rate	0.2 L.min^{-1}
	Duration	15 min up to and beyond 480 min
	Volume	3 L up to and beyond 96 L
Analysis	Sampling treatment	Methanol/H2SO4/toluene before evaporation, dissolution in toluene, evaporation and final dissolution in ACN
	Analytical technique	LC MS or MS-MS, C18 column, 50 mm x 1 mm ID, eluent : MeOH/water 50/50 or MeOH/water/ACN 20/50/30, 0.1 mL.min^{-1}
	Analytical parameters	C8 column, 75 mm x 4.6 mm ID, eluent: ACN/methanolic or acetate buffer, 1 mL.min^{-1}, UV 242 nm, fluo. Xenon lamp ex. 368 nm, em. 403 nm, deuterium lamp ex. 254 nm, em; 409 nm, electroch. +0.8 V vs Ag/AgCl

Table 20: Validation data of the method 7

Method 7 – Sampling – Sampling treatment – analysis		ISO 17734-1 (Solvent free sampler)
Working range		1.7 ng.m^{-3} to 333 mg.m^{-3} @ 3 L, 0.05 to 10.4 mg.m^{-3} @ 96 L
Sampling and recovery efficiencies		Atmosphere generated in a generator. The method comparison was conducted by comparing the results obtained with the denuder tube to those obtained with the sampler described in ISO 17734 Standard, impinger + filter. Aerosol + vapour : At 45 %RH, 2,4-TDI : 87.5 % at 24 µg.m^{-3}, 93.2 % at 4.4 µg.m^{-3}, 90.9 % at 1.1 µg.m^{-3} ; 2,6-TDI : 79.5 % at 39 µg.m^{-3}, 78.5 % at 24 µg.m^{-3}, 87 % at 3.9 µg.m^{-3}.At 95 %RH, 2,4-TDI : 76.2 % at 21 µg.m^{-3}, 87.5 % at 1.6 µg.m^{-3}; 2,6-TDI : 64.8 % at 37 µg.m^{-3}, 81.1 % at 5.3 µg.m^{-3}. Vapour: 100% adsorbed at the pumped flow rate of 50 mL.min^{-1}, 83 to 87% at 200 mL.min^{-1} (Marand et al. 2005).
Capacity limit		1 mg per air sample
Detector response linearity		Linear, 5 to 280 ng.mL^{-1}
Storage stability		4 weeks after sampling for supplier information, losses observed 2 to 7 days after sampling in the study of Marand, 2005 (Marand et al. 2005)
Environmental condition		RH% in the recovery study
Selectivity / Interfering		Studied and identified
Speciation		Given by derivatisation and HPLC separation
Conditions for the 8h-OEL or 15min-STEL determination	Estimated expanded uncertainty	24 % (Mass or Nitrogen detection)
	Detection limit	0.2 nM/sample equiv. 11.6 µg.m^{-3} @ 3L; 0.36 µg.m^{-3} @ 96 L (ISO17734-1)
	Quantification limit	LC-MS : 0.1 µg.m^{-3} @ 3L, 0.03 µg.m^{-3} @ 96L

Method 7 – Sampling – Sampling treatment – analysis		ISO 17734-1 (Solvent free sampler)
		LC-MS/MS : 0.03 ng per sample 0.001 µg.m^{-3} @ 3L; <0.1 ng.m^{-3} @ 96L (Sigma Aldrich reporter n. 53 p. 4)
Additional details		-

Annex 8: Public consultation

This report and the conclusions were the subject of a public consultation from 18/08/2019 to 18/10/2019

The following individuals or organizations provided comments during the consultation phase:

- Arkema
- DECOS
- Europur
- ISOPA

Annexe 9 : Following up of the modification of the report

Date	Version	Description de la modification
21 March 2019	V01	Validation by the committee for public consultation
25 June 2020	V02	Validation by the committee Modifications : EXPERTISE COLLECTIVE: SYNTHESE DE L'ARGUMENTAIRE ET CONCLUSIONS : - Description de l'étude de Vandenplas - Ajout d'informations dans le profil toxicologique et dans la construction des valeurs - Figure 1 - Résultat de l'expertise collective concernant les méthodes de mesure atmosphériques dans les lieux de travail / Conclusions et recommandations Part A : - Description of Vandenplas study - Additionnal information in the toxicological profile and derivation of values Part B : - Figure 4, - Table 10 and 14 in annex 7. - Conclusions and recommandation
10/12/2020	V03	Validation by the committee Modifications : EXPERTISE COLLECTIVE: SYNTHESE DE L'ARGUMENTAIRE ET CONCLUSIONS : - Addition of the evaluation part of the measurement methods with regard to the 8h-OEL. Part B : - Addition of the evaluation part of the measurement methods with regard to the 8h-OEL.

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