anses

Valeurs limites d'exposition en milieu professionnel

Endotoxines Effets sur la santé

Avis de l'Anses
Expert Appraisal Report

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Connaître, évaluer, protéger

Avis de l'Anses
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Le directeur général

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

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

Evaluation des effets sur la santé sur le lieu de travail pour les endotoxines

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

L'Anses a été saisie en juin 2018 par la Direction générale du travail (DGT) pour la réalisation de l'expertise suivante : évaluation des effets sur la santé des endotoxines en vue de la fixation de valeurs limites d'exposition à des agents chimiques en milieu professionnel.

1. CONTEXTE ET OBJET DE LA SAISINE

Dans le cadre du protocole d'accord entre l'Anses et le ministère du travail pour la mise en œuvre du programme de travail d'expertise scientifique en matière de valeurs limites atmosphériques et biologiques pour les expositions professionnelles, établi en juillet 2018 et renouvelé en 2023, la DGT a saisi l'Anses afin de mener les travaux d'expertise nécessaires à la fixation de valeurs limites d'exposition professionnelle (VLEP) fondées sur des considérations sanitaires pour les endotoxines.

Cette substance est inscrite au programme de travail VLEP suite à des recommandations du CES, soulignant l'utilité d'une VLEP pour de nombreuses activités, telles que la collecte et le traitement de déchets ménagers, des eaux usées, du compost, l'utilisation de fluides de coupe, dans les scieries, dans l'industrie du tabac, la fabrication de litières, le traitement de la pomme

de terre ou du café dans l'industrie agroalimentaire, la production de céréales et de fibres végétales (lin, coton, …), la production et le stockage de semences, l'élevage (volailles, bovins, porcins,…) et le traitement de l'air.

La France ne dispose à ce jour d'aucune valeur limite d'exposition professionnelle pour les endotoxines.

2. ORGANISATION DE L'EXPERTISE

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

L'expertise relève du domaine de compétences du comité d'experts spécialisé (CES) « Valeurs sanitaires de référence » (CES VSR).

L'expertise collective relative à l'évaluation des effets sanitaires des endotoxines a été réalisée par le CES VSR avec l'appui de quatre rapporteurs.

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

Le présent avis se fonde pour les aspects scientifiques sur le rapport intitulé « *Expert appraisal on recommending occupational exposure limits for chemical agents - Assessment of health effects for endotoxins* » *(Anses, 2024).*

Le rapport en anglais ainsi que la partie « Analyse et conclusions du CES » du présent avis en français ont été adoptés par le CES VSR le 27 juin 2024.

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

Le profil toxicologique des endotoxines a été élaboré à partir du rapport établi par le comité d'experts néerlandais sur la sécurité au travail (Dutch Expert Committee on Occupational Safety ou DECOS) du Conseil de santé des Pays-Bas publié en 1998 et actualisé en 2010. Une recherche bibliographique complémentaire a été effectuée dans les bases de données PubMed® et Scopus® afin de prendre en compte la littérature scientifique parue sur les endotoxines jusqu'en décembre 2023. Les détails de la recherche bibliographique (requête, principaux mots-clés, critères d'inclusion et d'exclusion) sont décrits dans l'annexe 1 du rapport.

3. ANALYSE ET CONCLUSIONS DU CES

3.1. Informations générales

Les endotoxines sont des molécules complexes libérées sous forme de particules dans l'air lors de la division cellulaire et de la mort de bactéries, et dans une moindre mesure lors de leur multiplication (Anses 2016). Ce sont des composants de la membrane externe de bactéries à gram négatif (par exemple, *Enterobacteriaceae* ou *Pseudomonadaceae*) et de cyanobactéries (Duquenne et al. 2012 ; Anses 2016). Ces molécules, composées de protéines, de lipides et de lipopolysaccharides (LPS), jouent un rôle important à la fois dans l'intégrité cellulaire et dans l'interaction de la cellule avec l'environnement extracellulaire (Liebers et al. 2008 ; Duquenne et al. 2012).

Les termes « endotoxines » et « LPS » sont souvent utilisés de manière interchangeable dans la littérature. Cependant, le terme « LPS » devrait se référer aux lipopolysaccharides chimiques purs, exempts de tout autre composant de la membrane cellulaire des bactéries, tandis que le terme « endotoxines » devrait se référer aux lipopolysaccharides attachés à d'autres composants de la membrane bactérienne (Géhin et Le Bacle 2011 ; Duquenne et al. 2012).

Les LPS sont composés d'une partie d'ancrage membranaire hydrophobe appelée lipide A et d'un oligosaccharide central non répétitif couplé à un polysaccharide distal (antigène O) qui s'étend depuis la surface bactérienne. Il existe une multitude de variantes structurelles naturelles de LPS qui sont principalement dues à la grande diversité de la composition chimique de la région polysaccharidique (noyau et antigène O), mais aussi à des variations considérables de la structure du lipide A (Raetz et Whitfield 2002).

Les endotoxines (ou LPS) sont quasiment omniprésentes dans la nature, mais des concentrations élevées sont retrouvées dans certains environnements industriels et agricoles (élevages, silos à grains, industrie du coton et du lin, industrie de l'alimentation animale, stations d'épuration des eaux usées et de compostage des eaux usées, etc.) (MacIntosh et al. 2000 ; Health Council of the Netherlands 2010). En effet, les bactéries gram-négatives dans l'environnement professionnel peuvent être issues de matrices solides (poussières déposées, déchets ménagers, compost, grains et plantes) ou liquides (eaux usées, fluides d'usinage des métaux, etc.) contenant de la matière organique (Duquenne et al. 2012). La présence d'endotoxines dans l'air ambiant est liée à la présence de bactéries gram-négatives ou de fragments de parois cellulaires de ces bactéries dans les particules de poussières organiques en suspension dans l'air (Health Council of the Netherlands 2010).

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

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

Le comité d'experts néerlandais sur la sécurité au travail (DECOS) du Conseil de santé des Pays-Bas est la seule organisation scientifique ayant à ce jour publié une recommandation de valeur limite d'exposition professionnelle pour les endotoxines. En 2010, le DECOS a

recommandé une valeur limite d'exposition sur 8 heures pour les endotoxines de 90 UE/m^3 (soit 9 ng/m^3)[1] afin de prévenir leurs effets aigus et chroniques (Health Council of the Netherlands 2010).

3.3. Synthèse des données toxicologiques

■ Données de toxicocinétique

Les données de toxicocinétique sur les endotoxines sont peu nombreuses dans la littérature scientifique. La taille des aérosols ou des particules en suspension dans l'air contenant des composants bactériens tels que les endotoxines leur permet de se déposer à chaque niveau de l'arbre respiratoire (Health Council of the Netherlands 1998). Au cours du processus d'inflammation, des altérations de la perméabilité paracellulaire et transcellulaire de l'épithélium peuvent se produire, permettant aux endotoxines présentes dans les poussières organiques de franchir cette barrière. Les endotoxines liées aux cellules inactives, déposées dans diverses parties du poumon, peuvent être libérées et devenir biologiquement actives par divers mécanismes : lyse des bactéries par les antibiotiques ou le complément, phagocytose des bactéries par les macrophages et les leucocytes polymorphonucléaires et pendant la reproduction des bactéries (Morrison et al. 1985).

Il n'existe pas de données disponibles sur la distribution précise dans l'organisme des endotoxines après inhalation.

Aucune donnée n'est disponible sur le métabolisme des endotoxines chez l'Homme. Des études menées sur des rats ont montré que les LPS restent chimiquement inaltérés dans la circulation sanguine. Une désacylation partielle des LPS se produit dans divers organes (Freudenberg et Galanos 1990). La majorité des LPS est distribuée dans le foie (25%), de petites quantités sont distribuées dans la rate (1%) et les ganglions lymphatiques (0,5%) (Morrison et al. 1985).

Il n'existe pas de donnée précise dans la littérature sur les mécanismes exacts d'élimination des endotoxines. Le foie serait cependant le principal organe impliqué dans la clairance des LPS (Yao et al. 2016 ; Akiba et al. 2020). Les macrophages hépatiques (cellules de Kupffer) semblent être les principales cellules impliquées dans la clairance des LPS (Akiba et al. 2020).

■ Données de toxicité

- *Toxicité aigue*

  - **Effets respiratoires et pulmonaires**

La plupart des études de toxicité aiguë par inhalation d'endotoxines chez l'Homme disponibles rapportent des résultats d'explorations fonctionnelles respiratoires, examens permettant d'évaluer la fonction respiratoire. Les effets rapportés après inhalation consistent principalement en une diminution du volume expiratoire maximal par seconde (VEMS)

[1] Les concentrations d'endotoxines sont de plus en plus souvent déterminées par la mesure indirecte de l'activité enzymatique présentée en unités d'endotoxines par mètre cube d'air (UE/m^3), remplaçant les mesures en nanogrammes par mètre cube (ng/m^3) (Géhin et Le Bacle 2011 ; Liebers, Brüning et Raulf 2020). Le facteur de conversion varie en fonction de l'espèce bactérienne qui produit les endotoxines. Pour simplifier la conversion, un facteur couramment accepté est 10, soit 1 ng/m³ = 10 UE/m³ (Géhin et Le Bacle 2011).

(Haglind et Rylander 1984 ; Rylander et al. 1985 ; Castellan et al. 1987; Donham et al. 1989 ; Zock et al. 1998 ; Kline et al. 1999 ; Donham et al. 2000 ; Cyprowski et al. 2015).

Des volontaires sains (fumeurs et non-fumeurs) en population générale (après exclusion des sujets asthmatiques, souffrant de bronchite chronique ou d'essoufflement à l'effort) ont été soumis à un test préalable d'exposition à 1 000 UE/m^3 (100 ng/m^3) de LPS, afin de sélectionner les sujets sensibles, c'est à dire les volontaires ayant répondu par une diminution du VEMS d'au moins 5% (mais inférieure à 30%) (n = 33, dont 16 fumeurs). Au cours de 108 sessions d'exposition différentes, les volontaires (24 - 35 sujets) ont été exposés à une concentration constante et contrôlée de poussières de coton pendant 6 heures, avec des concentrations d'endotoxines dans l'air allant de 60 à 7 790 UE/m^3 (6 à 779 ng/m^3). Une relation exposition-réponse a été mise en évidence entre le ΔFEV1 (%) et la concentration d'endotoxines (ng/m^3), à savoir : ΔFEV1 = 3,84 - 4,02 (10log endotoxine (ng/m^3)) ; r = 0,85 (r^2 = 0,72), p<0,0001. Les 66 autres sessions d'exposition des mêmes sujets à de l'air pur ont entraîné un ΔFEV1 moyen de ± 0 %. En utilisant un modèle de régression linéaire, le calcul du pourcentage zéro de variation du VEMS pendant l'exposition aux endotoxines était de 90 UE/m^3 (9 ng/m^3) (Castellan et al. 1987).

D'autres paramètres respiratoires ont été mesurés tels que la capacité vitale forcée (CVF) (Milton et al. 1995; 1996; Bønløkke et al. 2009; Mitchell et al. 2015; Cyprowski et al. 2015) et le débit expiratoire de pointe adulte (DEP)(Buck et al. 1986; Milton et al. 1995; 1996; Bønløkke et al. 2009). Certaines de ces études ont mis en évidence une diminution significative de la CVF (Mitchell et al. 2015) et du DEP (Milton et al. 1995; 1996) liée à une exposition aux endotoxines. Dans un modèle à effets mixtes, la CVF diminuait au cours d'un poste de travail de 24,5 mL (IC[2] 95 % : [-44,7 à -4,3] ; P = 0,018) avec log10 (endotoxines totales) (Mitchell et al. 2015). Aucune relation dose-réponse n'a été identifiée pour le DEP et l'exposition aux endotoxines.

Une étude basée sur des auto-questionnaires a montré une association statistiquement significative à des symptômes respiratoires et de la fièvre/frissons pour des sujets exposés à plus de 250 UE/m^3 (25 ng/m^3) d'endotoxines (Laitinen et al. 2001).

- **Syndrome toxique des poussières organiques (ODTS)**

Le syndrome toxique des poussières organiques (Organic Dust Toxic Syndrome ou ODTS) est une maladie de type grippale non infectieuse qui survient après une exposition par inhalation de poussières organiques contaminées par des microorganismes (par exemple, des bactéries gram-négatives). Certaines études, basées sur des questionnaires, ont analysé l'association entre l'exposition aux endotoxines et la survenue d'un ODTS (Boehmer et al. 2009 ; Smit et al. 2005 ; Smit et al. 2006 ; Basinas et al. 2012). Certaines de ces études identifient des NOAEL[3] pour l'apparition de l'ODTS à partir de différents niveaux d'exposition aux endotoxines : 200 UE/m^3 (20 ng/m^3) (Smit et al. 2005) et 78 000 UE/m^3 (7 800 ng/m^3) (Smit et al. 2006). Une autre étude identifie un LOAEL[4] de 100 UE/m^3 (10 ng/m^3) (Basinas et al. 2012).

[2] IC : Intervalle de confiance
[3] NOAEL = no observed adverse effect level ou niveau sans effet néfaste observé
[4] LOAEL = lowest observed adverse effect level ou niveau d'exposition le plus bas produisant un effet néfaste observé

- **Effets cardiovasculaires**

Seules deux études évaluant les effets cardiovasculaires aigus liés à l'exposition aux endotoxines ont été identifiées. Dans l'une de ces études, les concentrations d'endotoxines étaient associées à des augmentations de la pression artérielle 30 minutes après l'exposition ; chaque doublement de la concentration d'endotoxines était significativement associé à une augmentation de 1,73 mm Hg de la pression artérielle systolique (IC à 95 % : [0,2 - 3,18] ; p = 0,02) et à une augmentation de 2,07 mm Hg de la pression artérielle diastolique (IC à 95 % : [0,74 - 3,39] ; p = 0,003) (Zhong et al. 2015). Ces deux études ont également mis en évidence, rapidement juste après l'exposition, une augmentation du niveau du facteur de croissance de l'endothélium vasculaire (VEGF) dans l'urine et dans le sang en association à une exposition aux endotoxines (Zhong et al. 2015; Liu et al. 2015), suggérant respectivement selon les auteurs, une réponse adaptative aux augmentations de pression observées, ou une réponse systémique aiguë aux atteintes endothéliales.

- *Irritation des voies respiratoires supérieures, des yeux et de la peau*

Quatre études, basées sur des auto-questionnaires, rapportent des irritations du nez, des yeux, des voies respiratoires supérieures (Herr et al. 2004 ; Heldal et al. 2010 ; Heldal et al. 2015 ; Aghaei et al. 2020). Seules deux de ces études associent l'exposition des endotoxines à une augmentation de la fréquence des irritations de la peau lors de la manipulation de déchets organiques (Herr et al. 2004) et à des irritations nasales chez des égoutiers (Heldal et al. 2010).

- *Modulation de la réponse immunologique*

De faibles niveaux d'exposition aux endotoxines augmentent significativement la réponse inflammatoire à l'exposition aux allergènes chez les sujets sensibilisés atteints d'asthme (Reed et Milton 2001; Liu 2002). De petites quantités d'endotoxines (< 1 ng/mL) se lient aux récepteurs des macrophages et d'autres cellules, générant de l'IL-12, qui inhibe les réponses IgE. Elles génèrent également des cytokines telles que l'IL-1, le TNF-α et l'IL-8, qui provoquent une inflammation (Michel 2000 ; Reed et Milton 2001). Les sujets asthmatiques exposés aux endotoxines montrent une diminution significative de la fonction pulmonaire (Michel et al. 1996 ; Eldridge et Peden 2000 ; Nightingale et al. 1998 ; Douwes et al. 2000).

- *Toxicité chronique*

  - **Effets respiratoires et pulmonaires**

La plupart des études de toxicité chronique par voie respiratoire disponibles sur les endotoxines se sont focalisées sur la réalisation d'explorations fonctionnelles respiratoires. Les effets rapportés par inhalation consistent principalement en une diminution du VEMS (Smid et al. 1992 ; Post et al. 1998 ; Kirychuk et al. 1998 ; Vogelzang et al. 1998 ; Christiani et al. 1999 ; 2001 ; Wang, et al. 2005 ; Shi et al. 2010 ; Ghani et al. 2016) avec des effets significativement associés à l'exposition aux endotoxines pour certaines études (Smid et al. 1992 ; Post et al. 1998 ; Kirychuk et al. 1998 ; Vogelzang et al. 1998 ; Ghani et al. 2016). Une étude longitudinale sur 5 ans a mis en évidence que le niveau d'endotoxines était un facteur prédictif significatif de la variation annuelle du VEMS et que la variation du VEMS durant un poste de travail était un prédicteur significatif de la variation annuelle du taux de VEMS (p=0,01) (Kirychuk et al. 1998).

Une étude transversale portant sur 315 travailleurs employés dans 14 usines d'aliments pour animaux aux Pays-Bas a observé une exposition individuelle moyenne sur 8 heures aux poussières de céréales de 9 mg/m^3 (diamètre de coupure médian de 30 µm) et de 250 UE/m^3 (25 ng/m^3) pour les endotoxines (2 - 4 700 UE/m^3), sur la base de 530 mesures individuelles des poussières. Toutes les variables étudiées de la fonction pulmonaire (CVF, VEMS, DEP, MEF75, MEF50) ont montré des valeurs significativement réduites avec l'augmentation de l'exposition à la fois aux poussières et aux endotoxines (Smid et al. 1992).

Un suivi de la population de l'étude précédente a été mené sur 5 ans auprès de 140 travailleurs de l'industrie de la transformation des céréales et de l'alimentation animale. L'article de Post *et al.* indique que, lors de la première enquête, 520[5] mesures individuelles d'exposition avaient été réalisées et que 179 autres mesures ont été effectuées lors de la seconde enquête. Les expositions moyennes durant le poste de travail allaient de 36 à 990 UE/m^3 (3,6 à 99 ng/m^3) pour les endotoxines (Post et al. 1998).

L'étude transversale sur 315 travailleurs employés dans 14 usines d'aliments pour animaux aux Pays-Bas a permis d'estimer une diminution de 49,1 mL de VEMS par 100 UE/m^3 (-4,91 mL par ng/m^3) d'exposition aux endotoxines pour les travailleurs ayant un historique de travail moyen de 13 ans. L'étude a révélé une corrélation significative entre l'exposition cumulée estimée aux endotoxines et l'altération de la fonction pulmonaire (Smid et al. 1992). Selon les auteurs, la valeur estimée du LOAEL se situerait entre 30 et 75 UE/m^3 (3 et 7,5 ng/m^3) (Smid 1993). Le suivi de 5 ans de cette même population a permis de calculer une diminution du VEMS de 0,326 mL (SE = 0,139) par 10 UE/m^3 (ou 1 ng/m^3) d'exposition aux endotoxines par année d'exposition, après ajustement sur l'âge, la taille et le tabagisme (r^2 = 0,12) (Post et al. 1998).

D'autres paramètres respiratoires ont été étudiés tels que la capacité vitale forcée (CVF) (Smid et al. 1992 ; Kirychuk et al. 1998 ; Vogelzang et al. 1998 ; Wang, et al. 2005) et le débit expiratoire de pointe adulte (DEP) (Smid et al. 1992), les débits expiratoires 25-75 (MEF25-75) (Smid et al. 1992), les débits expiratoires 50 (MMEF) (Post et al. 1998). Certaines de ces études ont mis en évidence une diminution de la CVF (Vogelzang et al. 1998 ; Ghani et al. 2016) après exposition aux endotoxines.

Certaines études ont évalué les paramètres respiratoires par l'administration d'un questionnaire aux sujets afin qu'ils renseignent des symptômes respiratoires chroniques (Shi et al. 2010 ; Basinas et al. 2012 ; Lai et al. 2014 ; 2015 ; 2016 ; Carnes et al. 2017 ; Lim et al. 2019). Ces études révèlent des associations significatives entre l'exposition aux endotoxines et la byssinose (Shi et al. 2010), l'asthme (Carnes et al. 2017), la bronchite chronique (Shi et al. 2010 ; Basinas et al. 2012), la toux chronique (Shi et al. 2010), la respiration sifflante et la rhinoconjonctivite (Lim et al. 2019).

De nombreuses études transversales étudiant les effets respiratoires associés à l'exposition aux endotoxines ont été publiées. Cependant, elles ne peuvent pas être exploitées pour l'évaluation des effets respiratoires d'une exposition à long terme aux endotoxines, car elles ne permettent pas de distinguer les effets aigus des effets dus à une exposition répétée (Kennedy et al. 1987 ; Simpson et al. 1998 ; Oldenburg et al. 2007 ; Smit et al. 2008 ; Freitas et al. 2016 ; Anyfantis et al. 2017 ; Heldal et al. 2019 ; Shakri et al. 2020).

[5] L'article de Smid *et al*. décrit 530 mesures individuelles de poussières (Smid et al. 1992).

- **Effets neurologiques**

Très peu d'études se sont intéressées à l'association entre exposition aux endotoxines et effets neurotoxiques. Aucune d'elles ne met en évidence d'association significative avec la survenue de la maladie de Parkinson (van der Mark et al. 2014 ; Checkoway et al. 2018) ou de la sclérose latérale amyotrophique (Visser et al. 2019).

- **Effets cardiovasculaires**

Les études évaluant l'association entre l'exposition aux endotoxines et des effets chroniques cardiovasculaires sont très limitées. Aucune d'elles ne met en évidence d'association significative avec la mortalité cardiovasculaire (Gallagher et al. 2012), la fonction microvasculaire (Karottki et al. 2014) et les marqueurs sanguins de l'activation plaquettaire (Straumfors et al. 2018).

- **Effets génotoxiques**

Une seule étude portant sur les effets génotoxiques a été identifiée dans la littérature scientifique. Cependant, elle n'a pas mis en évidence d'association significative entre l'exposition aux endotoxines et une augmentation des micronoyaux ou d'autres anomalies du noyau (Wultsch et al. 2013).

- **Effets cancérogènes**

Selon le rapport du DECOS, dans les années 1970, les résultats de plusieurs études de mortalité dans des cohortes professionnelles ont suggéré des risques réduits de cancer du poumon chez les travailleurs du textile (Health Council of the Netherlands 2010). D'autres publications ont confirmé ces résultats en suggérant une relation dose-réponse inverse entre une exposition aux endotoxines et le cancer du poumon (Agalliu et al. 2011; Applebaum et al. 2013 ; McElvenny et al. 2011 ; Xu et al. 2016 ; Ben Khedher et al. 2017). Ainsi, une étude a rapporté une réduction dose-dépendante du risque de cancer du poumon dans une cohorte d'ouvrières de l'industrie textile à Shanghai (Astrakianakis et al. 2007). Une revue de 2009 indique également que les études épidémiologiques dans l'industrie textile du coton et dans d'autres secteurs professionnels exposés aux endotoxines ont systématiquement montré une réduction du risque de cancer du poumon (Lundin et Checkoway 2009). Toutefois, l'absence de données sur les facteurs de confusion potentiels constituait une limite à la plupart des études.

D'autres publications ont évalué l'association entre l'exposition aux endotoxines et la survenue d'autres types de cancer. Aucune association significative n'a été mise en évidence entre une exposition aux endotoxines et le lymphome non Hodgkinien (Wang et al. 2013), le cancer de l'œsophage (Gallagher et al. 2015) et le cancer du pancréas (Reul et al. 2016). Concernant le risque de cancer de l'estomac, des tendances statistiquement augmentées ont été observées avec l'augmentation des niveaux d'exposition aux endotoxines. Toutefois, l'ampleur de l'effet était faible et à la limite de la significativité pour le quartile le plus exposé (HR = 1,2 ; IC à 95 % : [1,0 - 1,5]) (Gallagher et al. 2015).

3.4. Éléments de proposition pour fixer des VLEP

3.4.1. Choix de l'effet critique

Les études humaines disponibles mettent en évidence des effets toxiques aigus et chroniques. Les études sur l'association entre l'exposition aux endotoxines et la survenue d'un cancer n'ont pas montré de relation significative avec le lymphome non Hodgkinien (Wang et al. 2013), le cancer de l'œsophage (Gallagher et al. 2015) et le cancer du pancréas (Reul et al. 2016). Une association de faible ampleur et à la limite de significativité a été identifiée pour le cancer de l'estomac (Gallagher et al. 2015). Les études n'ont pas montré d'effets neurotoxiques significatifs associés à l'exposition aux endotoxines (van der Mark et al. 2014 ; Checkoway et al. 2018 ; Visser et al. 2019). En ce qui concerne les effets cardiovasculaires, aucune association significative n'a été trouvée avec la mortalité cardiovasculaire (Gallagher et al. 2012), la fonction microvasculaire (Karottki et al. 2014) ou les marqueurs sanguins de l'activation plaquettaire (Straumfors et al. 2018).

Par conséquent, le poumon semble être le principal organe cible après une exposition aux endotoxines. Des études ont notamment mis en évidence des altérations de la fonction pulmonaire, telles que :

- une diminution du VEMS (Haglind et Rylander 1984 ; Rylander et al. 1985 ; Castellan et al. 1987 ; Smid et al. 1992 ; Post et al. 1998 ; Kirychuk et al. 1998 ; Vogelzang et al. 1998 ; Zock et al. 1998 ; Kline et al. 1999 ; Donham et al. 2000 ; Wang, et al. 2005 ; Shi et al. 2010 ; Cyprowski et al. 2015) ;
- une diminution de la CVF (Milton et al. 1995 ; 1996; Vogelzang et al. 1998 ; Mitchell et al. 2015 ; Ghani et al. 2016) ;
- la survenue de symptômes respiratoires rapportés par des questionnaires tels que la bronchite chronique (Shi et al. 2010 ; Basinas et al. 2012), la toux chronique (Shi et al. 2010), la respiration sifflante et la rhinoconjonctivite (Lim et al. 2019) ;
- l'ODTS, une maladie de type grippale non infectieuse qui survient après une exposition par inhalation à des poussières organiques contaminées par des microorganismes (par exemple, des bactéries gram-négatives) (Boehmer et al. 2009 ; Smit et al. 2005 ; Smit et al. 2006 ; Basinas et al. 2012).

Les seuls effets bien documentés associés à l'exposition aiguë et chronique aux endotoxines concernent les modifications des débits respiratoires et le syndrome toxique des poussières organiques (ODTS). De nombreuses études ont été menées pour caractériser la relation entre l'exposition aux endotoxines et la réduction des paramètres respiratoires. En ce qui concerne l'ODTS, les symptômes ont été rapportés par questionnaires. Sur la base des articles publiés, et en particulier du fait des méthodologies utilisées et de la grande variabilité interindividuelle (et intra-individuelle) de la sensibilité des sujets à l'apparition de l'ODTS, le CES VSR estime que l'ODTS ne peut pas être utilisé comme effet critique pour la construction de VLEP pour les endotoxines. D'autre part, les études sur la CVF ne montrent pas d'association forte entre l'exposition aux endotoxines et la réduction de la CVF. Sur la base des études disponibles, l'effet observé aux concentrations les plus faibles est la diminution du VEMS.

Étant donné qu'une diminution d'au moins 1% du VEMS à l'échelle collective peut être considérée comme un effet adverse, le CES VSR retient la diminution de 1% du VEMS comme effet critique pour la dérivation de la VLCT-15min et de la VLEP-8h.

Pour la plupart des effets non cancérogènes, il est considéré, par défaut et en l'état actuel des connaissances, que la toxicité ne s'exprime qu'au-delà d'un seuil de dose (Anses, à paraître). Par conséquent, cet effet critique est considéré comme résultant d'un mécanisme à seuil.

- **Valeur limite d'exposition professionnelle sur 15 minutes (VLCT-15min)**

  - *Choix de l'étude clé*

Après une exposition court terme aux endotoxines, certaines études suggèrent des relations dose-réponse entre la diminution du VEMS et l'exposition aux endotoxines (Haglind et Rylander 1984 ; Rylander et al. 1985 ; Donham et al. 1989 ; Donham et al. 2000). Cependant, ces études présentent des limites, telles que des conditions d'exposition aux endotoxines peu précises, en particulier dans les études transversales (Donham et al. 1989 ; Donham et al. 2000), une exposition élevée aux endotoxines (Haglind et Rylander 1984 ; Rylander et al. 1985), des relations dose-réponse non significatives (Haglind et Rylander 1984) et la non prise en compte de la co-exposition à d'autres contaminants susceptibles de provoquer des effets respiratoires (Rylander et al. 1985 ; Donham et al. 1989).
Parmi toutes les études établissant des relations dose-réponse sur la diminution de 1% du VEMS après une exposition aiguë aux endotoxines, l'étude de Castellan *et al*. de 1987 a été identifiée comme l'étude la plus pertinente et la plus robuste pour la dérivation de la VLCT-15min car :

- des volontaires (24 - 35 sujets) ont été exposés à de la poussière de coton pendant 6 heures, avec des concentrations d'endotoxines dans l'air allant de 60 à 7 790 UE/m^3 (6 à 779 ng/m^3) au cours de 108 sessions d'exposition différentes ;
- la concentration de l'exposition aux endotoxines a été très bien contrôlée : des concentrations de poussières constantes ont été maintenues pendant les sessions d'exposition en ajustant la proportion d'air contaminé par la poussière provenant de la salle de cardage du coton et pénétrant dans la salle d'exposition. Les concentrations de poussières en suspension dans l'air ont été contrôlées à l'aide d'un système de surveillance continue des aérosols et de mesures gravimétriques moyennes, environ toutes les heures, de la fraction inhalable des poussières en suspension dans l'air ;
- la population de volontaires excluait les sujets souffrant d'asthme, de bronchite chronique et d'essoufflement à l'effort, mais incluait une population sensible avec une réponse de diminution du VEMS d'au moins 5% et inférieure à 30% au pré-test d'exposition à 1 000 UE/m^3 (100 ng/m^3) de LPS (Castellan et al. 1987).

Le CES VSR retient donc l'étude de Castellan *et al.* de 1987 comme étude clé pour l'établissement de la VLCT-15min.

  - *Choix du point de départ (PoD)*

Dans l'étude de Castellan *et al*., les auteurs ont calculé que le pourcentage zéro de changement du VEMS pendant l'exposition aux endotoxines était de 90 UE/m^3 (9 ng/m^3) en utilisant un modèle de régression linéaire (Castellan et al. 1987).

Le CES VSR retient la concentration de 90 UE/m^3 (9 ng/m^3) comme NOAEL pour la construction de la VLCT-15min.

- *Choix des facteurs d'incertitude*

Le calcul de la VLCT-15min à partir du NOAEL retenu a été effectué à l'aide des facteurs d'incertitude (FI) suivants (Anses, à paraître) :

- Variabilité inter-espèces (FI_A)

Application d'un FI_A de 1 dans la mesure où l'étude clé utilisée pour établir la VLCT-15min est basée sur une population humaine.

- Variabilité inter-individuelle (FI_H)

Bien que les auteurs de l'étude clé aient sélectionné des sujets sensibles, ont été exclus les sujets souffrant d'asthme, de bronchite chronique et d'essoufflement à l'effort, ainsi que les volontaires présentant une diminution de plus de 30 % du VEMS lors du test de dépistage. Par conséquent, un FI_H de 5 est retenu conformément à la méthodologie.

- Type de PoD utilisé (BMDL, LOAEL ou NOAEL) (FI_L)

Application d'un FI_L de 1 étant donné que le PoD retenu pour la construction de la valeur est un NOAEL.

- Qualité de la base de données (FI_D)

Application d'un FI_D de 1 étant donné que les effets sur la réduction du VEMS, de la CVF et le syndrome toxique des poussières organiques (ODTS) sont bien documentés dans la littérature scientifique.

Le CES VSR retient un facteur d'incertitude global de 5 conduisant à une VLCT-15min calculée de 90 UE/m^3 /5, soit 18 UE/m^3 (1.8 ng/m^3) arrondie à 20 UE/m^3 (2 ng/m^3).

Par conséquent, le CES VSR recommande une VLCT-15min de 20 UE/m^3 (2 ng/m^3)[6] afin de prévenir une diminution de 1% du VEMS. Cependant, le respect de la valeur limite proposée ne garantit pas l'absence de survenue du syndrome toxique des poussières organiques.

Dans la mesure où le contrôle d'une VLCT-15min implique un mesurage lors des pics d'exposition et que cela peut parfois s'avérer difficile à contrôler sur la durée d'un poste de travail de 8 heures, les experts ont estimé 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. Cette nécessité est renforcée, dans le cas des endotoxines, par le fait qu'une réduction du VEMS au cours d'un seul poste de travail est prédictive d'une réduction du VEMS à long terme. En effet, Kirychuk *et al.* ont montré que la variation du VEMS par rapport à la ligne de base durant le poste de travail était un facteur prédictif significatif de la variation annuelle du VEMS (Kirychuk et al. 1998). En outre, Christiani *et al.* ont conclu à l'existence d'une corrélation significative entre la perte chronique de la fonction pulmonaire et la durée de l'exposition (années de travail dans les filatures de coton). Ces pertes fonctionnelles étaient significativement associées à l'exposition cumulative aux endotoxines. Les travailleurs ayant un niveau d'exposition cumulée aux endotoxines plus élevé présentaient des pertes de VEMS significativement plus importantes que ceux ayant un faible niveau d'exposition (Christiani et al. 2001).

[6] Dans la mesure où l'étude de Castellan de 1987 s'appuie sur des mesures de la fraction inhalable, c'est la mesure de la fraction inhalable qui est considérée pour la recommandation de la VLCT-15min.

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

  - *Choix des études clés et du point de départ (PoD)*

Quel que soit le type (transversal ou longitudinal) des études épidémiologiques publiées, la caractérisation d'une association dose-réponse entre l'exposition aux endotoxines et la diminution du VEMS est rendue difficile par les co-expositions à d'autres composants (particules, champignons, acariens) et parfois à d'autres agents toxiques (ammoniac, H_2S, aldéhydes, etc.), ces facteurs confondants n'étant généralement pas pris en compte dans les analyses.

De plus, les études longitudinales disponibles n'ont pas permis d'établir une VLEP-8h en raison de l'absence de relation dose-réponse significative ou d'association entre une exposition chronique aux endotoxines et la réduction du VEMS.

Les études transversales disponibles renseignant les effets respiratoires après une exposition à long terme présentent des limites supplémentaires pour la dérivation d'une VLEP-8h car elles ne permettent pas de différencier les effets chroniques de l'exposition des effets aigus.

Par conséquent, le CES VSR a décidé de retenir la concentration de 90 UE/m^3 (9 ng/m^3) correspondant au NOAEL identifié par l'étude de Castellan après 6 heures d'exposition pour la dérivation d'une VLEP-8h comme PoD, dans la mesure où :

- deux études portant sur la même cohorte de travailleurs du secteur de l'alimentation animale exposés à la poussière de céréales aux Pays-Bas (Smid et al. 1992 ; Post et al. 1998) ont permis d'évaluer comment le PoD de 90 UE/m^3 (9 ng/m^3) identifié par l'étude de Castellan affecterait la fonction pulmonaire au cours d'une vie professionnelle de 40 ans. Les deux équations de régression cohérentes identifiées dans ces études ont montré que 40 ans d'exposition aux endotoxines à 90 UE/m^3 (9 ng/m^3) entraîneraient une perte supplémentaire de 120 mL du VEMS, en plus de la diminution physiologique du VEMS avec l'âge. Par rapport à une perte naturelle moyenne du VEMS de 30 mL/an à partir de l'âge de 30 ans chez les sujets sains (Guénard et Rouatbi 2004 ; Lowery et al. 2013), une perte supplémentaire de 120 mL après 40 ans d'exposition ne semble pas entraîner d'effets individuels sur la santé. Par conséquent, le CES VSR, en accord avec le DECOS, considère qu'une réduction supplémentaire de 120 mL du VEMS après 40 ans d'exposition aux endotoxines n'est pas à considérer comme un effet adverse sur la santé (Health Council of the Netherlands 2010);
- une analyse groupée de quatre études de cohortes dans plusieurs pays et plusieurs industries identifie un LOAEL de 100 UE/m^3 (10 ng/m^3) pour le risque de bronchite chronique (auto-déclarée comme la persistance de la toux et des expectorations pendant au moins 3 mois, au cours des 12 mois précédant l'administration du questionnaire) (Basinas et al. 2012). Malgré la déclaration subjective de l'effet sur la santé, ce LOAEL proche du NOAEL de 90 UE/m^3 (9 ng/m^3)) est un argument supplémentaire en faveur du maintien de la même valeur de NOAEL pour les effets chroniques ;
- l'étude de Kennedy *et al.* rapporte que, même s'il y a une diminution du VEMS au cours d'un poste de travail, il n'y a pas d'association de la diminution du VEMS avec la durée d'exposition ou l'exposition cumulée. Les effets aigus sur le débit respiratoire seraient donc détectables avant l'apparition des effets chroniques (Kennedy et al. 1987).

Sur la base de l'ensemble de ces éléments, le CES VSR retient la concentration de 90 UE/m^3 (9 ng/m^3) comme NOAEL pour la construction de la VLEP-8h.

- *Choix des facteurs d'incertitude*

Le calcul de la VLEP-8h à partir du NOAEL retenu de 90 UE/m^3 (9 ng/m^3) a été effectué à l'aide des facteurs d'incertitude (FI) suivants (Anses, à paraître) :

- Variabilité inter-espèces (FI_A)

Application d'un FI_A de 1 dans la mesure où l'étude clé utilisée pour établir la VLEP-8h est basée sur une population humaine.

- Variabilité interindividuelle (FI_H)

Bien que les auteurs de l'étude clé aient sélectionné des sujets sensibles, les sujets souffrant d'asthme, de bronchite chronique et d'essoufflement à l'effort ainsi que les volontaires présentant une diminution de plus de 30 % du VEMS lors du test de dépistage ont été exclus. Par conséquent, un FI_H de 5 est retenu conformément à la méthodologie.

- Type de PoD utilisé (BMDL, LOAEL ou NOAEL) ($FI_{L/B}$)

Application d'un $FI_{L/B}$ de 1 étant donné que le PoD retenu pour l'établissement de la VLEP-8h est un NOAEL.

- Transposition d'une exposition moyen terme à une exposition long terme (FI_S)

Certaines études ont montré qu'une réduction du VEMS au cours d'un seul poste de travail est prédictive d'une réduction du VEMS à long terme (Kirychuk et al. 1998 ; Christiani et al. 2001). Elles démontrent aussi que des effets aigus sur la modification du débit respiratoire sont détectables avant l'apparition d'effets chroniques (Kennedy et al. 1987). De plus, le PoD de l'étude de Castellan est proche du LOAEL identifié pour l'ODTS (Basinas et al. 2012) et a été étudié pour l'effet de l'exposition à long terme (Smid et al. 1992 ; Post et al. 1998).
Dans la mesure où l'étude clé de Castellan *et al*. est également confortée par des études chroniques (Smid et al. 1992 ; Post et al. 1998 ; Kennedy et al. 1987 ; Kirychuk et al. 1998 ; Christiani et al. 2001), un FI_S de 1 est retenu.

- Qualité de la base de données (FI_D)

Application d'un FI_D de 1 étant donné que les effets de l'ODTS et de la réduction du VEMS et de la CVF sont bien documentés dans la littérature scientifique.

Le CES VSR retient un facteur d'incertitude global de 5 conduisant à une VLEP-8h calculée de 90 UE/m^3 /5, soit 18 UE/m^3 (1.8 ng/m^3) arrondie à 20 UE/m^3 (2 ng/m^3).

Par conséquent, le CES VSR recommande une VLEP-8h de 20 UE/m^3 (2 ng/m^3)[7] **pour prévenir une diminution de 1% du VEMS pour une exposition long terme aux endotoxines. Cependant, le respect de la valeur limite proposée ne garantit pas l'absence de survenue du syndrome toxique des poussières organiques.**

[7] Dans la mesure où l'étude de Castellan de 1987 s'appuie sur des mesures de la fraction inhalable, c'est la mesure de la fraction inhalable qui est considérée pour la recommandation de la VLEP-8h.

■ Mention « peau »

En l'absence de données quantitatives, la mention « peau » n'est pas recommandée pour les endotoxines.

■ Mention « bruit »

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

■ Conclusion et recommandations

Type de VLEP		**VLCT-15min**	**VLEP-8h**
VR	Organisme	Anses	Anses
	Année	2024	2024
	Valeur	20 UE/m^3 (= 2 ng/m^3) (fraction inhalable)	20 UE/m^3 (= 2 ng/m^3) (fraction inhalable)
Population cible		Travailleurs	Travailleurs
Effet critique		Diminution de 1% du VEMS	Diminution de 1% du VEMS
Etude clé	Référence	Castellan *et al.* 1987	Castellan *et al.* 1987
	Population de l'étude	Volontaires sains	Volontaires sains
	Exposition (temps, voie)	108 sessions de 6 heures, inhalation	108 sessions de 6 heures, inhalation
Etudes support		/	Smid *et al.* 1992 ; Post *et al.* 1998 ; Basinas *et al.* 2012 ; Kennedy *et al.* 1987 ; Kirychuk *et al.* 1998 ; Christiani *et al.* 2001
Point de départ (POD)		NOAEL = 90 UE/m^3 (= 9 ng/m^3)	NOAEL = 90 UE/m^3 (= 9 ng/m^3)
Ajustement temporel		Non	Non
Ajustement dosimétrique		Non	Non
Facteurs d'incertitude (FI)		FI_H = 5	FI_H = 5
Mentions (peau, bruit)		Non	Non

Dans la mesure où les valeurs recommandées ne permettent pas de garantir une protection contre la survenue du syndrome toxique des poussières organiques, le CES VSR recommande d'abaisser les niveaux d'exposition aux endotoxines aux niveaux les plus bas techniquement possibles.

4. CONCLUSIONS ET RECOMMANDATIONS DE L'AGENCE

Conformément aux conclusions de son Comité d'Experts Spécialisés (CES) « Valeurs Sanitaires de Référence », l'agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail recommande la fixation d'une VLCT-15min de 20 UE/m^3 (soit 2 ng/m^3) et d'une VLEP-8h de 20 UE/m^3 (soit 2 ng/m^3). Faute de données disponibles, ni la mention « peau », ni la mention « bruit » ne sont recommandées. Ces valeurs recommandées visent à prévenir une diminution de 1% du volume expiratoire maximal par seconde (VEMS), effet critique retenu, sans qu'elles ne puissent garantir l'exclusion du syndrome toxique de poussières organiques, maladie de type grippale non infectieuse.

Au regard du syndrome toxique de poussières organiques évoqué ci-dessus, l'agence recommande de limiter les expositions aux endotoxines aux niveaux aussi bas que raisonnablement possible dans la mesure où les données disponibles ne permettent pas de déterminer une valeur en dessous de laquelle sa survenue pourrait être exclue. Elle souligne, par ailleurs, l'existence d'une grande variabilité interindividuelle et intra-individuelle lors de la survenue de ce syndrome.

A titre d'élément de comparaison, l'agence indique qu'un rapport d'étude de 2007 de l'Ineris[8] (Institut national de l'environnement industriel et des risques) rapporte un bruit de fond moyen dans l'atmosphère en endotoxines totales variant entre 0.3 et 4.4 UE/m^3 en zone urbaine, entre 1.3 et 30 UE/m^3 en zone industrielle en amont des vents, une valeur moyenne de 9 UE/m^3 à 30 mètres en amont des vents d'élevage d'engraissement de truies et une valeur moyenne de 0.4 UE/m^3 pour des environnements naturels.

Pr Benoit Vallet

[8] Déléry, Laure. « Endotoxines. Eléments disponibles pour une évaluation des risques sanitaires en lien avec les émissions des installations classées pour la protection de l'environnement ». Ineris, 2007 https://www.ineris.fr/sites/ineris.fr/files/contribution/Documents/Rapport_DRC6_endotoxines.pdf

MOTS-CLÉS

VLEP, valeurs limites, milieu professionnel, agents chimiques, effets sur la santé, valeur de référence, endotoxines, LPS

KEY WORDS

OEL, limit values, occupational environment, chemical agents, health effects, reference value, endotoxin, LPS

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Anses. (2024). Avis de l'agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail relatif à l'expertise en vue de la fixation de valeurs limites d'exposition à des agents chimiques en milieu professionnel - Évaluation des effets sur la santé sur le lieu de travail des endotoxines. (saisine 2012-SA-0075). Maisons-Alfort : Anses, 16 p.

Expert appraisal on recommending occupational exposure limits for chemical agents

Assessment of health effects at the workplace for endotoxins

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

Évaluation des effets sur la santé sur le lieu de travail pour les endotoxines

Mission permanente VLEP/ OEL Permanent Mission
Saisine n° 2012-SA-0075 / Request n°2012-SA-0075

Rapport d'expertise collective
Collective expert appraisal report

Comité d'experts spécialisé « Valeurs sanitaires de référence »

Expert Committee on "health reference values"

June 2024

Juin 2024

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Mots clés

VLEP, valeurs limites, niveaux d'exposition, milieu professionnel, agents chimiques, effets sur la santé, lieu de travail, valeur de référence, endotoxines

Key words

OEL, limit values, exposure levels, occupational, chemical agents, health effects, workplace, reference value, endotoxins

Presentation of participants

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

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

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Acronyms and abbreviations

3-OHFA	3-hydroxy fatty acid
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 hyperresponsiveness
BP	Blood pressure
CAP	Concentrated ambient particle
Carsat	*Caisse d'assurance retraite et de la santé au travail*
Cramif	*Caisse régionale d'assurance maladie d'Ile-de-France*
sCD14	Soluble form of CD14 receptor
mCD14	Membrane of CD14 receptor
CI	Confidence interval
CO2	Carbon dioxide
COCT	Conseil d'orientation des conditions de travail (French Steering Committee on Working Conditions)
COPD	Chronic obstructive pulmonary disease
CRP	C-reactive protein
CT	Computed tomography
DECOS	Dutch Expert Committee of Occupational Safety
EBC	Exhaled breath condensate
ECP	Eosinophilic cationic protein
EDC	Electrostatic dust fall collector
EU	Endotoxin Unit
FEF25-75	Forced expiratory flow at 25 to 75% of forced vital capacity
FEF50	Forced expiratory flow at 50% of forced vital capacity
Fe NO	Fractional exhaled nitric oxide
FEV1	Forced expiratory volume in 1 second
ΔFEV1	(Across-shift) change in FEV over an exposure period of several hours
FVC	Forced vital capacity
GC-MS	Gas chromatography-mass spectrometry
GM	Geometric mean
GSD	Geometric standard deviation
H_2S	Hydrogen sulphide
HBROEL	Health-based recommended occupational exposure limit
HD	House dust
HDM	House dust mist
HR	Hazard ratio
HRV	Health Reference Values
ICAM-1	Intercellular adhesion molecule 1
ICARE	Investigation of occupational and environmental causes of respiratory cancers
IFNγ	Interferon-gamma
IgE	Immunoglobulin E
IL	Interleukin
INRS	*Institut national de recherche et de sécurité* (The French National Research and Safety Institute for the Prevention of Occupational accidents and Diseases)
IRSST	*Institut de Recherche Robert-Sauvé en Santé et en Sécurité du Travail*
KLARE	Kinetic limulus assay with resistant-parallel-line estimation
LAL	Limulus amebocyte lysate
LBP	Lipid-binding protein
LOAEL	Lowest observed adverse effect level
LOEL	Lowest observed effect level
LPS	Lipopolysaccharide
M	Mean
MD	Median
MMEF	Maximal mid-expiration flow (average flow over middle half of FVC)
MEF_{25}	Maximum expiratory flow rate at 25% of forced vital capacity

MEF_{50}	Maximum expiratory flow rate at 50% of forced vital capacity
MEF_{75}	Maximum expiratory flow rate at 75% of forced vital capacity
MIP-1α	Macrophage inflammatory protein-1 α
MPO	Myeloperoxidase
MRC	Medical Research Council
MVF	Microvascular function
NAL	Nasal lavage
NEL	No effect level
NF-kB	Nuclear factor kB
NHL	Non-Hodgkin lymphoma
NOAEL	No observed adverse effect level
NOEL	No observed effect level
NOS	Nitric oxide synthase
OEL	Occupational exposure limit
ODTS	Organic dust toxic syndrome
OR	Odds ratio
OSH	Occupational safety and health
PC	Provocative histamine concentration
PD	Parkinson disease
PEF / PEFR	Peak expiratory flow / Peak expiratory flow rate
PM	Particulate matter
$PM_{2.5}$	Particulate matter < 2.5 µm in aerodynamic diameter
PM_{10}	Particulate matter < 10 µm in aerodynamic diameter
PMN	Polymorphonuclear leukocyte (neutrophil)
R^2	Correlation coefficient
RAC	Risk Assessment Committee
RH	Relative air humidity
RR	Relative risk
SBP	Segmental bronchoprovocation
SD	Standard deviation
SEM	Standard error of the mean
SpD	Surfactant protein D
SPT	Skin prick test
SMR	Standard mortality ratio
STEL	Short-term exposure limit
T-cell	Thymus cell derived
Th1	T-helper type1
TLR	Toll-like receptor
TNF-α	Tumour necrosis factor alpha
TWA	Time-weighted average
VEGF	Vascular endothelial growth factor
WWW	Waste water workers

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

1.1 Background

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

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

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

1.2 Purpose of the request

In the scope of the protocol of agreement between ANSES and the Ministry of Labour for the implementation of the scientific expertise work programme on atmospheric and biological limit values for occupational exposure established in July 2018 and renewed in 2023, Directorate General for Labour (DGT) requested ANSES to carry out the necessary assessment for setting OELs for endotoxins.

To date, France has no OEL for endotoxins.

1.2.1 Procedure: means implemented and organisation

ANSES entrusted examination of this request to the Expert Committee on Health Reference Values (HRV Committee).

For the part relating to the recommendation of values, 4 Committee experts have been named rapporteurs. Several ANSES employees also contributed to this work and were responsible for scientific coordination of the different expert groups.

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

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

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

This collective expert appraisal work and its conclusions and recommendations were adopted by the HRV Committee in June 2024.

1.3 Prevention of risks of conflicts of interest

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

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

2 Method

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

The Committee recommends the use of three types of values:

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

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

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

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

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

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

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

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

The toxicological profile of endotoxins was based on the report drawn up by the Dutch Expert Committee on Occupational Safety (DECOS) of the Health Council of the Netherlands, published in 1998 and updated in 2010. An additional bibliographical search was carried out in the PubMed® and Scopus® databases to take into account the scientific literature published on endotoxins up to December 2023. Details of the bibliographic search (query, main keywords, inclusion and exclusion criteria) are described in Annex 1 of the report.

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

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

- identify the target organ(s) and the critical effect on the basis of the toxicological profile;

- identify the establishment assumption, with or without a dose threshold, depending on the substance's mode of action;

- select one (or more) key study(s) of good scientific quality, the most relevant among the epidemiological or toxicological studies enabling the establishment of a dose-response relationship;

- define a starting point (PoD) in humans or animals on the basis of this (these) study(s), and adjust it to humans if necessary in the case of a PoD obtained in animals;

- for a threshold OEL, apply uncertainty factors to this PoD;

- for an OEL without threshold, determine a slope and/or concentrations/doses associated with several risk levels.

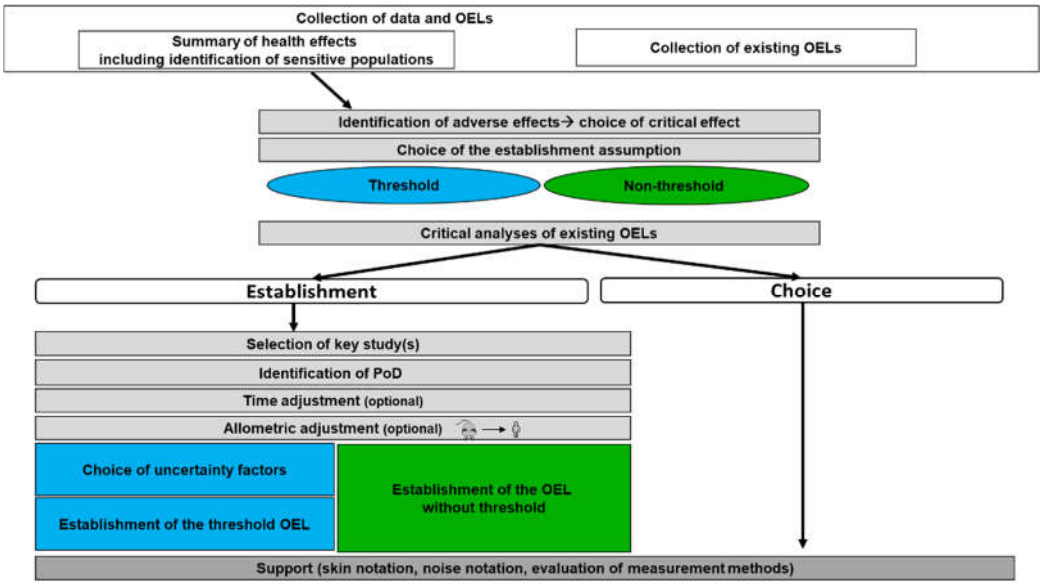

Figure 1 Steps for proposing an OEL.

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

3 Background information

3.1 Substance identification

Definition and structure

Endotoxins are toxic biological substances, components of the outer membrane of Gram-negative bacteria (e.g. *Enterobacteriaceae* or *Pseudomonadaceae*) and cyanobacteria (Duquenne et al. 2012; Anses 2016). They are released during lysis of these bacteria and, to a lesser degree, during their multiplication (Anses 2016). These complex molecules, composed of proteins, lipids, and lipopolysaccharides (LPS), are playing an important role in both cell integrity and interactions of the cell with the extracellular environment (Liebers et al. 2008; Duquenne, et al. 2012).

The terms "endotoxin" and "LPS" are often used interchangeably in the literature. However, the term “LPS” should refer to the pure chemical LPS, free of any other bacteria cell membrane components, whereas the term “endotoxin” should refer to the LPS attached to other components of the bacterial membrane (Géhin and Le Bacle 2011; Duquenne et al. 2012).

LPS consists of a hydrophobic membrane anchor portion known as lipid A and a non-repeating core oligosaccharide coupled to a distal polysaccharide (O-antigen) that extends from the bacterial surface (Figure 2) (Raetz and Whitfield 2002).

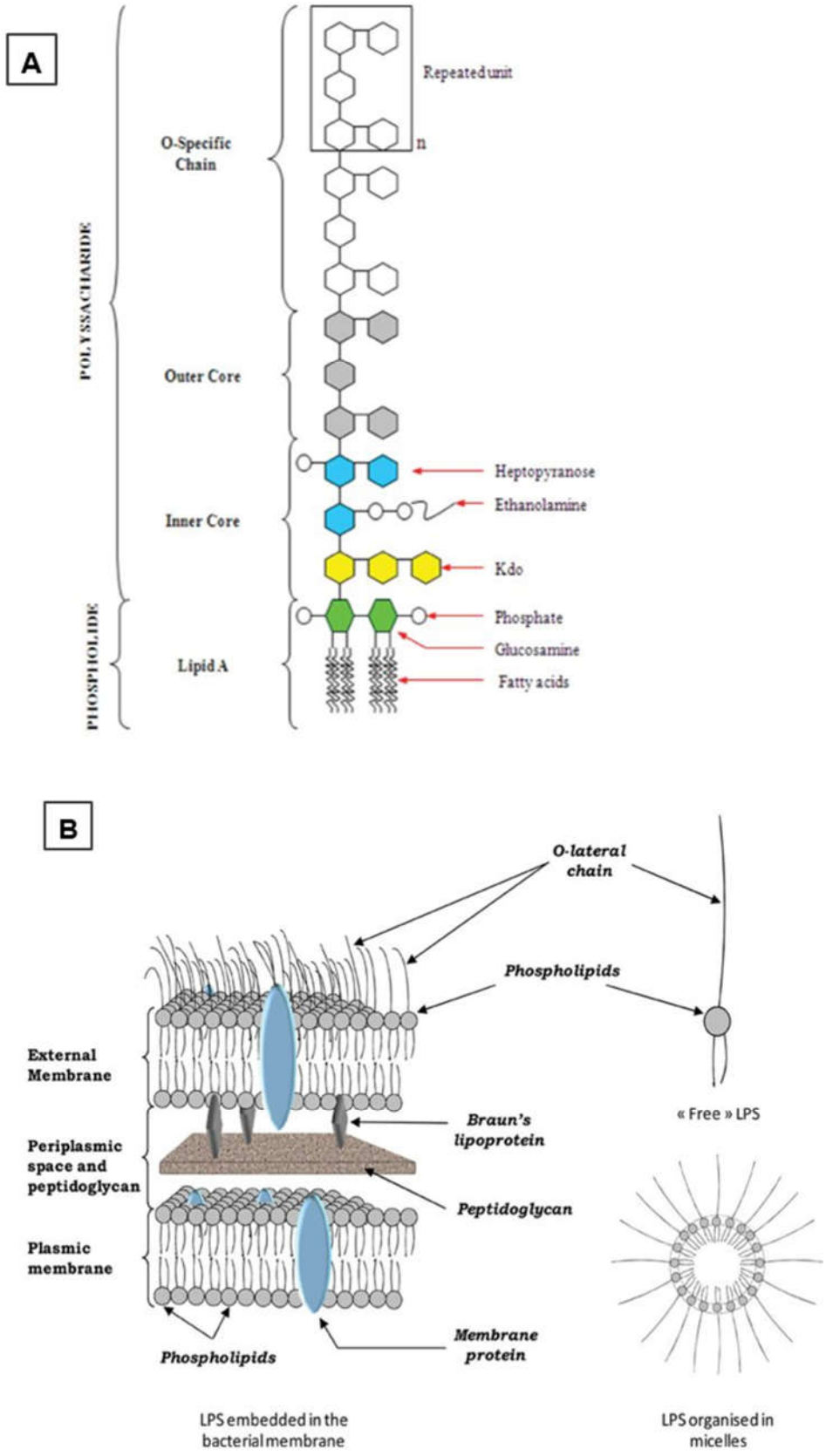

Figure 2 Endotoxin structure and organisation (according to Prescott *et al.* 2003 as cited in Duquenne et al. 2012) - (A) Chemical structure of LPS in *Salmonella typhimurium* (Kdo, 3-deoxy-d-manno-2 octulosonic acid ; (B) Location of LPS in outer membrane of bacteria.

There is a multitude of natural structural variants of LPS that are primarily due to the extended diversity in chemical composition of the polysaccharide region (core and O-antigen), but also due to considerable variations in the fine structure of lipid A (Raetz and Whitfield 2002). The O-specific chain structures, consisting of up to 50 repeating oligosaccharide units formed of 2–8 monosaccharide components, are characterized by extremely high structural variability even within a given bacterial species. These structures are at the origin of the serotyping, historically used to identify Gram-negative bacteria (Matsuura 2013; Caroff and Novikov 2020). The core oligosaccharide has less structural variation compared to the hypervariable O-polysaccharides and has limited variation within a given bacterial genus (Matsuura 2013). The *E. coli* lipid A type is relatively conserved in a wide variety of Gram-negative bacteria, although some bacterial species have different types of lipid A. Nonetheless, some variants of lipid A frequently coexist even in a single specie of bacteria, and their structures are sometimes modified under different environmental conditions (Raetz and Whitfield 2002; Matsuura 2013).

3.2 Physico-chemical properties

Endotoxins are complex amphiphilic high molecular weights molecules (about 10 to 20 kDa), with a tendency of forming very large insoluble aggregates in the form of micelles or vesicles in aqueous solution, as LPS (Harm et al. 2021). They are characterized by an ability of resisting to extreme temperatures and pH values (Miyamoto et al. 2009). Endotoxins are thermostable in the presence of moist heat and are not significantly destroyed by conventional autoclaving processes (Franco et al. 2018). It is well accepted that only dry-heat treatment is efficient in destroying endotoxins, requiring a temperature of 250°C for more than 30 min or 180°C for more than 3 hr (Miyamoto et al. 2009).

Airborne endotoxins exist in three main forms:

I) pure, small LPS molecules with molecular weights of 2 to 20 kDa;

II) pure LPS associated with other cell wall components (including LPS associated with an intact bacterial cell, called “bound endotoxins”)

III) endotoxins associated with other biological or non-biological aerosol particles, the size of these “agglomerates” varying according to the source of the aerosol sampled (Duquenne et al. 2012).

3.3 Classification

EU classification and labelling

Endotoxins are naturally occurring substances and as such, have not been classified and labelled by the European Union under Regulation (EC) No 1272/2008 on the classification, labelling and packaging of substances and mixtures (CLP Regulation). Endotoxins have not been classified by other agencies (IARC, ACGIH, DFG).

3.4 Sources of exposure

Endotoxins or LPS are practically ubiquitous in nature but occur in high concentrations in particular industrial and agricultural settings (e.g. pig, chicken, cow and horse farming, grain elevators, cotton and linen industry, potato processing industry, poultry slaughterhouse, animal feed industry, water sewage treatment and sewage composting plants, garbage handling facilities, organic waste compositing facilities, wood chip composting and timber storing facilities, grain harvest, stockage and grinding, sugar production, malting plants, onion trade....) (MacIntosh et al. 2000; Health Council

of the Netherlands 2010). Indeed, sources of gram-negative bacteria in the occupational environment can consist in either solid (settled dust, household waste, compost, grains, and plants) or liquid (wastewater, metalworking fluids, water from dental unit waterlines, etc.) matrices that contain organic matter (Duquenne et al. 2012). The presence of endotoxins in ambient air is related to the presence of gram-negative bacteria or cell wall fragments from such bacteria in airborne organic dust particles (Health Council of the Netherlands 2010). Reviews of exposure data in various occupational settings are described in the Health Council of the Netherlands report (Health Council of the Netherlands 2010), and several publications (Duquenne et al. 2012; Basinas et al. 2015).

The release of endotoxins or LPS occurs not only upon cell death (i.e. cell lysis) but also during growth and division (Miyamoto et al. 2009). Small amounts of LPS are secreted by the bacterial cells during replication, whereas substantial amounts may be released during the destruction of the cells, for example by heating bacteria-containing solutions or through solvents (Unger et al. 2014). Endotoxins can become airborne during work practices that create aerosols from matrices containing gram-negative bacteria. Given that, cell wall rupture is favoured by dehydration and mechanical impact, cell lysis is probably the most significant source of endotoxins in bioaerosols (Duquenne et al. 2012). Concentrations of airborne endotoxins at workplaces are governed by many factors including the production process, tasks to be performed, operating procedures, distance from the source and climatic parameters (Duquenne et al. 2012). A large variability in exposure levels between sectors, jobs, and tasks performed has been observed (van Duuren-Stuurman et al. 2018).

Endotoxin levels can be several thousand times higher in occupational settings than concentrations observed in non-exposed environments, such as outdoor air (Madsen 2006). House dust is a common and consistent source of endotoxin exposure as well (Carnes et al. 2017).

3.5 Measurement method

The most commonly used method for detecting endotoxins is the Limulus amebocyte lysate (LAL) assay. This test is based on the coagulation of the hemolymph of *Limulus polyphemus* or *Xyphosura* following the administration of endotoxins. Amebocytes, ovoid-shaped cells that are granulated and nucleated, are present in this hemolymph. Upon exposure to an agression, including the presence of endotoxins, amebocytes lose their ovoid shape, become polylobed, and aggregate to form a protective clot at the site of the aggression. Various methods enable semi-quantitative analysis (gelation method) or quantitative analysis (turbidimetric method or chromogenic method, the most commonly used) (Géhin and Le Bacle 2011; Liebers et al. 2020).

The endotoxin concentration results are increasingly determined as an indirect measure of the enzymatic activity presented in endotoxin units per cubic meter of air (EU/m^3), replacing the measurements in nanograms per cubic meter (ng/m^3) (Géhin and Le Bacle 2011; Liebers et al. 2020). The conversion factor varies depending on the bacterial species producing the endotoxins. To simplify conversion, a commonly accepted factor is 10: 1 ng/m^3 = 10 EU/m^3 (Géhin and Le Bacle 2011).

4 Summary of toxicological data

As animal health data is lacking, the toxicological profile is essentially based on human data.

4.1 Toxicokinetics

Where possible, the toxicokinetic properties of endotoxins are described hereafter for the inhalation route, which is the predominant route of interest for the workers.

It is unlikely that endotoxins can penetrate intact human skin (Anderson et al. 2007).

Ingestion of endotoxins may occur at the workplace. However, healthy individuals carry a large intestinal load of LPS with no harm, and it is generally accepted that the barrier function of the gut must firstly be compromised in order for a pathogenesis to occur, as for example in inflammatory bowel disease in man (Wellmann et al. 1986; Wallace et al. 2016).

4.1.1 Absorption

Few data are available about specific endotoxin absorption. Airborne aerosols or dust particles containing bacterial components such as endotoxins are of a size that can deposit at each level of the respiratory tree (Health Council of the Netherlands 1998). Whole bacteria have particle sizes of 1-3 µm and fragments of gram-negative bacteria range down to molecular aggregates. Such particles can be deposited in the trachea and large bronchi and are eliminated by mucociliary transport (Jacobs 1989). Smaller particles deposit in the deeper airways including small bronchi, bronchiole and alveoli where endotoxins can be phagocytosed by macrophages and induce inflammatory reactions. During the inflammation process, alterations in the paracellular and transcellular permeability of the epithelium may occur, enabling endotoxins in organic dust to cross this barrier. Macrophages are then drained into the lymphatic system. Inactive cell-bound endotoxins, deposited in various parts of the lung, can be liberated and become biologically active through various mechanisms: bacteria lysis by antibiotics or complement, phagocytosis of bacteria by macrophages and polymorphonuclear leucocytes (PMN) and during reproduction of bacteria (Morrison et al. 1985).

4.1.2 Distribution

No data is available for distribution of endotoxins. Sparse data about distribution of LPS were however obtained in mice. In mice, studies have shown that radiolabeled LPS intravenously injected in a lethal dose is distributed fast over extravascular spaces with a half-life of approximately 10 hr (Health Council of the Netherlands 1998). The majority of LPS was distributed in the liver (25%), small amounts were distributed in the spleen (1%) and lymph nodes (0.5%) (Morrison *et al.* 1985). This study did not indicate where the rest of LPS was distributed. Freudenberg *et al*. showed that LPS was found in Kupffer's cells and granulocytes (Freudenberg et al. 1984).

In 2010, the Dutch Expert Committee of Occupational Safety (DECOS) indicated not being aware of any data on the exact distribution of endotoxins after inhalation (Health Council of Netherlands 2010).

4.1.3 Metabolism

No data are available on endotoxins metabolism by the human organism (Health Council of the Netherlands 1998).

Studies in rats have shown that LPS remains chemically unaltered in the circulation. Partial deacylation of LPS occurs in various organs. Enzymes identified in granulocytes and macrophages are capable of cleaving the ester-bound fatty acids in LPS. However, studies in rats have shown that

chemical degradation of LPS in the liver does not lead to detoxification of the molecule (Freudenberg and Galanos 1990).

4.1.4 Excretion

No data are available on the exact elimination mechanisms (Health Council of the Netherlands 1998). Jacobs *et al.* assumed that most of the endotoxins deposited in the lung are eliminated by macrophage and polymorphonuclear leucocyte phagocytosis (Jacobs 1989).

The liver is the major organ involved in clearance of LPS (Yao et al. 2016; Akiba et al. 2020). Hepatic macrophages (Kupffer cells) appear to be the principal cells involved in the clearance of LPS (Akiba et al. 2020). Yao *et al*. observed that circulating LPS is rapidly cleared from the circulation, with a half-life between 2 and 4 min (Yao et al. 2016). According to Freudenberg, removal of LPS from the organism takes places slowly, over many weeks. Degraded LPS is excreted through the liver into the gut, in the bile, and can be detected in feces (Freudenberg and Galanos 1990).

A small amount of low molecular weight-degradation products of LPS is excreted in urine (Freudenberg and Galanos 1990).

4.2 Acute toxicity

High levels of endotoxins in blood circulation (i.e. endotoxemia), as observed during severe gram-negative bacterial infections (notably after application of antibiotics) or as caused by translocation of enterobacteria from the gut, lead to dramatic pathophysiological reactions such as fever, leukopenia, tachycardia, tachypnea, hypotension, disseminated intravascular coagulation, and multi-organ failure (Schletter et al. 1995). Therefore, intravenously (IV) administered drugs and surgical instruments must be endotoxin-free, to avoid severe effects due to contaminating LPS. Specific data about acute toxicity of endotoxins by inhalation are lacking.

4.2.1.1 Respiratory and pulmonary toxicity after endotoxins inhalation

In 2010, the Dutch Expert Committee on Occupational Safety (DECOS), which is part of the Health Council of the Netherlands (Health Council of the Netherlands 2010), summarised the studies on acute respiratory effects described in its previous report (Health Council of the Netherlands 1998). Three other publications were identified through literature research until December 2023 (Zock et al. 1998; Mitchell et al. 2015; Cyprowski et al. 2015).

The main studies are summarized below.

Haglind and Rylander studied changes in forced expiratory volume in 1 second (FEV1) and polymorphonuclear neutrophils (PMN) on nasal epithelium in 68 students and 39 cotton mill workers in an experimental cardroom. FEV1 was measured before and after 4 hr working session which for the cotton mill workers took place on a Monday. The amount of airborne endotoxins was determined using the LAL technique. The airborne endotoxins levels ranged from 800 to 120 600 EU/m^3 (80 to 12 060 ng/m^3). No significant correlation was observed between the group average ΔFEV1 and endotoxins exposure, but when smoking habits were kept as a constant variable, the correlation between the average ΔFEV1 and endotoxins exposures became significant: $r = -0.65$ ($p < 0.02$). The study demonstrated that a dose-related decrease in FEV1 (Δ FEV1 %) was more pronounced in smoking cotton mill workers, resulting in a threshold of 800 EU/m^3 (80 ng/m^3) versus 1 700 EU/m^3 (170 ng/m^3) in non-smoking (n = 13) students. This suggests an increased risk for smokers (Haglind and Rylander 1984).

In a study of Rylander *et al*., 15 cotton mill workers (of whom 8 persons had a history of byssinosis ie an airway hyperreactivity characterized by bronchoconstriction in cotton, hemp and linen workers) were exposed on Monday morning for 4 hr in an experimental cardroom to cotton dust containing gram-negative bacteria and their endotoxins. During the experiment, dust and endotoxins levels were determined. The amount of endotoxins in the cotton lint extract was determined using the LAL assay. Endotoxin concentrations ranged from 700 – 56 200 EU/m^3 (70 – 5 620 ng/m^3) (personal sampling). FEV1 and the neutrophil count before and after work were measured and the prevalence of symptoms of byssinosis was recorded. A correlation was found between endotoxins exposure and ΔFEV1; endotoxins exposure was significantly related to FEV1 decrease over the exposure period, when adjusted for the amount of dust ($r=-0.56$, $p<0.05$). The authors calculated an endotoxins concentration of 330 EU/m^3 (33 ng/m^3) at which average FEV1 changes were zero using individual FEV1 changes and ambient endotoxins concentrations in a regression analysis ($r=-0.56$, $p<0.05$). Moreover, a dose-response relationship was observed between endotoxins levels and the number of subjects with symptoms of byssinosis ($r=0.81$, $p<0.001$). Workers without previous symptoms of byssinosis experienced fever, chest tightness, and breathing difficulties at the end of the shift at the highest endotoxins levels (Rylander et al. 1985).

Buck et al. assayed crude and purified aqueous extracts of cotton bracts shown to cause airway constriction in naive subjects for the determination of endotoxins content. Pulmonary function measured by flow changes on partial expiratory flow volume curves was used to assess airway responses to the bract extracts after their inhalation by a panel of volunteers (number of volunteers not specified). While breathing normally, each subject inhaled the aerosol of cotton bract extract for 10 min. Constriction was assayed by comparing lung function values obtained from recordings of partial and maximum expiratory flow volume (PEFV, MEFV) curves before and at 30 min intervals for a two and a half hr to three hr period after the 10 min inhalation of the aerosolised extract. The LAL test was used to determine endotoxins content of bracts extracted. Crude aqueous extracts from various bracts harvested before and after senescence of the cotton plant displayed endotoxins concentrations ranging widely from 0.086 to 50 µg/mL. The partially purified extract contained less than 1 ng/mL of endotoxins. The panel of volunteers responded to this purified bract extract, however, with a decrease in pulmonary function which was more than 60% of that seen with the crude extract of bracts. The authors concluded that aqueous extracts of cotton bract contain an agent other than endotoxins that causes acute airway constriction in people. These responses were similar to the acute responses experienced by cotton textile workers (Buck et al. 1986).

In a study by Castellan *et al*., healthy volunteers (smoking and non-smoking) were selected from the general population. Subjects suffering from asthma, chronic bronchitis and exertional breathlessness were excluded. Volunteers were not occupationally exposed to substances known to affect airway response and had a FEV1 above 80 % of the predicted 'normal' value[1] (male subjects (16-35 years) 95th percentiles. In addition, the volunteers were pre-tested by exposure to 1000 EU/m^3 (100 ng/m^3) LPS, in order to select sensitive subjects; only volunteers that responded with a FEV1 decrease of at least 5% (and not more than 30%) were accepted for the main study (Castellan et al. 1987).

[1] Individual value calculated on the basis of the anthropometric characteristics of individuals, their age and their ethnic origin. A fall of at least 20% in FEV1 defines an obstructive syndrome when this parameter is used to define it (below 20%, no conclusion is possible at individual level, due to the inter- and intra-individual variabilities of the parameter).

The main study started with 61 subjects (including 34 smokers). However, during the 20-months study period, the number of participating subjects decreased to 33 (16 smokers) for a variety of reasons, none of which were related to the responsiveness to cotton dust according to the authors. In 108 different exposure sessions, volunteers (24-35 subjects) were exposed to cotton dust during 6 hr, with airborne endotoxins concentrations ranging from 60 – 7 790 EU/m^3 (6 to 779 ng/m^3). Each of the 108 separate sessions followed at least two full days without exposure. Nine study cottons were purchased from commercial brokers in bale form, 22 were grown and prepared as bales for the exposure studies with use of standard commercial methods, and one was a rebaled composite blend of cotton samples from hundreds of individual bales processed in a commercial yarn-production mill. The exposure system consisted of a commercial carding machine in a cardroom, an exposure room, and connecting duct work. The mass airborne concentration (mg/m^3) of dust was calculated for each exposure session as a time-weighted average with use of the weights of all dust samples collected by vertical elutriators. The concentration of endotoxins (nanograms of U.S. reference endotoxins / m^3) was calculated as a time-weighted average with use of the same dust samples, extracted and assayed for endotoxins activity with a modification of the LAL test. Spirometry tests were performed before and after each exposure. The group mean percentage change in FEV1 was calculated for each exposure session for the subjects exposed. The authors found an exposure-response relation between ΔFEV1 (%) and endotoxins concentration (ng/m^3) of ΔFEV1 = 3.84 - 4.02 ($_{10}$log endotoxin (ng/m^3)); r = 0.85 (r2 = 0.72), p<0.0001. Another 66 sessions of exposure of the same subjects to clean air resulted in a mean ΔFEV1 of ± 0%. Using linear regression modeling, the authors calculated the zero percentage change in FEV1 during exposure to endotoxins to be 90 EU/m^3 (9 ng/m^3). In contrast, total dust exposure concentration (instead of endotoxins air concentration) was not correlated with ΔFEV1. However, some limitations can be described. The threshold value was calculated on the basis of a linear derivation of a statistical model and does not allow for precise estimation of the exposure-response relation at either very high or very low doses. Moreover, the results described in the study are relevant only to a minority of the general population since only sensitive subjects were included ie only volunteers that responded with a FEV1 decrease of at least 5% and not more than 30% to 1 000 EU/ m^3 (100 ng/m^3) LPS. Only one-third of the volunteers was included.

The difference between the calculated zero-change level in Rylander's study (330 EU/m^3 (33 ng/m^3)) and the one in Castellan's study (90 EU/m^3 (9 ng/m^3)) might be due to different exposure times (4 vs 6 hr). In addition, in Castellan's study the responsiveness was enhanced for the assessment of acute airway responses by selecting responsive subjects during pre-screening. Furthermore, the population of Rylander's study consisted of cotton mill workers who had been occupationally exposed to the same agent for years. As long-term exposure might cause short-term tolerance for effects of endotoxins, this might obscure the actual dose-response relationship, as might also the healthy worker effect in Rylander's study. Finally, it cannot be ruled out that other constituents of cotton dust may also be of importance in the development of acute pulmonary effects. This was suggested by the results of a study performed by Buck et al. in which changes in lung function were demonstrated when subjects were exposed to an endotoxin-free eluate of cotton dust (Buck et al. 1986).

Donham *et al*. investigated the relation between the health of workers and the environment in swine confinement buildings in a study of 57 workers. Environmental measurements of airborne dust and microbes were performed inside 30 swine confinement buildings. Gravimetric methods were used to measure dust. Endotoxin analysis was performed on the total dust and respirable dust samples using the LAL assay. The 2 to 8 hr mean endotoxins exposure, characterized by area sampling of total dust, was 1 800 EU/m^3 (180 ng/m^3). The authors found a significant dose response relationship between endotoxins exposure and an across-shift decrement of FEV1 and the maximum expiratory flow rate at 25% of vital capacity (MEF25) in non-smoking swine confinement workers (n = 41). The threshold level of endotoxins inducing a FEV1 decrement was about 2 000 EU/m^3 (200 ng/m^3). Eighty five per cent of the individuals had decrements over the work shift if endotoxins values were superior to 2 000 EU/m^3 (200 ng/m^3), whereas 11% experienced decrements with exposure values inferior to

2 000 EU/m^3 (200 ng/m^3). A decrement in FEV1 over a work shift began at exposures of about 1 800 EU/m^3 (180 ng/m^3) (90% confidence limits = 0-0.44). A no effect level of 1 800 EU/m^3 (180 ng/m^3) was estimated (Donham et al. 1989). Endotoxins, however, were not the only hazardous substances in this environment as other environmental contaminants, including particulate matter, mites and fungi, are able to induce similar respiratory manifestations. These confounding factors were not taken into account in the analysis.

Milton *et al*. measured peak expiratory flow (PEF) and workplace exposure to endotoxins, phenolic resin, and formaldehyde to investigate asthma symptoms and medication use among employees in a fiberglass wool manufacturing plant. Each participant wore a personal sampling pump on 5 or 6 work days while keeping a PEF diary. FEV1, forced vital capacity (FVC) and PEF were analysed for each employee before and after work. Baseline lung function was measured on the first day back to work following 2 or more days off work. Spirometry was also performed on 2 other working days. Endotoxins were analysed by the kinetic limulus assay with resistant-parallel-line estimation (KLARE) method using the kinetic turbidimetric LAL. Endotoxins measurements on samples from the fiberglass wool plant were extensively cross-validated by comparison with levels of LPS determined by 3-hydroxy fatty acid (3-OHFA) measurement using gas chromatography-mass spectrometry (GC-MS). The extracts were also analysed for phenolic resin content by spectrophotometric scanning. The dinitrophenylhydrazine-coated glass filter was analysed for formaldehyde using the HPLC method. The subjects were divided among four exposure groups, based on preliminary area and personal sampling, and current work location and job title: “basement” (high endotoxins and phenolic resin exposures, moderate formaldehyde exposure), “forehearth” (moderate endotoxins exposure, high phenolic resin, and highest formaldehyde exposures), “oven” (low endotoxins and phenolic resin exposures, moderate formaldehyde exposure), and “other” (low exposure to all measured air pollutants). The authors showed a dose-response relationship with across-shift changes over 4 hr in self-recorded PEF of 37 fibreglass workers exposed to 4 – 7 590 EU/m^3 (0.4 - 759 ng/m^3) endotoxins (personal sampling). An effect on across-shift changes in FEV1 was also suggested but was not as strong as that demonstrated for PEF. In the medium endotoxins level exposure group (geometric mean = 84 EU/m^3 (8.4 ng/m^3), range 40-150 EU/m^3 (4 - 15 ng/m^3)), acute decline effects on PEF were measured. No details were provided in the article about the effect of endotoxins exposure on FVC. Phenolic resin exposure was not consistently associated with decrements of respiratory volumes or flows, and formaldehyde was not associated with decrements in lung function. Therefore, according to DECOS report (Health Council of the Netherlands 2010), Milton *et al.* defined 84 EU/m^3 (8.4 ng/m^3) as the LOEL (lowest-observed--effect-level) and 17 EU/m^3 (1.7 ng/m^3) as the NOAEL for endotoxins in this study (Milton et al. 1995; 1996).

Zock *et al*. studied work-related respiratory symptoms, acute lung function changes and personal endotoxins exposure in 61 workers from a potato processing plant. Sixty-eight of the 149 available dust filters (46%; randomly chosen but at least one measure per worker) were analysed using a kinetic modification of the LAL assay to evaluate endotoxins exposure. Based on the average level of endotoxins exposure at their workstation, workers were divided into low (arithmetic mean (AM) = 21 EU/m^3 (2.1 ng/m^3)) and high (AM = 56 EU/m^3 (5.6 ng/ m^3)) exposure categories. Lung function measurements were performed in two periods: before the start of the processing season (from 6 to 14 August 1990) and during the processing season (from 15 to 31 October 1990). A total of 148 across-shift lung function changes were measured before and after work during three consecutive afternoon shifts. The mean FEV1 showed a decrease equal to 0.06 - 0.12 L over the work shift, being largest on the first working day after a 3-day absence from work. The initial FEV1 values are not specified in the article. Workers exposed to high endotoxins levels showed a larger across-shift decrease in lung function than workers exposed to low endotoxins exposures. On the first day after a three-day absence from work, mean lung function decrease in workers exposed to high endotoxins concentrations (53–60 EU/m^3 (5.3-6 ng/m^3)) was equal to 5% for the FEV1. The results suggest that endotoxins related effects on across-shift lung function change can be expected above 53 EU/m^3 (5.3 ng/m^3) over 8 hr (Zock et al. 1998).

In a study by Kline *et al*., 72 healthy volunteers (non-atopic, non-asthmatic, non-smoking) were exposed several hours in sequence to increasing single doses of nebulised LPS: 0.5, 1.0, 2.0, 3.0, 5.0, 10 and 20 µg LPS/person by inhalation challenge (the precise experimental protocol was not specified by the authors). Lung function test was performed 1, 10, 20 and 30 min after inhalation of each dose. The inhalation challenge was continued with the next dose of LPS when, 30 min (or more) after exposure, the subject's FEV1 had decreased by less than 20%. The authors observed marked differences in the response to inhaled LPS: eight 'sensitive' subjects had at least a 20% decline in FEV1 after inhaling 6.5 µg LPS or less per person (cumulative dose) and eleven 'hyposensitive' persons maintained a FEV1 > 90% after inhaling 41.5 µg LPS/person. The three most sensitive responders reached a FEV1 decrease of 20% at the second dose (1.5 µg/person cumulative) (Kline et al. 1999).

Donham *et al*. observed in poultry workers (n = 257) statistically significant dose-response relations between lung function decrement (FEV1 and forced expiratory flow between 25 and 75 percent of lung volume (FEF25-75)) over a work shift (2 to 4 hr), and each quartile of exposure to endotoxins and dust levels (both total and respirable fractions). Personal sampling was conducted for total and respirable dusts, total and respirable endotoxins and ammonia. Total and respirable samples of endotoxins were analysed using the LAL assay. Multiple logistic regressions were performed by using environmental parameters as main predictor variables, with each environmental parameter analysed individually (controlling for age, years worked in poultry industry, gender, smoking status and education) in relation to cross-shift lung function decrease (FEV1 and FEF25-75 of 3%, 5% or 10%). Mean total endotoxins and respirable endotoxins exposure were respectively 1 589.1 ± 3 394.1 EU/m^3 (158.9 ± 339.4 ng/m^3) and 58.9 ± 97.3 EU/m^3 (5.89 ± 9.73 ng/m^3). The exposure-response correlations were weak; the correlation coefficients (r) were 0.16 (r^2 = 0.026) and 0.19 (r^2 = 0.036) for respirable and total endotoxins respectively. These low coefficients indicate that only 3-4% of the variation in lung function is explained by exposure to endotoxins. Correlation and multiple regression were used to calculate the levels at which a 3% across-shift change in FEV1 was statistically significant; this was the case at concentrations of 2.4 mg/m^3 total dust, 0.16% respirable dust, 614 EU/m^3 (61.4 ng/m^3) endotoxins and 0.35 EU/m^3 (0.035 ng/m^3) respirable endotoxins. The relatively arbitrary NOEL's in combination with weak correlations limit the usefulness of this study (Donham et al. 2000).

Laitinen *et al*. investigated the associations between self-reported symptoms and exposure to endotoxins of workers in several industries. Air samples for endotoxins and peptidoglycan assays were collected on the first working day after at least a 2-day absence from work (no information available on the granulometry of samples). Sampling time varied from 1–8 hr, depending on workers' activities in a bioaerosol-contaminated area during the work shift. Biologically active endotoxins were analysed using the endpoint chromogenic LAL assay and total amount of endotoxins were analysed with the GC-MS assay. Among 77 workers, the number of workers with respiratory complaints or fever/shivering was statistically significantly higher when the concentration of biologically-active endotoxins in the air was over 250 EU/m^3 (25 ng/m^3). Likewise, the reporting of eye symptoms and chest tightness increased when the airborne concentration of biologically active endotoxins surpassed 1 500 EU/m^3 (150 ng/m^3). Notably, excluding workers with atopy or symptoms of chronic bronchitis from the analysis did not change the results (Laitinen et al. 2001). The division of exposed workers into 2 groups (> or < 250 and 1 500 EU/m^3 (25 and 150 ng/m^3)) seemed arbitrary, as the study did not provide any statements about the origin of these limits.

Heldal and Eduard conducted the monitoring of endotoxins exposure during waste collection through personal sampling. The sampling duration encompassed an entire work shift, equivalent to approximately 4–6 hr. Total dust was determined gravimetrically in an air conditioned weighing room. Endotoxins was analysed in duplicate using the quantitative kinetic procedure of the LAL test (Kinetic-QCL kit, Bio Whittaker). The prevalence of nasal irritation was significantly increased ($p < 0.05$) in a small waste collectors population (n = 6) exposed to endotoxins at a level of 4.5 EU/m^3

[0.2 - 17 EU/m^3] (0.45 ng/m^3 [0.02-1.7 ng/m^3]), when compared to less exposed waste collectors (N = 12; 1.4 EU/m^3 [0 - 4.6 EU/m^3] (0.14 ng/m^3 [0-0.46 ng/m^3]). Eye irritation was also reported by almost half of the waste collectors of the study (10 of 22 workers), but it was not significantly associated with the exposure level to endotoxins. The influence of possible confounders (age and smoking) was studied for the symptoms significantly associated to exposure. Smokers were more highly exposed to endotoxins than non-smokers, and more smokers than non-smokers had nose and eye irritation (Heldal and Eduard 2004).

Bonlokke *et al*. investigated the health effects in swine farm workers during summer and winter. Twenty-four workers underwent lung function testing (FEV1, FVC, FEV1/FVC, PEF, FEF25, FEF50, FEF75 and MMEF) and blood sampling before and after work. On visit days the workers were equipped with personal samplers and instructed to carry them from entry into the swine houses until end of work within these houses. Endotoxins measurements were performed using the endpoint chromogenic LAL assay. The mean endotoxins exposure of the workers was highest during winter (25 690 versus 6 553 EU/m^3 (2 569 vs 655.3 ng/m^3); p = 0.004). No difference in lung function was found between the seasons although exposure to endotoxins varied between the seasons (Bønløkke et al. 2009). Some studies also found seasonal differences in endotoxins levels in pig houses (Preller et al. 1995) and in intensive livestock production (Schulze et al. 2006). On the other hand, Seedorf *et al.* did not observe a significant seasonal variation in airborne endotoxins concentrations for cattle, pigs and poultry farms (Seedorf et al. 1998).

Mitchell *et al*. tried to assess respiratory exposures and lung function in a cross-sectional study of California dairy workers. Exposure of 205 dairy and 45 control (vegetable processing) workers to particulate matter and endotoxins was monitored. Two samplers were attached to a backpack at shoulder level in the personal breathing zone. Total endotoxins exposure was determined as the 3-hydroxy fatty acid (3-OHFA) endotoxin components analysed using gas chromatography-mass spectroscopy. Samples were analysed for biologically active endotoxins using the recombinant factor C assay, which detects the activation of Factor C by utilizing a fluorogenic substrate. Mixed-effects multiple linear regression modeling was used. Where baseline (long-term effects) modeling was conducted, covariates included age, height, and smoking status (current vs former/never). Covariates considered for cross-shift (acute effects) modeling included the monitoring day's measurements of the following: self-reported time in dust, time at start of shift (AM or PM), work shift length, wearing of personal protective equipment, and whether they smoked that day. Endotoxins concentrations were 329 EU/m^3 or 1122 pmol/m^3 and 13.5 EU/m^3 or 110 pmol/m^3, respectively, for dairy and control workers. In a mixed-effects model, forced vital capacity (FVC) decreased across a work shift by 24.5 mL (95% confidence interval, -44.7 to -4.3; P = 0.018) with log10 (total endotoxins) and by 22.0 mL (95% confidence interval, -43.2 to -0.08; P = 0.042) per hour worked. No significant association was observed for FEV1 decrease. Modern California dairy endotoxins exposures were associated with a mild acute decrease in FVC (Mitchell et al. 2015).

Cyprowski *et al*. assessed endotoxins exposure among sewage treatment plant workers (STPW) and its effect on across-shift changes in respiratory airflow. A group of 78 STPW from a large sewage treatment plant was studied. Across-shift spirometric measurements were performed on Mondays, after 2-days absence from work, with the use of portable spirometer. FVC, and FEV1 parameters were analysed. Endotoxins concentration was assayed with the LAL test. The concentration of inhalable dust and endotoxins ranged from 0.01 to 1.38 mg/m^3 and from 0.68 to 214 EU/m^3 (0.068-21.4 ng/m^3), respectively. Endotoxins exposure was characterized with the skewed distribution (AM = 38.8 EU/m^3 (3.88 ng/m^3), geometric mean (GM) = 15.4 EU/m^3 (1.54 ng/m^3), geometric standard deviation (GSD) = 4.21). Through the use of multifactor analysis, which adjusted on the main confounders (inhalable dust concentration and current and previous smoking habit), it was found that, despite low levels measured, endotoxins exposure had a significant impact on the observed across-shift decline in FEV1 (p = 0.044). The regression slope was additionally calculated (r = -

0.017, p = 0.071) between endotoxins concentrations and percentage of changes in FEV1. No significant association was observed for FVC decrease (Cyprowski et al. 2015).

DECOS underlined that effects after acute exposure to endotoxins might differ between workers who have been exposed in the past and healthy volunteers who have not been as much exposed before, because of the tolerance phenomena that is observed for acute clinical effects after long-term or multiple exposure to endotoxins (Greisman et al. 1969). Even if this tolerance might disappear within a few days, DECOS considered that it may obscure the actual dose-response and that it should be taken into consideration for the interpretation of the studies (Health Council of the Netherlands 2010).

Table 1. Summary of studies concerning the effects on lung function of acute exposure to endotoxins

Reference	Design	Endotoxin exposure	Assessment	Results
Haglind and Rylander (1984)	Experimental study	800-120 600 EU/m^3 (80-12 060 ng/m^3)	Changes in respiratory function (FEV,) and polymorphonuclear neutrophils (PMN) on nasal epithelium were studied in 68 students and 39 cotton mill workers in an experimental cardroom Exposure to 800-120 600 EU/m^3 (80-12 060 ng/m^3) endotoxins for 4 hr	Dose-related decrease in FEV1 was more pronounced in smoking cotton mill workers resulting in a threshold of 800 EU/m^3 (**80 ng/m^3**) versus 1700 EU/m^3 **(170 ng/m^3)** in non-smoking workers (n = 13)
Rylander *et al*. (1985)	Experimental exposure	700 – 56 200 EU/m^3 (70-5 620 ng/m^3)	15 cotton mill workers exposed in an experimental card room to cotton dust for 4 hr Exposure to 700 – 56 200 EU/m^3 (70-5620 ng/m^3) endotoxins for 4 hr	At endotoxins concentration of **330 EU/m^3 (33 ng/m^3)** : average FEV1 changes were zero using individual FEV1 changes and ambient endotoxins concentrations in a regression analysis (r=-0.56, p<0.05)
Buck *et al*. (1986)	Experimental study	0.086 to 50 µg/mL	Pulmonary function measured by flow changes on partial expiratory flow volume curves in naive volonteers exposed to crude and purified aqueous extracts of cotton bracts Constriction assayed by comparing lung function values obtained from recordings of partial and maximum expiratory flow volume (PEFV, MEFV) curves before and at 30 min intervals for a 2h30 to 3h period after the 10 min inhalation of the aerosolised extract.	Volunteers' pulmonary function measured were similar to the acute responses experienced by cotton textile workers. Nevertheless, the authors concluded that aqueous extracts of cotton bract contain an agent(s) other than endotoxins that causes acute airway constriction in people.
Castellan *et al*. (1987)	Experimental study	60 – 7 790 EU/m^3 (6-779 ng/m^3)	33 healthy volunteers exposed to cotton dust during 6 hr in 108 different exposure sessions Measurement of FEV1 variations	At endotoxins concentration of **90 EU/m^3** (**9 ng/m^3**): zero percentage change in FEV1 during exposure to endotoxins using linear regression modeling Exposure-response relation between ΔFEV1 and endotoxins concentration of: % ΔFEV1 = 3.84 - 4.02 ($_{10}$log endotoxins (ng/m^3)); r = 0.85 (r2 = 0.72), p<0.0001.
Donham *et al*. (1989)	Cross-sectional study	Mean 1 800 EU/m^3 (180 ng/m^3)	57 workers on 30 swine farms in southern Sweden and 55 matched controls FEV1 measurements before and at the end of a shift	The threshold level of endotoxins relative to FEV1 decrement was about 2000 EU/m^3 (200 ng/m^3) A no-effect level of 1 800 EU/m^3 (180 ng/m^3) was estimated. Significant dose response relationship between endotoxins exposure and an across-shift decrement of FEV1 and the maximum expiratory flow rate at 25% of vital capacity (MEF25) in non-smoking swine confinement workers (n = 41)

Milton *et al.* (1995 and 1996)	Cross-sectionnal study	4 – 7 590 EU/m^3 (0.4 - 759 ng/m^3)	Among employees in a fiberglass wool manufacturing plant. Measurement of peak expiratory flow (PEF), FEV1 and FVC before and at the end of a shift Simultaneous measurement of workplace exposure to endotoxins	The authors defined 84 EU/m^3 (8.4 ng/m^3) as the LOAEL (lowest-observed-effect-level) and 17 EU/m^3 (1.7 ng/m^3) as the NOAEL for across-shift PEF decrease. VEMS decrement was a less sensitive indicator. No significant variation was observed for FVC
Zock *et al.* (1998)	Cross-sectionnal study	Low exposure : 21 EU/m^3 (2.1 ng/m^3) High exposure : 56 EU/m^3 (5.6 ng/m^3)	148 across-shift lung function changes were measured in 61 workers from a potato processing plant.	The mean FEV1 showed a decrease equal to 0.06-0.12L over the work shift On the first day after a three-day absence from work, mean lung function decrease in workers exposed to high endotoxins concentrations (53–60 EU/m^3) was equal to 5% for the FEV1. The exposure related effects suggest that endotoxins related effects on across-shift lung function change can be expected above 53 EU/m^3 (5.3 ng/m^3) over 8 hr.
Kline *et al.* (1999)	Experimental study	LPS: 0.5, 1.0, 2.0, 3.0, 5.0, 10 and 20 µg LPS/person	72 healthy volunteers (non-atopic, non-asthmatic, non-smoking) were exposed (within several hours) in sequence to increasing single doses of nebulised LPS (0.5-20 µg)	Eight 'sensitive' subjects had at least a 20% decline in FEV1 after inhaling 6.5 µg LPS or less per person (cumulative dose) and eleven 'hyposensitive' persons maintained a FEV1 > 90% after inhaling 41.5 µg LPS/person. The three most sensitive responders reached a FEV1 decrease of 20% at the second dose (1.5 µg/person cumulative).
Donham *et al.* (2000)	Cross-sectionnal study	Mean total endotoxins exposure: 1 589.1 EU/m^3 (SD:3 394.1) (158.9 ng/m^3) Mean respirable endotoxins exposure: 58.9 EU/m^3 (SD: 97.3) (5.89 ng/m^3)	257 poultry workers over a work shift (2 to 4 hr) Measurements of air endotoxins concentrations (both total and respirable) FEV1 measurements before and after a work shift	Correlation and multiple regression were used to calculate the levels at which a 3% across-shift change in FEV1 was statistically significant; this was the case at concentrations of 2.4 mg/m^3 total dust, 0.16% respirable dust, **614 EU/m^3 (61.4 ng/m^3)** endotoxins and 0.35 EU/m^3 (0.035 ng/m^3) respirable endotoxins
Laitinen *et al.* (2001)	Cross-sectional study	2 groups (> or < 250 and 1500 EU/m^3 (25 and 150 ng/m^3))	Self-reported symptoms of 77 workers in several industries Air endotoxin measurements (no indication of the PM fraction considered)	Number of workers with respiratory complaints or fever/shivering was statistically significantly higher when the concentration of biologically-active endotoxins in the air was over 250 EU/m^3 (25 ng/m^3). Likewise, the reporting of eye symptoms and chest tightness increased when the airborne concentration of biologically active endotoxins surpassed 1500 EU/m^3 (150 ng/m^3).
Bonlokke *et al.* (2009)	Observational study	Mean endotoxins exposure: During winter: 25 690 EU/m^3 (2 569 ng/m^3) During summer: 6 553 EU/m^3 (655.3 ng/m^3)	24 swine farm workers underwent lung function testing (before and after a work shift) and blood sampling before and after work. Air endotoxins measurements	No difference in lung function were found between the seasons although exposure to endotoxins varied between the seasons
Mitchell *et al.* (2015)	Cross-sectionnal study	Endotoxins concentrations for dairy workers = 329 EU/m (32.9 ng/m^3) For control workers = 13.5 EU/m (1.35 ng/m^3)	Exposure of 205 dairy and 45 control (vegetable processing) workers to particulate matter and endotoxins was monitored FEV1 and FVC measurements before and after a work shift	In a mixed-effects model, forced vital capacity decreased across a work shift by 24.5 mL (95% confidence interval, -44.7 to -4.3; P = 0.018) with log10 (total endotoxins) and by 22.0 mL (95% confidence interval, -43.2 to -0.08; P = 0.042) per hour worked. Modern California dairy endotoxins exposures and shift length were associated with a mild acute decrease in forced vital capacity.

				No significant variation observed for FEV1
Cyprowski *et al*. (2015)	Cross-sectional study	0.68 - 214 EU/m^3 (0.068-21.4 ng/m^3)	Across-shift spirometric measurements (FEV1 and FVC) performed on Mondays, after 2-days absence from work, on a group of 78 sewage treatment plant workers	The regression slope was additionally calculated (r = -0.017, p = 0.071) between endotoxins concentrations and percentage of changes in FEV1. Relatively low levels of endotoxins among sewage treatment plant workers may cause small, but significant across-shift declines in FEV1. No significant variation observed for FVC

4.2.1.2 Organic Dust Toxic Syndrome

Organic dust toxic syndrome (ODTS) is a non-infectious influenza-like illness that occurs after exposure by inhalation of organic dust that is contaminated with microorganisms (e.g., Gram-negative bacteria and fungi). ODTS is characterized by acute onset of fever, chills, myalgia, non-productive cough, dyspnea, chest tightness, malaise and headache; symptoms typically begin 4 – 12 hr after exposure and last for 1 – 3 days (Boehmer et al. 2009; Paris 2014).

Boehmer *et al*. conducted an epidemiologic study of landscape employees from three divisions (forestry, parks, and parkways). A questionnaire was designed to assess symptoms of illness since July 1, 2007, underlying health conditions (including asthma and allergies), cigarette-smoking behavior, and occupational exposure to organic material, including mulch, grass or grass clippings, compost, and hay. Medical records were reviewed for the ill employees who had sought medical care. The mean endotoxins concentration was 32170 EU/g of mulch. Five (12%) of 43 employees experienced respiratory illness compatible with ODTS. Illness was associated with prolonged mulch exposure (≥ 6 vs < 6 hr/day; RR = 24.7; 95% confidence interval = 3.3 – 184.9). Mulch samples contained high levels of Aspergillus spores and endotoxins. Contaminated mulch was implicated as the source of presumed ODTS among landscape workers (Boehmer et al. 2009).

Smit *et al*. investigated work-related symptoms in wastewater treatment workers. A questionnaire developed specifically for bioaerosol related health effects in the waste recycling and composting industry was evaluated for 468 employees from 67 sewage treatment plants. The questionnaire included questions about respiratory symptoms, diarrhea, allergies, and flu-like symptoms resembling ODTS during the past 12 months. Personal endotoxins exposure (8-hr measurements; n = 460) was measured in 216 workers from 40 wastewater treatment plants. One to six samples per worker were collected during three different periods (June – July, August –October, and November 2003). Inhalable dust was sampled and endotoxins extraction and analysis were performed by LAL assay. Endotoxins exposure ranged from 0.6 to 2 093 EU/m^3 (0.06-209.3 ng/m^3), the geometric mean exposure was 27 EU/m^3 (2.7 ng/ m^3). Factor analysis yielded three clusters of correlated symptoms: "lower respiratory and skin symptoms," "flu-like and systemic symptoms," and "upper respiratory symptoms." ODTS was more prevalent in workers exposed to endotoxins levels higher than 200 EU/m^3 (20 ng/m^3) (prevalence ratio = 2.03; 95% CI 1.20–3.44) (Smit et al. 2005).

Smit *et al*. studied levels of personal exposure to endotoxins among workers in a grass seed quality inspection laboratory and in the agricultural seed processing industry after episodes of severe work-related health problems resembling ODTS. Inhalable dust and endotoxins levels were assessed in 101 samples from 57 workers in grass, cereal, and vegetable seed plants who were handling mainly grass seeds as bulk product, and horticulture seeds in smaller quantities. Endotoxins concentrations in seed extracts were determined by LAL assay. Geometric mean of endotoxins concentrations in personal samples were 1800 EU/m^3 (180 ng/m^3), particularly high in the grass seed quality inspection lab where endotoxins levels up to 274 000 EU/m^3 (27 400 ng/m^3). Exposure to high levels of endotoxins (up to 78 000 EU/m^3 (7 800 ng/m^3) was most likely associated with episodes of ODTS resembling symptoms in a grass seed quality inspection lab, and with respiratory symptoms in an agricultural research institute. The authors concluded that occupational exposure to inhalable agricultural seed dust can induce inflammatory responses, and is a potential cause of ODTS (Smit et al. 2006).

Basinas *et al*. tested the hypotheses that current endotoxins exposure is inversely associated with allergic sensitisation and positively associated with non-allergic respiratory diseases in four

occupationally exposed populations using a standardised analytical approach. Data were pooled from four epidemiological studies including 3883 Dutch and Danish employees in veterinary medicine, agriculture and power plants using biofuel. Endotoxins exposure was estimated by quantitative job-exposure matrices specific for the study populations. Dose-response relationships between exposure, IgE-mediated sensitisation to common allergens and self-reported health symptoms were assessed using logistic regression and generalized additive modeling. Adjustments were made for study, age, sex, atopic predisposition, smoking habit and farm childhood. Heterogeneity was assessed by analysis stratified by study. Endotoxins exposure was a dose-dependent risk factor for both ODTS and chronic bronchitis from exposure levels of 100 EU/m^3 and higher (Basinas et al. 2012).

4.2.1.3 Cardiovascular toxicity after endotoxin inhalation

Zhong *et al*. investigated the association between endotoxins exposure and blood pressure (BP). A single-blind, randomised, trial of controlled human exposure to concentrated ambient particles (CAPs) was conducted to investigate the physiological cardiovascular response to particle-associated-endotoxins. During the exposure, particles were collected on polycarbonate membrane filters and, subsequently, were analysed for their endotoxins content. The endotoxins concentrations were analysed using LAL test. Fifty healthy non smoking volunteers were successively exposed for 130 min to: 250 mg/m^3 fine CAPs (0.1-2,5 µm), 200 µg/m^3 coarse CAPs (2.5-10 µm)), filtered air, or medical air. Each exposure was followed by a minimum 2-week washout period before the next exposure. During the study period, the endotoxins levels varied from 0.3 to 213 EU/m^3 (0.03 to 21.30 ng/m^3) with a median of 25 EU/m^3 (2.50 ng/m^3)[2]. Beta-1,3-glucan was simultaneously measured and its concentration ranged from 0.02 to 124.58 ng/m^3 with a median of 5.53 ng/m^3. Fine CAPs contained the highest endotoxins (median 7.07 ng/m^3; IQR: 7.09 ng/m^3) and beta-1,3-glucan concentrations (median 10.49 ng/m^3 ; IQR : 16,29 ng/m^3). Endotoxins concentrations were associated with increases in BP at 0.5-hr post-exposure; every doubling in endotoxins concentration was significantly associated with 1.73 mm Hg higher systolic BP (95% CI: 0.2 - 3.18; p = 0.02) and with 2.07 mm Hg higher diastolic BP (95% CI: 0.74 - 3.39; p = 0.003).Beta-1,3-glucan concentrations were also associated with BP variations : every doubling was associated with 0.80 mm Hg (95 % CI: -0.07-1,67 mmHg) and 0.88 mm Hg (95 % CI: 0.09-1.66 mm Hg) increases in 0.5 hr-post exposure systolic and diastolic BP, respectively. As CAP mass varied from an exposure to another, results were adjusted for the total exposure mass concentration. These adjustments did not modified the associations. No adjustments were performed to take into account the simultaneous exposure to endotoxins and beta-1,3-glucan. No adjustments were made for PM10 and PM2.5 concentrations. In addition, an increased level of urine vascular endothelial growth factor (VEGF) was also observed immediately after exposure; it increased with endotoxins exposure but not with beta-1,3-glucan exposure. As this post-exposure VEGF elevation attenuated the association between endotoxins and the 0.5-hr post-exposure BP, the authors suggest that it might constitute an adaptive response to the observed cardiovascular-related outcome (Zhong et al. 2015).

In a similarly-designed study, Liu *et al*. investigated the association between exposure to particle-associated-endotoxins and changes in blood or urine VEGF and blood endothelin-1, as biomarkers of vascular function. The endotoxins concentrations were analysed using LAL test. Endotoxins in coarse CAPs (mean ± SD endotoxins concentration of 2.0 ± 1.1 ln(ng/m^3)) was significantly

[2] Results are presented for CAPs in general. No distinction was made according to the type of PM.

associated with increased blood VEGF at 1 hr post-exposure, whereas the endotoxins in fine CAP (mean (SD) endotoxins concentration of 2.0 ± 0.6) ln(ng/m^3) was significantly associated with increased urinary VEGF at 1 hr post-exposure. These changes were transitory (lasting < 21 hr post-exposure). Based on the results, the authors suggest that endotoxins in ambient particles may contribute to vascular mediator changes, and that increased VEGF levels may represent an acute systemic response to endothelial injury during exposure to PM and endotoxins (Liu et al. 2015).

4.3 Upper airways, eye and skin irritation after external exposure to endotoxins

Skin irritation and skin diseases have been reported at workplaces when organic waste is handled, which are possibly due to dermal contact with irritating microbial contaminants, e.g. endotoxins. More frequent self-reported skin irritation was observed in a cross-sectional study for the group of subjects with the longer indoor waste storage that, according to the authors of the study, might be attributed to the endotoxins contamination (Herr et al. 2004).

Heldal *et al*. investigated the associations between dust, endotoxins and health effects in sewage workers. Exposure of 19 workers handling dry sludge and 25 other sewage workers was measured. Controls were office workers from compost and sewage plants. Self-reported nose irritation were associated with exposure to endotoxins with ORs of 4.0 (95% CI : 1.5-10) (Heldal et al. 2010).

Self-reported symptoms as nose, eye or upper airway irritation were not significantly associated with endotoxins exposure (median: 3 EU/m^3 (0.3 ng/m^3); range: 0 - 730 EU/m^3 (0-73 ng/m^3)) among compost workers (N = 47), when compared with controls (N = 37) (Heldal et al. 2015).

Exposure to endotoxins and the presence of subjective respiratory and clinical symptoms were studied at a large scale composting plant, as reported by Aghaei *et al*. While the prevalence of most of the symptoms including cough, wheezing, eye irritation, and runny eyes were higher in highly and moderately exposed groups (respectively 5 400 and 3 192 EU/m^3 (540 and 319 ng/m^3) mean exposure level) compared to low exposed workers (112 EU/m^3 (11.2 ng/m^3) mean exposure level), the difference was not statistically significant (Aghaei et al. 2020).

A LPS nasal challenge (50 µg/nostril; LPS from *Escherichia coli*) model by investigating the effect of the CXCR2 inhibitor AZD8309 on neutrophilic inflammation of 18 healthy volunteers induced only minimal local irritation (and no signs of systemic inflammation) according to Virtala *et al*. (Virtala et al. 2012).

4.4 Modulation of immunologic response

Low levels of endotoxins exposure significantly increased the inflammatory response to allergen exposure in sensitised subjects with asthma (Reed and Milton 2001; Liu 2002). Small amounts of endotoxins (< 1 ng/mL) binding to receptors on macrophages and other cells generates the production of IL-12, which inhibits IgE responses. It also generates the production of other cytokines like IL-1, TNF-α, and IL-8, which cause inflammation (Michel 2000; Reed and Milton 2001). Hunt *et al*. used segmental bronchoprovocation (SBP) with allergen in an attempt to study BAL cytology modifications 24 hr after challenge. Due to endotoxins contamination of the allergen extracts, neutrophils (rather than eosinophils) were recruited in the bronchoalveolar lavage (BAL) fluids in the first four patients (Hunt et al. 2012).

Low levels of endotoxins exposure can also enhance the inflammatory reaction to allergens in subjects with allergic rhinitis (Eldridge and Peden 2000), as well as skin test wheal-and-flare response to allergens (Michel et al. 1991). Michel *et al*. performed skin prick tests in 20 patients with house dust mite (HDM) allergy. They used LPS (E coli 026: B6 serotype) at concentrations of 1, 10, 100 micrograms/mL, HDM extracts at different dilutions, and solutions of LPS mixed with HDM. LPS

had a synergistic effect on the HDM skin response both in terms of flare [27.4 +/- 2.8 mm, 26.6 +/- 3.3 mm, 28.1 +/- 2.5 mm versus 20.7 +/- 3.6 mm, respectively in presence of LPS 1 ($P < 0.02$), 10 ($P < 0.05$), 100 ($P < 0.01$) micrograms/mL] and wheal response [6.4 +/- 1.0 mm, in presence of LPS 100 micrograms/mL versus 4.9 +/- 1.1 mm in absence of LPS ($P < 0.05$)] (Michel et al. 1991).

Asthmatic subjects exposed to endotoxins show a significant decrease in lung function (Michel et al. 1996; Eldridge and Peden 2000; Nightingale et al. 1998; Douwes et al. 2000). In subjects (n = 37) exposed to a high level of HDM allergen, the severity of asthma was related to concomitant exposure to endotoxins in HD, since the concentration of HD endotoxins was significantly and inversely correlated with FEV1 ($p < 0.05$) and FEV1/FVC ($p < 0.02$) (Michel et al. 1996).

4.5 Chronic toxicity

4.5.1.1 Respiratory and pulmonary toxicity

Smid *et al*. performed a cross-sectional study involving 315 workers employed in 14 animal feed mills in the Netherlands. Gravimetric dust concentrations and endotoxins levels (using the LAL test) were determined. The average 8 hr personal inhalable dust (<30 µm) exposure was 9 mg/m^3 for grain dust (range 0.2 - 150 mg/m^3) and 250 EU/m^3 (25 ng/m^3) for endotoxins (range 2 - 4700 EU/m^3) based on 530 personal dust samples. A short self-administered questionnaire was used to collect information on respiratory symptoms, including chronic cough and chronic phlegm (daily at least 3 months during the previous 2 years), shortness of breath, ever wheezing, frequent wheezing (at least 1 week during the previous 2 years) and chest tightness. Forced expiratory lung function measurements were conducted after a period of at least 48 hr without exposure to organic dust, between 11:00 A.M. and 3:00 P.M. An external control group was selected without exposure to agents that may affect the respiratory system. However, this group was not used in the epidemiologic analyses due to differences in variables other than exposure. Subsequent analyses were then conducted with only exposed workers and internal control subjects who were non-production animal feed workers. Adjustments for confounders such as age, height and smoking habits were made in the analyses, but there was no adjustment for PM effect. For the calculation of the estimated cumulative exposure, the authors assumed that current dust and endotoxins levels were representative of historic exposure levels. The dust sampled was characterized by a 50% cut-off diameter of 30 µm, resembling the inspirable dust fraction that passes the mouth and nostrils when inhaled. All studied lung function variables (FVC, FEV1, PEF, MEF75, MEF50)[3] showed significantly reduced values with increasing current exposure to both dust and endotoxins. The estimated cumulative exposure to endotoxins appeared to be more strongly related to lung function variables than were the cumulative dust levels. The stronger association with endotoxins exposure was further supported by lower p-values than those for dust exposure. Mean current exposure levels per job title ranged from 60 to 680 EU/m^3 (6 to 68 ng/m^3) for endotoxins and from 1.7 to 29.7 mg/m^3 for dust. The dose-response relation for current endotoxins exposure and FEV1 was calculated to be -49.1 mL per 100 EU/m^3 (-4.91 mL per ng/m^3) for workers with a mean work history of 13 years. No clear differences in respiratory symptom prevalence existed between different exposure groups. The study

[3] PEF is peak expiratory flow rate ; MEF75 and MEF50 are mean expiratory flow rates at 75% and 50%, respectively, of vital capacity.

indicated a significant correlation between the estimated cumulative exposure to both dust and endotoxins and lung function impairment (Smid et al. 1992).

Smid et al. calculated a safe threshold level between 30 - 75 EU/m^3 (3 and 7.5 ng/m^3) based on the previous study performed on animal feed industry (Smid 1993). Both acute and chronic lung function effects were observed in the intermediate exposure group (400 EU/m^3 or 40 ng/m^3) compared to the low exposure group (<150 EU/m^3 or <15 ng/m^3). The LOAEL was set as the upper limit of the lower exposure group. Regression models estimated that 40 years of exposure to 150 EU/m^3 (15 ng/m^3) could result in a decrease in FEV1 of approximately 200 mL (equivalent to approximately 5% FEV1). For MEF75, the effect would be 1200 mL/s (approximately 16%). The author suggested that the NOEL would be below 150 EU/m^3 (15 ng/m^3). Considering selection and attenuation leading to downward bias, the author applied a safety factor on the LOAEL, proposing a 'safe' level between 30 and 75 EU/m^3 (3 and 7.5 ng/m^3).

Post *et al*. conducted a study involving 140 workers in the grain processing and animal feed industry during 5 years. This study was a follow-up of the study population of the previously described cross-sectional study by Smid et al. (1992). In the first survey 520 personal exposure[4] samples were collected (Smid et al. (1992)), and another 179 samples were gathered during the second survey. Mean exposures per job title ranged from 36 to 990 EU/m^3 (3.6 to 99 ng/m^3) for endotoxins. The annual decline in FEV1 and in maximal mid-expiration flow (MMEF, the average expiratory flow over the middle half of the FVC) were measured on Mondays at the beginning of the study and approximately 5 years later. Inhalable dust and endotoxins (with the LAL test) concentrations were measured in the samples. The annual decline in FEV1 and MMEF (both adjusted for age, height and smoking) showed statistically significant associations with occupational exposure to both dust and endotoxins. A FEV1 decrease of 0.326 mL (SE = 0.139) per 10 EU/m^3 (or 1 ng/m^3 endotoxin) per year of exposure was calculated (r2 = 0.12). Over the 5-year period, 14% of workers had a rapid (>90 mL/y) annual decrease in FEV1. Workers with an endotoxins exposure >200 EU/m^3 (>20 ng/m^3) had a statistically significantly higher risk (OR = 3.3; 95% CI: 1.02 - 10.3) of rapid decline in FEV1. Increasing working years was related to decreasing annual decline in FEV1 (-18 mL) for over 20 years of working and fewer people with rapid decline in FEV1 (Post et al. 1998). The data presented do not allow to determine the respective responsibilities of endotoxins and dust in the respiratory effects reported.

Kirychuk et al. conducted a longitudinal study spanning 5 years, focusing on 42 swine-confinement workers. ΔFEV1, annual rate change in FEV1 and FVC, and only the respirable fraction of personal endotoxins exposure were measured at baseline and after 5 years. Respirable dust was measured with a personal sampler worn by the subject during work over an 8 hr period. Arithmetic means were calculated to estimate average exposures in the barn for both across-shift and follow up studies. To estimate the average annual exposure, the arithmetic mean for the number of zones in the barn was calculated, and then the arithmetic mean of the means for summer and winter measurements was calculated. Endotoxins analyses were performed using the LAL assay, chromogenic method. The mean exposure to respirable endotoxins was about 65 EU/m^3 (6.5 ng/m^3). Air ammonia was also measured; mean concentrations were 14-16 ppm in winter and 5-7 ppm in summer. The mean annual rate change between baseline and follow-up for FEV1 was -54 mL ± 62 mL/year (-1.2 ± 1.4%) and for FVC, it was -49 ± 72 mL/year (-0.9 ± 1.3%). After adjusting for age, height, smoking, ammonia and hours spent in the barn, the endotoxins level was a significant predictor of annual rate change

[4] The article by Smid *et al*. mentions that 530 samples were collected (Smid et al. 1992).

for FEV 1 ($\beta = 0.09$; (SD = 0.04; p = 0.03) but not FVC($\beta = 0.08$ (SD = 0.05; p = 0,10). After adjusting for age, height, smoking and hours spent in the barn, the baseline across-shift changes in FEV1 and FVC were a significant predictor of annual rate change in FEV1 (p=0.01) and FVC (p=0.02), respectively. Co-exposures (to ammonia, particulate matter, etc.) were not taken into account in these analyses (Kirychuk et al. 1998).

Vogelzang *et al*. conducted a 3-yr follow-up study involving 171 pig farmers (89 asymptomatic and 82 with one or more chronic respiratory symptoms), selected from a larger group of pig farmers. Measurements of exposure were carried out on the farms of all participants during full work shifts of on average 8.3 hr on 2 days in summer 1991 and winter 1992. Personal exposure to inhalable dust was determined using a dust sampler and endotoxins were measured with a modified kinetic LAL test. Due to considerable day-to-day variations in dust exposure in individual participants compared to the variations of exposure in the entire group, the long-term average exposure was predicted by a mathematical modeling technique: long-term average exposure to dust and endotoxins of each individual farmer was estimated using data on farm characteristics and time spent on activities in pig farming of all cohort members combined. Estimated long-term average exposure to inhalable dust and to endotoxins was 2.63 mg/m^3 (geometric mean) and 1 050 EU/m^3 (105 ng/m^3), respectively. The study revealed a decrease in baseline FEV1 of 73 mL/year (compared to a normal age-related decrease of 29 mL/y)[5] and a FVC decrease of 55 mL/y. After adjusting for age, baseline FEV1 or FVC and smoking, decline in FEV1 during the 3-yr period was significantly associated with endotoxins exposure alone, whereas decline in FVC was associated with both endotoxins exposure and inhalable dust exposure. In a subsequent publication (Vogelzang et al. 2000), bronchial responsiveness was measured. Provocative histamine concentrations (PC's) were measured for a 10% and a 20% fall in FEV1. Both symptomatic and asymptomatic groups experienced a decrease in PC10 and PC20 within 3 years of additional exposure to ammonia and dust (Vogelzang et al. 1998). As co-exposure to other respiratory irritants (e.g. dust, ammonia) were not taken into account in the analyses, the changes observed cannot be attributed to endotoxins exposure alone.

Christiani *et al*. performed an 11-year follow-up study of 2 cohorts of cotton workers (n = 349) and silk workers (n = 319), including both active and retired individuals. Area samples were collected over the three surveys in the yarn preparation areas of the two mills. Measurements of typical work activities were taken during a 4-month period (September–December) for the first two surveys, and expanded over a four-season (12-month) period during the third survey. FEV1 measurements were performed 5, 6 and 11 years after the start of the study, and compared with measurements before the work periods. In the two mills, exposure data, including both dust and endotoxins, were tabulated by work area and survey. Endotoxins concentrations were categorized into ''high'' and ''low'' areas, with ''high'' defined as ≥ 1 500 EU/m^3, and ''low'' as < 1 500 EU/m^3, based arbitrarily on the approximate midpoint in the distribution of data. Endotoxins assays were performed by the same laboratory on the dust samples using the LAL assay, chromogenic method. In all three surveys, pulmonary function tests were performed before the subjects entered the work area on the first day of work after a 2-day rest. Changes in pulmonary function were measured over 5, 6, and 11 years using pre-shift values from all three surveys. To estimate chronic effects of long-term dust and endotoxins exposure, a cumulative dust and endotoxins variables for each subject in the two mills was constructed. Cumulative exposure index was calculated by the addition of the products of the years worked in each work area by the geometric mean concentrations of dust and endotoxins over

[5] Data from article "Official statement of the European Respiratory Society." Quanjer, P. H. 1993. Eur. Respir. J. 6(Suppl. 16):1–40.View Full R"

the periods In the cotton work areas, the mean exposure levels (area sampling) ranged from 0.2 to 1.6 mg/m^3 for dust and 27 to 12 038 EU/m^3 (2.7 - 1204 ng/m^3) for endotoxins. When individual cumulative exposure values were estimated, the median cumulative exposure for cotton dust was 15.6 mg/m^3 years and for endotoxins 4,020 EU/m^3 years. In contrast, silk workers had a mean exposure of 0.2 mg/m^3 for dust and no detectable (<1 EU/m^3) for endotoxins. A total of 730 air samples were collected over the 11-year survey period, and workers had an average of 25 years of employment at the study's end. The average annual FEV1 loss was the same for cotton and silk workers. During the first 5 years of follow-up, FEV1 loss in cotton workers was higher (though statistically insignificantly) at 40 mL/yr compared to 30 mL/yr in silk workers. However, in the last 6 years of the study, the FEV1 loss reduced to 18 mL/yr in cotton workers and 27 mL/yr in silk workers. The total FEV1 decrease over 11 years was 0.31 L in both cotton and silk workers. After adjusting for confounders (age, height, gender and smoking (pack-year)), the 11-year loss in FEV1 was associated with cumulative exposure to dust but not with endotoxins exposure (Christiani et al. 1999).

Christiani *et al*. published the results of an additional 4-year follow-up, bringing the total duration to 15 years for the same cohort. Endotoxins assays were performed on the dust samples using the LAL assay, chromogenic method. Over the 15-year survey period, a total of 802 air samples were collected, with a median cumulative endotoxins exposure of 48 000 EU/m^3·years (4800 ng/ m^3·yr) and a median cumulative dust exposure of 14.2 mg/m^3.yr. A small but statistically significantly higher annual FEV1 loss was found in cotton workers (-32.3 ± 1.0 mL, or 1.1%) compared to silk workers (-29.4 ±1.0 mL or 1.0%). However, the annual decrease in FEV1 in this study remained relatively similar to the results of the previous study, while the FEV1 remained 100% of the predicted value. It can therefore be assumed that despite the significantly higher decrease in FEV1 in cotton workers, the FEV1 will still not be significantly lower than 100% of predicted. A difference was found only in smokers (-43.7 (2.0) mL/yr for cotton workers and -39.3 (2.1) mL/yr for silk workers), as non-smokers had similar annual FEV1 losses in both groups (-40.4 (3.2) mL/yr for cotton workers and -40.1 (2.5) mL/yr for silk workers). Statistically higher decreases of both FEV1 and FVC were observed in workers highly exposed to endotoxins (> 4800 ng/ m^3·yr) compared to those with lower exposure. A statistically significant relation ($p<0.001$) was found between the change in FEV1 and the across-shift change in FEV1 (ΔFEV1). Additionally, a statistically significant association was observed between accelerated chronic loss in FEV1 and byssinosis or chest tightness at work (Christiani et al. 2001). Authors concluded that there was a significant correlation between chronic loss of pulmonary function and length of exposure (years worked in cotton mills). These functional losses were significantly associated with cumulative endotoxin exposure. The workers with a higher level of cumulative endotoxins exposure had significantly greater losses of FEV1 and FVC than did those with a low level. Conversely, a relationship of cumulative cotton dust exposure was not detected in the workers. Nevertheless, the data presented do not allow to determine the respective responsibilities of endotoxins and other dust components, in the respiratory effects reported.

Wang *et al*. investigated the chronic effects of long term exposure to cotton dust on respiratory health, and lung function (annual decline FEV1 and FVC). The follow-up study spanned five surveys conducted over a 20-year period and included 447 cotton and 472 silk textile workers recruited in Shanghai, China. Inhalable air samplings on airborne cotton dust in the various work areas were measured. Endotoxins assays were performed on the dust samples using the LAL assay, chromogenic method. A standardized questionnaire on work history, respiratory symptoms/diseases (byssinosis, chest tightness, chronic bronchitis, chronic cough and dyspnea) and smoking history was administered. Spirograms were performed at each survey. The values of lung function measured at each period were outcome variables, whereas age, sex, height, smoking status, pack-years, years since last worked (*i.e.* time away from work-related exposure) and exposure to cotton dust were predictive variables. Cotton workers exhibited a cumulative endotoxins exposure of 49 122.60 (± 45 284) EU/m^3-years (4912.2 ± 45 284 ng/m^3-years). In comparison with silk workers, cotton workers had greater annual declines in FEV1, either as a whole group, or as sex- or smoking-specified groups. Similar trends were seen for FVC). The authors concluded that long-term exposure to cotton dust may result in excessive chronic annual loss in FEV1, and in higher proportions of persistent respiratory symptoms or diseases, all of which were more highly related to exposure to

cotton dust-associated endotoxins (Wang et al. 2005). As workers were exposed to cotton dust, respiratory changes cannot be attributed to endotoxins exposure alone.

Shi *et al*. examined temporal aspects of the exposure-response relationship between airborne endotoxins exposure, longitudinal change in FEV1, and respiratory symptoms in a cohort of Chinese cotton textile workers. The prospective cohort study followed 447 cotton textile workers from 1981 to 2006 at approximately 5-year intervals. Stationary measurements of airborne cotton dust were performed with a vertical elutriator in the two cotton textile mills, in six work areas where yarn was prepared. Endotoxins assays were performed on the cotton dust sample filters to measure airborne endotoxins concentrations using the LAL assay, chromogenic method. The granulometry of the dust sampled is not specified. Cumulative exposure to endotoxins (EU/m^3-years) estimated for each participant was derived from work area samples and from detailed work histories. Sampling occurred during the first four surveys. The authors used a generalized estimating equations approach to model FEV1 level and respiratory symptoms as a function of past exposure (cumulative exposure up to the start of the most recent 5-year survey interval) and cumulative exposure (within the most recent interval) to endotoxins, after adjusting for other covariates. All models for FEV1 level and symptoms were adjusted for age (year), height (centimeters), sex (female as reference), smoking status (current or former smoker with never smoker as reference), work status (active vs. retired) and years since cessation of exposure. No adjustments were made for concomitant particulate matter exposure. Models were stratified by active versus retired work status and by years employed before the baseline survey (< 5 and ≥ 5 years). Cumulative endotoxins exposure of the participants was 52 820 ± 45 507 EU/m^3-years (5 282 ± 4 550.7 ng/m^3-years). Past exposure to endotoxins was associated with reduced FEV1 level among retired cotton workers. Among all cotton workers, past exposure was more strongly associated with reduced FEV1 for those hired < 5 years before baseline than for those who were hired ≥ 5 years after baseline. Recent endotoxins exposure was significantly associated with byssinosis, chronic bronchitis, and chronic cough. As cotton workers were exposed to cotton dust, the respiratory changes observed cannot be attributed to endotoxins exposure alone (Shi et al. 2010).

Several adult population studies have shown that early life exposure to farming and bacterial agents decreased the risk of allergic sensitisation and asthma throughout life. There is evidence suggesting that the protective effect may extend beyond childhood exposure. Studies in adult populations have shown that farmers and rural dwellers have a reduced risk of asthma and allergic sensitisation. Basinas *et al*. tested the hypothesis that current endotoxins exposure is inversely associated with allergic sensitisation and positively associated with non-allergic respiratory diseases in four occupationally exposed populations using a standardized analytical approach. Data were pooled from four epidemiological studies including 3883 Dutch and Danish employees in veterinary medicine, agriculture and power plants using biofuel. Personal endotoxins exposure was estimated for every worker by means of quantitative job-exposure matrices developed from measurements and the available questionnaire information in each of the participating studies. Dose-response relationships between exposure, IgE-mediated sensitisation to common allergens (assessed with skin prick tests in the Danish studies and through serological testing of specific IgE using enzyme immunoassays) and self-reported health symptoms were assessed using logistic regression and generalised additive modeling. Median estimated current average endotoxins exposure was 219 EU/m^3 (21.9 ng/m^3) (range from 0.01 to 10 645 EU/m^3). Endotoxins exposure was a risk factor for organic dust toxic syndrome (even in the subgroup with the lowest endotoxins exposures), and levels above 100 EU/m^3 (10 ng/m^3) significantly increased the risk of chronic bronchitis ($p<0.0001$). Current endotoxins exposure was dose-dependently associated with a reduced prevalence of allergic sensitisation (ORs of 0.92, 0.81 and 0.66 for low mediate, high mediate and high exposure) and hay fever (ORs of 1.16, 0.81 and 0.58) (Basinas et al. 2012).

Lai *et al*. investigated the effect of long-term exposure to endotoxins in cotton dust on health, and determined whether these effects differ by gender. In the Shanghai Textile Worker Study, 447 cotton and 472 control silk textile workers were followed from 1981 to 2011 with repeated measures of occupational endotoxins exposure, spirometry and health questionnaires. Endotoxins from collected

dust (particle size not specified) was measured in a single laboratory using the chromogenic LAL assay. Impaired lung function was defined as a decline in FEV1 to less than the 5th centile of predicted value. Total follow-up time and cumulative endotoxins exposure calculated for cotton workers was 37 688 [15 517-65 554] EU/m^3-years (3 768.8 ng/m^3-years). Hazard Ratios (HRs) for the composite end point of impaired lung function or death was 1.47 (95% CI 1.09 to 1.97) for cotton versus silk workers and 1.04 (95% CI 1.01 to 1.07) per 10 000 EU/m^3-years (or 1 000 ng/m^3-years) increase in exposure. HRs for all-cause mortality were 1.36 (95% CI 0.93 to 1.99) for cotton versus silk workers and 1.04 (95% CI 0.99 to 1.08) per 10 000 EU/m^3-years (or 1 000 ng/m^3-years). The risk associated with occupational endotoxins exposure was elevated only in men. The concomitant exposure to particulate matter and other components of textile dust were not taken into account in the analyses. As cotton mill workers were exposed to cotton dust, not to endotoxins alone, the reported effects cannot be attributed to the latter alone (Lai et al. 2014).

Lai *et al*. attempted to evaluate whether the transient FEV1 improvement noted after cessation of occupational dust exposure due to worker retirement was sustained and to identify factors that modify FEV1 improvement based on the Shanghai Textile Worker Study. Spirometry was performed at 5-year intervals. The effect of work cessation on FEV1 was modeled using generalized additive mixed effects models to identify the trajectory of FEV1 recovery. Linear mixed effects models incorporating interaction terms were used to identify modifiers of FEV1 recovery. Generalized additive mixed models identified a non-linear improvement in FEV1 for all workers after exposure cessation, with no plateau noted 25 years after retirement. Linear mixed effects models incorporating interaction terms identified prior endotoxins exposure (p=0.01) and male gender (p=0.002) as risk factors for impaired FEV1 improvement after exposure cessation (Lai et al. 2015).

Lai *et al*. identified the relative contributions of smoking and occupational endotoxins exposure to parenchymal and airway remodeling as defined by quantitative computed tomography (CT) based on the Shanghai Textile Worker Study. Occupational endotoxins exposure was associated with a decrease (–1.3%) in percent emphysema (LAAI-950), a 3.3-Hounsfield unit increase in 15th percentile density, an 18.1g increase in lung mass, and a 2.3% increase in wall area percent. Lung mass was the only measure associated with FEV1 decline, with each 10 g increase in lung mass associated with an additional loss (–6.1 mL) of FEV1 (p = 0.001) between 1981 and 2011. The effects of occupational endotoxins exposure appear to persist even after the cessation of exposure (Lai et al. 2016).

Ghani *et al*. examined the effects of airborne endotoxins on lung function impairment in exposure-response relationships among the workers of textile industry. This cross-sectional study was conducted at Lahore College for Women University, Pakistan, from January to August 2014, and recruited textile mill workers. A microbial air sampler was used to collect air samples from two industries each on glass fibre filter to recognise probable causes of endotoxins exposure. Airborne endotoxins concentrations were measured by using the LAL process through chromogenic method on inhalable dust. The data was analysed to determine the correlation between the endotoxins exposure duration and pulmonary function test parameters. Two hundred subjects were subdivided into 100 each in exposed and control groups. Overall, 160 (80%) subjects were not aware of safety measures and the remaining 40 (20%) were partially practising. The endotoxins concentration was between 12 EU/m^3 and 300 EU/m^3 (1.2-30 ng/m^3) with a mean range concentration of 40-300 EU/m^3 (4-30 ng/m^3) in work area. Changes in pulmonary function due to endotoxins exposure were significantly decreased FVC, FEV1, FEV1/FVC and PEF (p<0.05), especially among 30 to 40 year-old participants (p<0.001) (Ghani et al. 2016).

Carnes *et al*. investigated the association of house dust endotoxins levels with asthma and related phenotypes (wheeze, atopy, and pulmonary function) in a large U.S. farming population. Dust were collected from the bedrooms (n = 2,485) of participants enrolled in a case-control study of current asthma (927 cases) nested within the Agricultural Health Study. Outcomes were measured by questionnaire and spirometry. Vacuum dust sample from bedroom floors and sleeping surfaces was collected and endotoxins levels were measured using the LAL assay. The overall geometric mean

endotoxins concentration of the bed and bedroom floor house dust was 30.4 EU/mg. Endotoxins level in dust was significantly associated with current asthma (odds ratio [OR], 1.30; 95% confidence interval [CI], 1.14-1.47), and this relationship was modified by early-life farm exposure (born on a farm: OR, 1.18; 95% CI, 1.02-1.37; not born on a farm: OR, 1.67; 95% CI, 1.26-2.20; P = 0.05). Significant positive associations were seen with both atopic and nonatopic asthma. Endotoxins level was not related to either atopy or wheeze. Higher endotoxin levels were related to lower FEV1/FVC in asthma cases only (P = 0.01) (Carnes et al. 2017).

Lim *et al*. examined the associations between endotoxins in office dust and respiratory symptoms and airway inflammation. The study population was 695 office workers in Malaysia. Health data were collected using a standardized questionnaire which contained questions on age, sex, ethnicity, smoking habits, respiratory symptoms and home environment developed according to the European Community Respiratory Health Survey, and the International Study of Asthma and Allergies in Childhood survey. In addition, sensitisation testing and measurement of fractional exhaled nitric oxide (Fe NO) were performed. Indoor temperature, relative air humidity (RH) and carbon dioxide ($CO_{2)}$ were measured in the offices and settled dust was vacuumed and analysed for endotoxins content. Concentration of endotoxins was determined using the LAL chromogenic method. The median level of endotoxins in office dust was 11.3 EU/mg (1.13 ng/mg). Overall, 9.6% of the workers had doctor-diagnosed asthma, 15.5% had wheeze, 18.4% had daytime attacks of breathlessness and 25.8% had elevated Fe NO (≥25 ppb). After adjusting for personal and home environment factors, endotoxins concentration in dust was associated with wheeze (P = 0.02) and rhinoconjunctivitis (P = 0.007) (Lim et al. 2019).

Table 2. Summary of studies concerning the effects on lung function after chronic exposure to endotoxins

Reference	Design	Endotoxin exposure	Assessment	Results
Smid *et al*. (1992)	Cross-sectional study	<150, 300-400 and 670 EU/m^3 (range 2-4700) (<15, 30-34 and 67 ng/m^3 (range 0.2-470))	315 workers employed in 14 animal feed mills in the Netherlands	Regression: annual FEV1 β (ng/m^3 or 10 EU/m^3) endotoxins exposure) = -0.34 mL FEV1 Calculated level : 75 EU/m^3 (7.5 ng/m^3) The authors set the LOAEL as the upper limit of the lower exposure 'safe' level between 30 and 75 EU/m^3 (3 and 7.5 ng/m^3)
Post *et al*. (1998)	5 year follow-up	36-990 EU/m^3 (3.6-99 ng/m^3)	140 workers in the grain processing and animal feed industry during 5 years (follow-up of the population of the study of Smid *et al*. (1992))	Regression: annual FEV1 β (ng/m^3 endotoxins exposure) = -0.326 SE 0.139, R^2=0.12 Calculated level[6] : 75 EU/m^3 (7.5 ng/m^3)
Kirychuk *et al*. (1998)	Longitudinal study	Mean exposure to respirable endotoxins: 65 EU/m^3 (6.5 ng/m^3)	ΔFEV1, annual rate change in FEV1 and FVC, and only the respirable fraction of personal endotoxins exposure were measured for 42 swine-confinement workers at baseline and after 5 years	Mean annual rate change between baseline and follow-up for FEV1 = -54 mL ± 62 mL/year (-1.2 ± 1.4%) and for FVC= -49 ± 72 mL/year (-0.9 ± 1.3%). After adjusting for age, height, smoking, ammonia and hours spent in the barn, the endotoxins level was a significant predictor of annual rate change for FEV 1 but not FVC. After adjusting for age, height, smoking and hours spent in the barn, the baseline across-shift change in FEV1 and FVC was a significant predictor of annual rate change in FEV1 and FVC.
Vogelzang *et al*. (1998)	3 year follow-up	1 050 EU/m^3 (105 ng/m^3)	3-yr follow-up study involving 171 pig farmers	After adjusting for age, baseline FEV1 or FVC and smoking, decline in FEV1 during the 3-yr period was significantly associated with endotoxins exposure alone, whereas decline in FVC was associated with both endotoxins exposure and inhalable dust exposure. Not possible to calculate level because extrapolation is outside the curve.
Christiani *et al*. (1999)	11 year follow-up	32 000 EU/m^3 .y = 1 500 EU/m^3 [42-12 038 EU/ m^3] (150 ng/m^3)	11-year follow-up study involving cotton workers (n = 349) and silk workers (n = 319), including both active and retired individuals	No cumulative endotoxins effect could be detected on FEV1.

[6] According to the DECOS

Christiani *et al.* (2001)	15 year follow-up	Median cumulative: 48,000 EU/m^3 .y ≈ 1,500 EU/m^3 (150 ng/m^3)	Additional 4-year follow-up, bringing the total duration to 15 years for the previously described cohort (Christiani et al. 1999)	9% excess annual FEV1 decrease (i.e. 1.1% instead of 1.0% in control group); correlation between ΔFEV1 and annual FEV1 decline. Significant correlation between chronic loss of pulmonary function and length of exposure (years worked in cotton mills). These functional losses were significantly associated with cumulative endotoxins exposure. The workers with a higher level of cumulative endotoxins exposure had significantly greater losses of FEV1 and FVC than did those with a low level. Findings independent from endotoxins exposure.
Wang *et al.* (2005)	Follow-up study	Cumulative endotoxins exposure: 49 122.60 (± 45 284) EU/m^3 -years (4 912 ng/m^3-years)	Five surveys conducted over a 20-year period and included 447 cotton and 472 silk textile workers recruited in Shanghai, China	The annual decline in FEV1 was higher among cotton workers (32.4 ± 1.0 mL/yr) compared to silk workers (27.3 ± 0.9 mL/yr).
Shi *et al.* (2010)	Prospective cohort study	Cumulative endotoxins exposure = 52 820 ± 45 507 EU/m^3-years (5 282 ng/ m^3 ± 4 551 ng/ m^3)	447 chinese cotton textile workers from 1981 to 2006 at approximately 5-year intervals	Among all cotton workers, past exposure (cumulative exposure up to the start of the most recent 5-year survey interval) was more strongly associated with reduced FEV1 for those hired < 5 years before baseline than for those who were hired ≥ 5 years after baseline. Recent endotoxins exposure was significantly associated with byssinosis, chronic bronchitis, and chronic cough.
Basinas *et al.* (2012)	Data pooled from 4 epidemiological study	Median estimated current average endotoxins exposure = 219 EU/m^3 (21.9 ng/m^3) (range from 0.01 to 10 645 EU/m^3)	Personal endotoxins exposure estimated by means of quantitative Job-Exposure Matrices for 3 883 Dutch and Danish employees in veterinary, medicine, agriculture and power plants using biofuel, and questionnaire information. Symptoms were collected using a questionnaire.	Endotoxins exposure was a risk factor for organic dust toxic syndrome, and levels above 100 EU/m^3 (10 ng/m^3) significantly increased the risk of chronic bronchitis ($p<0.0001$).
Lai *et al.* (2014 ; 2015 ; 2016)	Follow-up study	Total follow-up time and cumulative endotoxins exposure = 37 688 [15 517-65 554] EU/m^3-years (3768.8 [1 551-6555] ng/m^3-years)	447 cotton and 472 control silk textile workers were followed from 1981 to 2011 with repeated measures of occupational endotoxins exposure, spirometry and health questionnaires.	HRs for the composite end point of impaired lung function or death was 1.47 (95% CI 1.09 to 1.97) for cotton vs silk workers and 1.04 (95% CI 1.01 to 1.07) per 10 000 endotoxins units (EU)/m^3-years increase in exposure. Occupational endotoxins exposure was associated with a decrease (–1.3%) in percent emphysema (LAAI-950), a 3.3-Hounsfield unit increase in 15th percentile density, an 18.1-g increase in lung mass, and a 2.3% increase in wall area percent.
Ghani *et al.* (2016)	Cross-sectional study	Endotoxins concentration between 12 EU/m^3 and 300 EU/m^3 (1.2-30 ng/m^3); mean range concentration = 40-300 EU/m^3 (4-30 ng/m^3)	Textile mill workers at Lahore College for Women University, Lahore, Pakistan, from January to August 2014	Changes in pulmonary function due to endotoxins exposure showed decreased force vital capacity, flow rate and peak expiratory flow parameters significantly different ($p<0.05$, $p<0.001$).
Carnes *et al.* (2017)	Case-control study	Geometric mean endotoxin concentration of the bed and	Dust collected from the bedrooms (n = 2,485) of participants enrolled in a case-control study of current asthma (927 cases) to study the association of house dust endotoxins levels	Endotoxins level was significantly associated with current asthma (odds ratio [OR], 1.30; 95% confidence interval [CI], 1.14-1.47), and this relationship was modified by early-life farm exposure (born on a farm: OR, 1.18; 95% CI, 1.02-1.37; not born on a farm: OR, 1.67; 95% CI, 1.26-2.20; P = 0.05). Significant

		bedroom floor house dust = 30.4 EU/mg	with asthma and related phenotypes (wheeze, atopy and pulmonary function)	positive associations were seen with both atopic and nonatopic asthma. Endotoxins level was not related to either atopy or wheeze. Higher endotoxins levels were related to lower FEV1/FVC in asthma cases only (P = 0.01).
Lim *et al*. (2019)	Cross-sectional study	Median level of endotoxins = 11.3 EU/mg (1.13 ng/mg)	695 office workers in Malaysia; Health data were collected using a questionnaire, sensitisation testing and measurement of fractional exhaled nitric oxide (Fe NO)	Endotoxins concentration in dust was associated with wheeze (p = 0.02) and rhinoconjunctivitis (p = 0.007).

Several cross-sectional studies of respiratory effects associated with endotoxins exposure are published. They cannot be used for the evaluation of the respiratory effects of long-term endotoxins exposure, as they do not allow to distinguish between acute effects and effects due to repeated exposure. These studies are described above and are summarized in Table 3.

Kennedy *et al.* conducted a cross-sectional study investigating the relationship between endotoxins and dust exposure and lung disease in 443 cotton workers and 439 control subjects from a silk mill. Each worker's pre- and post-shift FVC and FEV1 were determined. Questionnaire administration and pulmonary function testing were carried out on each worker before entering the workshop after a 48-hr rest period. Function tests were repeated at the end of the shift. Airborne dust was collected using vertical elutriators designed to collect particles less than 15µm aerodynamic diameter. Endotoxins assays were performed on the dust samples using the LAL assay, chromogenic method. Endotoxins concentrations ranged from 10 to 9 200 EU/m^3 (1-920 ng/ m^3) and dust concentrations varied from 0.15 to 2.5 mg/m^3 in 130 area samples (<15 µgm). The cotton worker population was categorized into 4 groups according to current exposure to endotoxins (median endotoxins exposures of 20, 1 000, 2 300 and 5 200 EU/m^3 (2, 100, 230 and 520 ng/m^3)). Then, these groups were compared for FEV1, FVC, FEV1/FVC%, across-shift ΔFEV1 and prevalences of chronic bronchitis and byssinosis. All analyses were adjusted for confounding factors such as age, height and smoking habits. Cumulative exposures to dust and endotoxins were estimated for each worker by assigning an exposure level to each work area and summing the product of exposure level times years employed in that area for all work areas reported by the worker. The authors found a dose-response trend with the current endotoxins level and FEV1, change in FEV1 over the shift and prevalence of chronic bronchitis and byssinosis, except for the highest exposure level group in which a reversal of the trend was seen most likely to be caused by a 'healthy workers effect'. The dose-response relation for current exposure was statistically significant for measured pre-shift FEV1 and was calculated to be -2.4 mL per 100 EU/m^3 (10 ng/m^3) ($p<0.10$). Excluding the highest endotoxins exposure category, the coefficient for workers with a mean work history of 15 years increased to -7.8 mL per 100 EU/m^3 (10 ng/m^3) ($p<0.01$). No correlation coefficient was given. Mean pre-shift FEV1 in group 1 (median 20 EU/m^3 (2 ng/m^3)) and group 2 (median 1 000 EU/m^3 (100 ng/m^3)) were higher than FEV1 in the control group of silk workers (FEV1 set on 100%); FEV1 in group 3 (median 2 300 EU/m^3 (230 ng/m^3)) was 96.7% and in group 4 (median 5 200 EU/m^3 (520 ng/m^3)) 98.5% for non-smokers, with no statistically significant difference from the control group. The authors tried to assess a threshold level of endotoxins exposure by comparing the silk workers (control group) with cotton workers who had always worked in an area with 'low endotoxins' levels (below 200 EU/m^3 (20 ng/m^3)). Although no difference in baseline (= pre-shift) spirometry was found, the increased prevalence of byssinosis and chronic bronchitis and the augmented across-shift change in FEV1 led the authors to suggest that even exposure to the lowest level of endotoxins at 10 to 200 EU/m^3 (1-20 ng/m^3) constitutes an adverse respiratory health effect (Kennedy et al. 1987) It should be noted that workers in this study, were actually exposed to cotton dust and that the co-exposure to particulate matter and to components of dust other than endotoxins were not taken into account in the analyses.

In a large study of Simpson *et al.,* symptoms prevalence and endotoxins exposure levels were assessed among 1 032 workers in several occupations and industries. Lower respiratory tract symptoms, including cough, phlegm, shortness of breath, wheeze and chest tightness, were

recorded. ODTS[7] was identified in individuals reporting recurrent episodes of at least two of the following symptoms: fever, shivering, malaise, weakness and joint or muscle pain. Byssinosis, work related chronic bronchitis and eye and nasal irritation were also registered. A representative sample of the workforce at each site was selected to record personal total dust exposures during a typical work shift. To normalise the data, the natural log for both current endotoxins and dust exposures were ascribed to individual people. Each occupational group specific to site and room were accredited with the average value for the samples taken. The highest endotoxins exposures for both the range and median exposures were found in the poultry (median 120 000 EU/m^3 (12 000 ng/m^3); highest 720 000 EU/m^3 (72 000 ng/m^3)), swine confinement (median 6 000 EU/ m^3 (600 ng/m^3) ; highest 149 000 EU/ m^3 (14 900 ng/m^3)) and cotton spinning (median 4 000 EU/m^3 (400 ng/m^3); highest 69 000 EU/m^3 (6 900 ng/m^3)) industries (Simpson et al. 1996). The study demonstrated a relation between prevalence of symptoms and the exposure level to endotoxins. The authors showed a figure with percentage of workers with lower respiratory tract symptoms plotted against the mean endotoxins level for that group of workers, with a log-linear regression line drawn. However, an examination of the plotted data suggests that an exponential curve might offer a better fit and that symptoms are noticeably increased when endotoxins levels exceed approximately 500 EU/m^3 (50 ng/m^3). Unfortunately, as raw data were not available, no calculation could be performed, and quantitative conclusion could not be drawn. The study revealed that workers with symptoms had consistently higher exposures to dust and endotoxins compared to their counterparts in the same occupations, although the difference was not statistically significant. Poultry workers presented the highest prevalences of lower respiratory tract symptoms and nasal and eye symptoms. Despite exposure to high levels of endotoxins (up to 500 000 EU/m^3 (50 µg/m^3)) only 1.3% of all workers suffered from ODTS (Simpson et al. 1998).

In a cross-sectional study involving 114 male employees of a cotton mill in western Germany (Latza et al. 2004), airborne endotoxins exposures were categorized as low (< 100 EU/m^3 (<10 ng/m^3)), medium (>100-450 EU/m^3 (10-45 ng/m^3)) and high (>450 EU/m^3 (>45 ng/m^3)), on the basis of endotoxins activity in the LAL assay. The article gives no indication on the timing of the measurements and on sampling granulometry. Crude risk estimates were adjusted for age (yr), smoking status (i.e., current smoker/former smoker/non-smoker), and pack-years. There was no adjustment for co-exposure to PM and other components of cotton dust. A dose-response relationship was observed between endotoxins exposure and the prevalence of wheezing (medium exposure group: OR = 2.15; 95% CI: 0.48 - 9.62); high exposure group OR = 5.49; 95% CI: 1.17 - 25.81) and cough (medium exposure group: OR = 2.11; 95% CI: 0.59 - 7.56); high exposure group OR = 3.93; 95% CI: 1.02 - 15.12) during the last 12 months, with statistical significance noted for the highest exposure group (> 450 EU/m^3 (> 45 ng/m^3)). The results suggested that there was a dose-dependent increase in bronchial symptoms, with significant effects occurring at exposures that exceeded 450 EU/m^3 (45 ng/m^3).

Oldenburg *et al*. performed a cross-sectional study involving 150 employees (114 male and 36 female) of the same German cotton spinning mill, subjecting them to lung function testing. During the period of 1999 to 2001 and concurrent to the repeated lung function testing, numerous inhalable and respiratory dust samples were collected in different areas of the cotton spinning mill. The duration of each dust sampling was 2 hr. The amount of endotoxins was measured using the

[7] Organic dust toxic syndrome

chromogenic version of the LAL assay. Airborne endotoxins exposures were categorized as low (< 100 EU/m^3 (< 10 ng/m^3)), medium (>100-450 EU/m^3 (10-45 ng/m^3)) and high (>450 EU/m^3 (> 45 ng/m^3)). A significant dose-response relationship was identified between current endotoxins exposure and the prevalence of an obstructive ventilation pattern (FEV1/FVC% < predicted FEV1/FVC%) for the highest exposure group (OR = 11.2; 95% CI: 1.03 - 121.2) after adjusting for sex, age, smoking status, and packyears. However, no significant deviation was observed in mean lung function parameters in the different exposure groups (Oldenburg et al. 2007).

Smit *et al*. investigated exposure-response relationships in Dutch farmers and agricultural industry workers, specifically examining the associations between current endotoxins exposure and allergic and respiratory symptoms in adults. Farming exposures during childhood were considered. The cross-sectional study, conducted in 2006, involved 877 participants, and a job-exposure matrix was created based on 249 full-shift personal airborne endotoxins samples to assign exposure levels. Endotoxins were analysed on inhalable dust samples by the quantitative kinetic chromogenic LAL assay. The average endotoxins exposure levels of the study population were practically identical between both workers who grew up on a farm and those who did not (GM = 265 EU/m^3 (26.5 ng/m^3)). Multiple logistic regressions were employed to analyse the associations between endotoxins concentration exposure and questionnaire data on symptoms. Adjusted OR for an interquartile range increase in endotoxins levels were elevated for respiratory symptoms such as wheezing (OR = 1.41 (95% CI: 1.16 - 1.72)), wheezing with shortness of breath (OR = 1.50 (95% CI: 1.18-1.90)) and daily cough (OR = 1.29 (95% CI: 1.03 - 1.62)). In contrast, endotoxins exposure was strongly associated with a decreased prevalence of hay fever (OR = 0.62 (95% CI: 0.49 - 0.78)). While workers who had grown up on a farm showed a lower prevalence of hay fever, there was no evidence of effects modification by farm childhood. Smit *et al*. concluded that occupational endotoxins exposure in adulthood was associated with an increased risk of asthma-like symptoms but a reduced prevalence of hay fever (Smit et al. 2008).

Freitas *et al*. performed a cross-sectional study in workplaces of two universities. Floor dust samples were collected from laboratories and animal facilities housing rats, mice, guinea pigs, rabbits or hamsters, and also from workplaces without animals. Volunteers were workers or students with 412 subjects exposed to laboratory animals (exposed group) and 339 subjects without exposure (non-exposed group). Floor dust samples were collected at both groups workplaces. One square meter was sampled for 2 min using a vacuum cleaner equipped with a fiberglass filter with a pore size of approximately 1 µm. Endotoxins concentration was measured using the kinetic chromogenic LAL assay. High endotoxins concentrations included values above the median (20.4 EU/mg) and low endotoxins concentrations were defined as ≤ 20.4 EU/mg. An association between endotoxins exposure and wheezing in the last 12 months, asthma defined by self-reported diagnosis and asthma confirmed by bronchial hyperresponsiveness (BHR) to mannitol was investigated. Dust samples were obtained at 145 workplaces, 92 with exposure to animals and 53 with no exposure. In the exposed group, the median concentration of endotoxins was 34.2 EU/mg of dust; and, in the non-exposed group, it was 10.2 EU/mg of dust ($p < 0.001$). The high concentration of endotoxins (above whole sample median, 20.4 EU/mg) was associated with increased wheezing prevalence ($p<0.001$), i.e., 61 % of workers exposed to high endotoxins concentration reported wheezing in the last 12 months compared to 29 % of workers exposed to low endotoxins concentration (≤ 20.4 EU/mg). A significant decrease for FEV1/FVC rate was detected in association with increasing exposure to airborne endotoxins measured in 22 different workplaces of the cotton-spinning mill, but details of the spirometric scores reduction are not given in the article. Co-exposures to particulate matter or components of cotton dust other than endotoxins were not taken into account in the analyses (Freitas et al. 2016).

Anyfantis *et al*. performed a secondary analysis from a cross-sectional study on respiratory symptoms and lung function of cotton industry workers in Greece (n=256). Endotoxins levels were

measured using LAL endpoint Method. The granulometry of dust sampling is not specified. The endotoxins level was categorized into "<100," "100–1000," "1000–2000,"and ">2000" EU/m^3 (<10, 10-100, 100-200 and > 200 ng/m^3). High exposure levels were recorded, near the ginning machines (>2000 EU/m^3 (>200 ng/m^3)) and the blending machines, before the spinning process (>2000 EU/m^3(>200 ng/m^3)). Statistical analysis revealed a significant (p=0.02) linear negative correlation between endotoxins levels and FEV1/FVC among studied workers. Indeed, it was found that exposure to high levels of airborne endotoxins (>2000 EU/m^3 (>200 ng/m^3)) was significantly (p<0.001) associated with reduced spirometry scores. However, details of the spirometric scores reduction are not given in the article. A high correlation was also identified between endotoxins exposure and presence of dyspnea (p<0.001), chronic cough (p=0.016), and spitting (p<0.001). Co-exposures to particulate matter or components of cotton dust other than endotoxins were not taken into account in the analyses (Anyfantis et al. 2017).

In a cross-sectional study, Heldal *et al*. investigated whether airborne exposure to endotoxins, hydrogen sulphide (H_2S), and inhalable particles negatively impacts the respiratory system and inflammatory blood proteins in sewage plants and sewer net system workers and further, to determine dose-response relationships between exposure and health outcomes. In total, 148 waste water workers (WWWs) from urban and rural sewage plants and the sewer net system participated. One hundred and twenty-one workers were exposed to sewage, 46 from sewage plants and 75 from the sewer net system. Twenty-seven workers were characterized as little or not exposed and served as an internal reference group. All exposure measurements were carried out from the beginning of the day and until lunchtime (sampling time 4–5 hr) while most of the practical work was performed. Throughout the sampling period, the workers recorded their work operations, the use of personal protective device and breaks. Exposure to endotoxins, dust particles, and bacteria were assessed in samples collected with inhalable personal air samplers and endotoxins were quantified by kinetic chromogenic LAL assay. Workers in sewage plants were exposed to significantly higher levels of endotoxins compared to workers in the sewer net system with respectively a median 55 EU/m^3 [4–262 EU/m^3] (5.5 [0.4-2.62] ng/m^3) and median 27 EU/m^3 [1–304 EU/m^3] (2.7 [0.1-30.4] ng/ m^3). The exposed workers had significantly higher C-reactive protein (CRP) levels, compared to the referents [1.2 µg/mL (0.1-19.0 µg/mL) and 0.8 µg/mL (0.1-5.0 µg/mL), respectively] and lower FEV1% [92.6%, standard deviation (SD) 14.6 and 102.0%, SD 10.1, respectively]. The serum CRP concentration was significantly and negatively associated with FEV1% (β = -7.7, R2 = 0.05) and forced vital capacity % (β = -8.5, R2 = 0.08), and serum concentration of Intercellular adhesion molecule 1 (ICAM-1) was associated with the estimated exposure to H_2S (β = -19.9, R2 = 0.07). The absolute value of FEV1 (3.39 L ± 0.1) was inversely associated with endotoxins exposure (β = −0.22, R2 = 0.18, p < 0.05), and this association was close to significance regarding FEV1% of predicted value (p = 0.07). No significant interaction between the exposure to endotoxins and the H_2S index was observed on the association with any of the analysed lung function variables. No adjustment was made for co-exposure to other components of sewage plants emissions (PM, infectious agents, etc.). Despite moderate levels of endotoxins and H_2S exposure, the results indicated an impact of these agents on lung function and the adhesion molecule ICAM-1, and a low-grade systemic inflammation was indicated in increased levels of CRP (Heldal et al. 2019).

Shakri *et al.* performed a comparative cross-sectional study that aimed to associate the endotoxins levels in inhalable rice dust and the lung function decline among rice millers. The study was conducted at 12 rice mills in different states across Peninsular Malaysia, recruiting 79 rice millers and 51 non-exposed subjects (administrative staff) from the Health Campus, Universiti Sains Malaysia. The endotoxins level in inhalable dust for both area and personal samplings were collected. The endotoxins concentrations were analysed using LAL Chromogenic Endpoint assay. The mean concentration of endotoxins for 28 areas of rice mills in Malaysia was 0.26 (standard deviation (SD) = 0.12) EU/m^3 (0.026 ng/m^3) whereas the mean personal inhalable endotoxins level among the rice millers was 0.29 (SD = 0.15) EU/m^3 (0.029 ng/m^3). Post-shift lung function tests showed lower FEV1/FVC among rice millers (54%) compared to non-exposed workers (62%), but

the difference was not statistically significant ($p = 0.31$). However, there were significant correlations between endotoxins concentration and post-shift lung function test parameters of measured FVC, FEV1 and Peak Expiratory Flow Rate (PEFR) ($p<0.05$). These variables were linearly related, nevertheless none of the parameters showed a strong correlation between the concentration of endotoxins with the lung function tests. It should be noted that duration of exposure and cumulative exposure were not considered in this study and that components or rice mill dust, other than endotoxins were not taken into account in the analyses (Shakri et al. 2020).

Table 3. Summary of studies not distinguishing acute and chronic pulmonary effects

Reference	Design	Endotoxin exposure	Assessment	Results
Kennedy *et al.* (1987)	Cross-sectional study	20, 1 000, 2 300 and 5 200 EU/m^3 (2, 100, 230 and 520 ng/m^3)	Pre- and post-shift FVC and FEV1 determined for 443 cotton workers and 439 control subjects from a silk mill.	Regression: annual FEV1 β (ng/m^3 or 10 EU/m^3) endotoxin exposure = -0.016 to -0.052 mL FEV1 Calculated level[8] : 750-1500 EU/m^3 (75-150 ng/m^3)
Simpson *et al.* (1998)	Cross-sectional study	Highest exposures: Poultry: median =120 000 EU/m^3 (12 000ng/m^3) Swine confinement: median = 6000 EU/m^3 (600 ng/m^3) Cotton spinning: median = 4000 EU/m^3 (400 ng/m^3)	Lower respiratory tract symptoms, including cough, phlegm, shortness of breath, wheeze and chest tightness, were recorded for 1 032 workers in several occupations and industries	Workers with symptoms had consistently higher exposures to dust and endotoxins compared to their counterparts in the same occupations, although the difference was not statistically significant. Poultry workers presented the highest prevalences of lower respiratory tract symptoms and nasal and eye symptoms. Despite exposure to high levels of endotoxins (up to 500 000 EU/m^3(50 000 ng/m^3) only 1.3% of all workers suffered from ODTS
Oldenburg *et al.* (2007)	Cross sectional study	Low: < 100 EU/m^3 (<10 ng/m^3) Medium: 100-450 EU/m^3 (10-45 ng/m^3) High: >450 EU/m^3 (>45 ng/m^3)	150 employees (114 males and 36 females) of the same German cotton spinning mill subjected to lung function testing	Significant dose-response relationship was identified between current endotoxins exposure and the prevalence of an obstructive ventilation pattern (OR = 11.2; 95% CI: 1.03 - 121.2) for the highest exposure group. However, no significant deviation was observed in mean lung function parameters in the different exposure groups.
Smit *et al.* (2008)	Cross-sectional study	GM = 265 EU/m^3 (26.5 ng/m^3)	877 Dutch farmers and agricultural industry workers; job-exposure matrix was created based on 249 full-shift personal airborne endotoxins samples to assign exposure levels	Adjusted OR for an interquartile range increase in endotoxins levels were elevated for respiratory symptoms such as wheezing (OR = 1.41 (95% CI: 1.16 - 1.72)), wheezing with shortness of breath (OR = 1.50 (95% CI: 1.18-1.90)) and daily cough (OR = 1.29 (95% CI: 1.03 - 1.62)).
Freitas *et al.* (2016)	Cross-sectional study	High endotoxins concentrations > 20.4 EU/mg); low endotoxins concentrations means ≤ 20.4	Workers or students with 412 subjects exposed to laboratory animals (exposed group) and 339	The high concentration of endotoxins (above whole sample median, 20.4 EU/mg) was associated with increased wheezing prevalence (p<0.001), i.e., 61 % of workers exposed to high endotoxins concentration reported wheezing

[8] According to DECOS

		EU/mg; median endotoxins concentration (exposed group) = 34.2 EU/mg of dust; non-exposed group = 10.2 EU/mg of dust ($p < 0.001$).	subjects without exposure (non-exposed group)	in the last 12 months compared to 29 % of workers exposed to low endotoxins concentration (≤ 20.4 EU/mg).
Anyfantis *et al*. (2017)	Cross-sectional study	endotoxins level was categorized into <100 EU/m^3 (<10 ng/m^3), 100–1000 EU/m^3(10-100 ng/m^3),1000–2000 EU/m^3 (100-200 ng/m^3) and >2000 EU/m^3 (>200 ng/m^3)	Cotton industry workers in Greece (n=256)	Statistical analysis revealed a significant ($p=0.02$) linear correlation between endotoxins levels and FEV1/FVC among studied workers. Exposure to high levels of airborne endotoxins (>2000 EU/m^3) was significantly ($p<0.001$) associated with reduced spirometry scores. A high correlation was also identified between endotoxins exposure and presence of dyspnea ($p<0.001$), chronic cough ($p=0.016$), and spitting ($p<0.001$).
Heldal *et al*. (2019)	Cross-sectional study	Workers in sewage plants: median 55 EU/m^3 [4–262 EU/m^3] (5.5 ng/m^3); Workers in the sewer net system: median 27 EU/m^3 [1–304 EU/m^3] (2.7 ng/m^3).	148 waste water workers (WWWs) from urban and rural sewage plants and the sewer net system participated	The absolute value of FEV1 (3.39 L ±0.7) was inversely associated with endotoxins exposure ($\beta = -0.22$, $R2 = 0.18$, $P < 0.05$), and this association was close to significant regarding FEV1% of predicted value ($P = 0.07$) The exposed workers had significantly higher C-reactive protein (CRP) compared to the referents [1.2 µg mL-1 (0.1-19.0 µg/mL) and 0.8 µg/mL (0.1-5.0 µg/mL), respectively] and lower FEV1% [92.6%, standard deviation (SD) 14.6 and 102.0%, SD 10.1, respectively], with numbers given as mean and SD.
Shakri *et al*. (2020)	Comparative cross-sectional study	Mean concentration of endotoxins = 0.26 (standard deviation (SD) = 0.12) EU/m^3 (0.026 ng/m^3) Mean personal inhalable endotoxins concentration = 0.29 (SD = 0.15) EU/m^3 (0.029 ng/m^3)	Endotoxin level in inhalable dust and personal samplings were collected from 79 rice millers and 51 non-exposed subjects	Significant correlations between endotoxins concentration and post-shift lung function test parameters of measured FVC, FEV1 and Peak Expiratory Flow Rate (PEFR). These variables were linearly related, nevertheless none of the parameters showed a strong correlation between the concentration of endotoxins with the lung function tests.

4.5.1.2 Neurotoxicity

In its 2010 report, DECOS stated that no evidence was found for possible adverse effect of chronic inhalatory exposure to endotoxins on neurological parameters (Health Council of the Netherlands 2010).

From the literature search until December 2023, several studies were identified.

Using data from a hospital-based case-control study (444 patients with Parkinson disease and 876 age- and sex-matched controls), van der Mark *et al*. studied the associations of Parkinson disease with occupational exposure to pesticides, but also to airborne endotoxins (exposure assessment through a general-population job-exposure matrix based on job codes (DOM-JEM)). For each confirmed patient with Parkinson disease, two matched controls were selected from persons who were seen at the department of neurology between January 2006 and December 2011 for non-neurodegenerative symptoms. The authors refered to previous work, in particular from Lange and coworkers (Niehaus and Lange 2003; Lange et al. 2006), having posited that endotoxins exposure may increase the risk of Parkinson disease by inducing inflammation-mediated neurodegeneration. This postulate was based on either human case-studies with acute high-dose exposures to LPS through an open wound or by injection, or by animal experimentation with injection of LPS into the central nervous system. The results for cumulative exposure to endotoxins and Parkinson disease risk, based on DOM-JEM, do not support a possible association between endotoxins exposure and Parkinson disease (van der Mark et al. 2014).

Checkoway *et al*. investigated the association between exposures to endotoxins and neurologist-determined parkinsonism, using data from the Shanghai women textile cohort (39 prevalent parkinsonism cases and 784 non-cases). The evaluation of endotoxins exposure was outlined in historical estimations in support of an epidemiological study of cancer incidence regarding the exposure to cotton dust and endotoxins, which were evaluated for individuals selected from a group of 267 400 female textile workers. Estimates of exposure were approximated based on measurements of the endotoxin content of airborne cotton dust collected in four studies from several Shanghai textile factories over 15 years and also from one previous study performed by the same research group (Astrakianakis et al. 2006). Average endotoxins content of airborne dust for seven specific processes was derived from a total of 765 samples from five factories obtained during these five surveys. Parkinsonism cases were determined by a score>=15 to the Unified Parkinson's Disease Rating Scale Part III (UPDRS3). No statistically significant association of endotoxins exposure was observed with parkinsonism risk, severity, or progression in their analysis (Checkoway et al. 2018).

Using data from a large, multicenter, population-based case-control study, Visser *et al*. investigated the association between endotoxins exposure (assessed through a general-population job-exposure matrix) and amyotrophic lateral sclerosis. In the single occupational exposure model, a positive statistically significant ($p<0.05$) association between the risk of amyotrophic lateral sclerosis and high-level exposure to endotoxins was observed. However, when adjusted on exposures to other particulates (silica, organic dust) or diesel motor exhaust, the association no longer remained significant (Visser et al. 2019).

4.5.1.3 Cardiovascular toxicity

DECOS stated that no evidence of cardiovascular effects has been reported following long-term exposure to endotoxins (Health Council of the Netherlands 2010).

Complementary bibliographical search from 2010 to 2023 allowed to identify new studies.

Gallagher *et al*. examined cardiovascular mortality over the period 1989-2000 in a cohort of 264 700 female textile workers in Shanghai, in relation with occupational exposures to dusts, endotoxins and

chemicals. Individual level exposure quantitative data for endotoxins and cotton dust were available only for a subcohort of 3188 women. For women in this subcohort, job and time period-specific cotton dust exposure levels were determined based on 2 400 historic cotton dust measurements made by Chinese factory inspectors in 56 cotton factories between 1975 and 1999. Dust estimates were converted to endotoxins estimates using measurements made in three Shanghai factories in 2002. Exposure estimates for all jobs that each woman held throughout the textile industry work history were summed to obtain cumulative exposure estimates for both types of exposures. Analyses were adjusted for age at baseline (continuous) and smoking (ever/never). Slightly elevated risks, of borderline statistical significance, were observed for ischemic stroke (HR = 1.12; 95% CI: 0.97 - 1.31) and hemorrhagic stroke (HR = 1.12; 95% CI: 1.02 - 1.23) for the cotton sector. Thus, no clear evidence of a potential role of endotoxins in the cardiovascular mortality in this cohort can be deducted from this study (Gallagher et al. 2012).

Karottki *et al*. conducted a cross-sectional study to explore whether microvascular function (MVF) was inversely associated with exposure to real-life levels of PM and PM-related endotoxin in a population-based sample of 58 residences (80 non-smoking volunteers). Indoor settled dust was collected by an electrostatic dust fall collector (EDC) at ≥1 m above the floor level over 28 days and endotoxins concentration was measured (median = 1354 EU/m^2; P5 - P95 = 236 - 6105). Models were adjusted for age, gender, BMI and in sensitivity analyses for intake of vasoactive drugs or statins or use of candles as categorical variable. Furthermore, adjustment for the time the home was unoccupied (on average 20% of the total time) was included as an estimate of time spent outside in sensitivity analyses of the significant associations found. Results did not show any association between MVF and endotoxins exposure (Karottki et al. 2014). The similarly-designed study of Karottki *et al*. did not show any association between indoor PM-related endotoxins exposure (measured in settled dust from 27 apartments) and MVF in 48 subjects either (Karottki et al. 2015).

Straumfors *et a*l. investigated if blood markers of platelet activation could indicate early signs of cardiovascular risk due to exposure to grain dust-containing endotoxins. Blood samples were collected from 102 grain elevator and compound feed mill workers, among which 68 exposed to grain dust and 38 non-exposed. Endotoxins exposure was evaluated through personal sampling over a work-shift, for two consecutive days, followed by LAL test. A high exposure to endotoxins was observed (GM = 777 EU/m^3 (77.7 ng/ m^3); GSD =5.53). The concentrations of the measured platelet activation markers sCD40L (soluble CD40 ligand contained in platelet granules) and sP-selectin (soluble P-Selectin released from platelets and the endothelial cell surface, with pro-thrombotic and proinflammatory properties) were similar between the exposed workers and the controls, indicating that neither grain dust nor any other exposure, including endotoxins, had any significant role in platelet activation as indicators of increased risks of cardiovascular effects in the study population (Straumfors et al. 2018).

4.6 Genotoxicity

Human data

There are no data identified in the scientific literature concerning mutagenic effects associated with exposure to endotoxins.

Wultsch *et al*. investigated nuclear anomalies reflecting genotoxic effects by assessing the frequency of micronuclei, binuclei and nuclear buds in exfoliated oral and nasal cells, in relation to occupational exposures to airborne endotoxins (3-day personal air sampling mean = 382 EU/m^3 (38.2 ng/m^3); 3-day stationary air sampling mean = 620 EU/m^3 (62 ng/m^3)) in workers (N = 25) employed at a power plant processing chicken manure in comparison with controls (N = 21). Results indicated that

occupational exposure to poultry manure was not associated with a significant increase in micronuclei and of other nuclear anomalies (Wultsch et al. 2013).

Animal data

There are no animal study available to study the effects of endotoxins and enable a quantitative assessment of the hazard based on the dose-response relationship.

4.7 Carcinogenicity

In this chapter, human studies specifically looking for an association of cancer risk with endotoxins exposure are taken into account. Studies carried out in populations occupationally exposed to endotoxins but not specifically taking these exposures into account are not considered.

According to DECOS, in the 1970's, findings in several occupational cohort studies suggested reduced risks in mortality studies of lung cancer risk among textile workers (Health Council of the Netherlands 2010). Other publications confirmed this finding by suggesting an inverse dose-response relationship between exposure to endotoxins and lung cancer. Astrakianakis *et al.*, for example, observed a dose-dependent reduction in lung cancer risk in a cohort of female textile workers in Shanghai (Astrakianakis et al. 2007). A review by Lundin *et al*. concerning the relation between exposure to endotoxins and cancer concluded that epidemiological studies of cotton textile and other endotoxins exposed occupational groups have consistently reduced lung cancer risks (Lundin and Checkoway 2009). However, absence of data on potential confounding factors has been a limitation of most studies.

Lung cancer

Agalliu *et al*. have reanalysed the lung cancer data from a case-cohort study of Shanghai female textile workers (n = 602 lung cancer cases, n = 3038 controls). This study aimed to estimate the separate effects of past and recent periods of cumulative exposure to endotoxins on lung cancer risk, using contiguous windows of exposures. Occupational endotoxins exposures due to cotton dust were reconstructed from historic cotton dust measurements and use of a job specific endotoxins survey. Cumulative endotoxins exposure was partitioned into two windows: ≥ 20 and < 20 years before occurrence of lung cancer. An inverse association between lung cancer and endotoxins exposure ≥ 20 years before occurrence of lung cancer (p-trend = 0.02) was observed, providing evidence that endotoxins exposure that occurred 20 years of more before risk is associated with the strongest protection against lung cancer (Agalliu et al. 2011).

Applebaum *et a*l. examined the left truncation bias on these same subcohort data, by comparing lung cancer rates in those hired longer ago with those hired more recently among unexposed subjects. An inverse dose–response between endotoxins and lung cancer remained for all subjects except those hired longest ago (> 50 years). The authors concluded that this finding is consistent with molecular and medical research showing that endotoxins can have a dual role in protecting against cancer and later increasing risk of cancer through production of immunotherapeutic agents for cancer. In addition to the association of LPS with tumour regression, evidence supports that tumour progression may also occur (Applebaum et al. 2013).

Checkoway *et al*. updated a case-cohort study nested within a cohort of 267 400 female textile workers in Shanghai, China. Authors compared exposure histories of 1 456 incident lung cancers cases diagnosed during 1989-2006 with those of a reference subcohort of 3 022 workers who were free of lung cancer at the end of follow-up. Endotoxins exposures were estimated quantitatively by applying a validated job–exposure matrix to factory and job assignment data spanning complete work history experience. No associations between cumulative endotoxins exposure and lung cancer was observed, only the analyses by exposure time windows revealed a non-significant elevated relative risk (RR =1.27 [0.93-1.73]) at the highest three exposure quintiles for exposures that

occurred > 15 years since first exposure. The authors concluded in a possible lung cancer promotion effect of endotoxins with increasing time since first exposure (Checkoway et al. 2014).

The studies of McElvenny *et al*. and Ben Khedher *et al*. add further weight to the evidence from a protective effect of endotoxins against lung cancer. McElvenny *et al*. updated the mortality data of a cohort of 3 551 workers who were employed in the British cotton industry. From their job title at the time of recruitment, subjects were assigned to three work areas: opening, carding and ring room/ winding. A simple job-exposure matrix was then created, in which airborne endotoxins concentrations were assigned to the three work areas, according to whether the mill spun predominantly coarse/medium or fine cotton. Risk of lung cancer tended to decrease with higher cumulative exposure to endotoxins, the trend being statistically significant in men ($p = 0.05$) and in both sexes combined ($p = 0.005$). No such trend was apparent for the combination of all cancers other than lung cancer (McElvenny et al. 2011).

Ben Khedher *et al*. have investigated the association between lung cancer and occupational exposure to endotoxins in a large French population-based case–control study entailing a large range of industries (ICARE, Investigation of occupational and environmental causes of respiratory cancers) (N = 2926 cases, 3555 matched controls) (Ben Khedher et al. 2017). The evaluation of exposure to endotoxins was based on a report by The French National Research and Safety Institute for the Prevention of Occupational accidents and Diseases (INRS) (Duquenne et al. 2012), whose authors identified published studies on the measurement of airborne endotoxins, and classified several work environments by providing lower and upper levels of exposure. An overall statistically significant ($p \leq 0.05$) inverse association between exposure to endotoxins and lung cancer was found (OR = 0.80; 95% CI: 0.66 - 0.95). This finding was corroborated by the statistically significant trends with duration and cumulative exposure (exposure periods > 14 years decreased lung cancer risk by > 40%, while the quartile of subjects with the highest cumulative exposure had an almost 60% risk decrease). The risk was also significantly decreased in workers who were exposed to endotoxins until 21 years before interview. Lung cancer risk was particularly reduced among workers highly exposed (≥ 1000 EU/m^3 (≥ 100 ng/m^3)), but a risk decrease was also observed in those weakly exposed (< 1000 EU/m^3 (< 100 ng m^3)).

The meta-analysis conducted by Xu *et al*. to assess the relationship between occupational exposure to endotoxins and the risk of lung cancer among workers in cotton textile mills (14 studies included) but also agriculture (20 studies included) supports that endotoxins high-level exposure in these two sectors is associated with decreased lung cancer risk. Pooled RR between endotoxins exposure and lung cancer was 0.94 (0.79 - 1.11) for textile workers and 0.70 (95% CI: 0.59 - 0.84) for agricultural workers. However, deficient information about smoking histories for participants in most studies makes difficult to state whether adjustment for smoking would create great influence on the overall risk (Xu et al. 2016).

The large pooled case-control study on lung cancer conducted by Peters *et al*. does not support the findings on a possible protective effect of endotoxins, especially among farmers (be it with or without correction for smoking habits). According to the authors, exposure misclassification (endotoxins exposure was assigned using a general population job-exposure matrix for biological exposures) may only partly explain the lack of association observed (Peters et al. 2012).

The US population-based case-control study conducted by Bhatti *et al*. to investigate the association between wood dust exposure and lung cancer (n = 440 lung cancer cases, n = 845 matched controls) suggest an inverse association, however with a modest non-significant decreased risk. The authors refer to the possible role of exposure to endotoxins present in wood dust to explain this finding. However, further studies would be necessary to explore this hypothesis (Bhatti et al. 2011).

Other types of cancer

Wang *et al*. evaluated the association between household dust endotoxins level and non-Hodgkin lymphoma (NHL). Measurements of endotoxins concentration were performed in dust from 618 cases and 460 controls providing vacuum cleaner bags. Analysis was performed using a modification

of the kinetic chromogenic LAL assay with a 12-point standard curve. This analysis revealed no evidence of an association between household dust endotoxins content and NHL risk (Wang et al. 2013).

Fang *et al*. investigated cancer mortality in relation to cotton dust and endotoxins exposure in the Shanghai textile workers cohort. Cotton textile workers (N = 444) and unexposed silk workers (N = 467) were followed for 30 years (26 777 person-years) and HRs for all cancers combined (with and without lung cancer) and gastrointestinal cancer were estimated. Endotoxins were measured from collected cotton dust sample filters using LAL, chromogenic method. Models using categories of cumulative cotton dust exposure while adjusting for time-varying pack-years smoked, work years and age were fitted. Risks of mortality from gastrointestinal cancers and from all cancers combined when excluding lung cancer were significantly increased in endotoxins highly-exposed cotton workers compared to silk workers. Findings from this study indirectly support that endotoxins may be protective against lung cancer, whereas also suggest that conversely, endotoxins may be carcinogenic at other sites in the body. The underlying biological mechanism is thus likely to be local rather than systemic (Fang et al. 2013).

Gallagher *et al*. specifically explored the associations between stomach and oesophageal cancer and occupational exposures (including endotoxins) in the Shanghai female textile workers case-control sub-cohort (N = 1374 stomach cancer cases; N = 190 oesophageal cancer cases; N = 3187 age-stratified non-cases from the cohort). Industrial hygienists estimated cotton dust exposures based on historical measurements from 56 factories. Concentrations of endotoxins were modeled based on predicated cotton dust estimates, surveys of textile job-specific endotoxins exposure (Christiani et al. 1993; Christiani et al. 1999) and measurements collected by University of Washington at three factories in Shanghai. All analyses were adjusted for age as a continuous variable and smoking status (ever/never) ascertained at baseline. Only modest non-significant decreasing trends in risk of oesophageal cancer were observed with increasing levels of exposure to both cotton dust and endotoxins. Regarding the risk of stomach cancer, statistically increasing trends were observed with increasing levels of exposure to endotoxins. However, the magnitude of the effect was small and only borderline significant for the most exposed quartile (HR = 1.2; 95% CI: 1.0 - 1.5) (Gallagher et al. 2015).

Reul *et al*. studied associations between pancreatic cancer and occupational exposures, including endotoxins, in a follow-up study of a sub-cohort of the Shanghai female textile workers (N = 481 pancreatic cancer cases, n = 3191 non-cases among the cohort). Cotton dust concentration measurements gathered by local industrial hygienists from 1975-1999 in 56 factories were assembled (Reul et al. 2016). Endotoxins data generated by investigators from the University of Washington and Harvard University was used to correlate cotton dust concentration to endotoxins concentration (Reul et al. 2016). In a prior case-cohort study of occupational risk factors for pancreatic cancer nested within the Shanghai textile workers, an inverse exposure-response trend for endotoxins exposure was observed (Li et al. 2006). In this analysis however, no evidence of a relationship between endotoxins exposure and pancreatic cancer was found, irrespective of the degree to which recent endotoxins exposure was discounted through lagged analysis.

5 Inventory of existing occupational exposure limits

In Europe, there is no recommendation of an occupational exposure limit (OEL) for endotoxins from the Scientific Committee on Occupational Exposure Limits (SCOEL) or the Risk Assessment Committee (RAC) of the European Chemical Agency (ECHA) available to date.

To date, only one health-based OEL scientific recommendation for endotoxins from DECOS of the Health Council of the Netherlands could be identified. In 2010, this committee recommended a health-based occupational exposure limit (HBROEL) (an 8 hr time-weighted average value) for endotoxins of 90 EU/m^3 (9 ng/m^3) to protect against acute, short-term and chronic effects (Health Council of the Netherlands 2010). The report released in 2010 is an update of a previous report from 1998 (Health Council of the Netherlands 1998). In 2010, DECOS based its value on three studies:

- Castellan *et al.* (1987): using linear regression modeling on the observed exposure-response relationship, showed that a zero % fall in FEV1 for a 6-hr exposure would occur at a level of 90 EU/m^3 (9 ng/m^3);
- Smid *et al.* (1992): in a cross-sectional study of chronic effects on lung function in animal feed workers, the authors, using regression models estimated that 40 years of exposure to 150 EU/m^3 (15 ng/m^3) could result in a decrease in FEV1 of approximately 200 mL (equivalent to approximately 5% FEV1);
- Post *et al.* (1998): in a 5-year follow-up study on the same workers as in the Smid *et al.* study, a FEV1 decrease of 0.326 mL (SE = 0.139) per 10 EU/m^3 (or 1 ng/m^3 endotoxins) per year of exposure was calculated.

In the 1998 report, No Effect Levels (NELs)[9] for inhalatory endotoxins exposure ranging from approximately 90 -1 800 EU/m^3 (9 to 180 ng/m^3) were calculated based mainly on experimental endotoxins exposure studies (Health Council of the Netherlands 1998). Calculated NELs for chronic and acute respiratory effects based on epidemiological studies in occupationally exposed populations are comparable. A starting point of 90 EU/m^3 (9 ng/m^3) was considered for the establishment of an 8 hr health-based recommended OEL, based on acute respiratory effects obtained from the large and well designed (according to the Health Council of the Netherlands) experimental exposure study by Castellan *et al*. from 1987, in which non-symptomatic subjects from the general population (smoking and non-smoking) were exposed to endotoxins contaminated cotton dust (Castellan et al. 1987). A safety factor of 2 was applied to the 90 EU/m^3 NOEL calculated by the authors, in order to compensate for increased risks for certain groups of workers and also taking into account that endotoxins may have chronic pulmonary effects at levels which may be lower than for acute respiratory effects. The resulting value was rounded to 50 EU/m^3. An OEL of 50 EU/m^3 (5 ng/m^3) measured as an 8h-TWA was recommended by the Committee (Health Council of the Netherlands 1998).

In 2010, the DECOS published an updated report with evidence from human volunteers and workplace studies published prior to January 2010 and having measured immunological responses

[9] Insofar as DECOS does not consider the reduction in FEV1 as an adverse effect, they describe a NEL in the 1998 report then a NOEL in the 2010 report.

due to endotoxins in biological samples such as blood, sputum, or broncho-alveolar or nasal lavage fluid. The 8h-OEL of 90 EU/m^3 (9 ng/m^3) proposed in this report is still based predominantly on evidence from the study of Castellan *et al*. (Castellan et al. 1987). In this study, volunteers having consistent ventilatory responses to the screening exposures, with falls in FEV1 between 5 - 30%, were accepted for the main study and exposed to airborne endotoxins exposures ranging from 60 – 7 790 EU/m^3 (6 to 779 ng/m^3) for 6-hr periods. One hundred and eight different dust exposure sessions were performed over a 20-months period.

The authors found the following exposure-response relation between the group mean ΔFEV1 (as determined from pre and post-exposure spirometry) and the endotoxins airborne concentration: % ΔFEV1 = 3.84 - 4.02 x (^{10}log endotoxin (ng/m^3)); r = 0.85, p < 0.0001. Another 66 sessions of exposure of the same subjects to clean air resulted in a mean ΔFEV1 of ± 0%.

Using linear regression modeling on the observed exposure-response relationship, the authors calculated that a zero % fall in FEV1 for a 6-hr exposure would occur at a level of 90 EU/m^3 (9 ng/m^3).

This level of exposure to endotoxins, thus being considered as a safe level for preventing acute workplace effect, DECOS considered additional studies to assess the effects after long-term endotoxins exposure, i.e. how this level would translate into lung function loss over a 40-year working life. Two studies involving the same cohort of animal feed workers (exposed to grain) in the Netherlands were considered : Smid *et al.*, a cross-sectional study on 315 subjects working in 14 animal feed mills (Smid et al. 1992) and Post *et al.*, a 5-year follow-up study of 140 of these workers (Post, Heederik, and Houba 1998). Smid *et al.* calculated a value of 0.34 mL FEV1 decline per year-ng/m^3 and Post *et al.* found an annual FEV1 decline of 0.33 ± 0.14 mL (standard error) per 10 EU/m^3 (or 1 ng/m^3) endotoxins exposure, confirming the Smid *et al.* dose-response relationship between annual decline in FEV1 and endotoxins exposure. Based on this correlation, 40 years of endotoxins exposure at 90 EU/m^3 (9 ng/m^3) would equate on average to an extra 120 mL loss of FEV1 (in addition to the physiological decrease of FEV1 with age).

This additional FEV1 loss of 120 mL after 40 years of exposure to endotoxins was not considered an adverse effect by the DECOS. This statement is substantiated by Sin *et al.*, the review part of the paper suggesting that an additional FEV1 loss of 200 - 300 mL is not related with cardiovascular or other health effects (Sin et al. 2005). Consequently, a temporal adjustment of the identified NOEL of 90 EU/m^3 (9 ng/m^3) identified in volunteers after a 6-hr exposure was not deemed necessary to afford adequate protection against the effects of prolonged exposure to endotoxins.

This is in keeping with estimates from another study, where a predicted excess fall of 200 mL of FEV1 was calculated for a 40-year working exposure at 150 EU/m^3 (15 ng/m^3) (Smid 1993). To put this into context, DECOS estimate would represent an excess decline of around one tenth of the normal age-related FEV1 decline over that period of approximately 1 000-1 200 mL.

No extrapolation factor was applied to the selected NOEL to take into account individual sensitivity variations. Indeed, DECOS considered the exposed volunteers in Castellan *et al*. as a sensitive group, as these were pre-selected on the basis of their sensitivity to endotoxins. Thus, the DECOS health-based recommended OEL for both acute and chronic endotoxins exposure is 90 EU/m^3 (9 ng/m^3) (8-hr TWA) (Health Council of the Netherlands 2010).

DECOS states that an exposure limit of 90 EU/m^3 (9 ng/m^3) over an 8-hr period (as opposed to 6 hr in Castellan *et al*.) should therefore protect all workers, as the study volunteers were pre-selected as being sensitive to endotoxins. This assumption is however likely to be limited, as Castellan *et al*. screened out the most sensitive individuals from participating in the study (Castellan et al. 1987). Any volunteer who had an acute fall in FEV1 of over 30% during the pre-screening exposure to 1 000 EU/m^3 (100 ng/m^3) was considered to be ineligible due to safety concerns. Given the additional exclusion of volunteers with pre-existing respiratory conditions, the evidence-base for the exposure

limit is also not applicable to those with asthma or chronic obstructive pulmonary disease (COPD). The final limitation to consider is that Castellan *et al*. noted that the level of FEV1 response might have been higher if individuals had been asked to exercise during the exposures, as would expected to be the case in the cotton industry. The basis for the DECOS NOEL is therefore based on experimental acute inhalation challenge tests on volunteers rather than data from workplace studies (Health Council of the Netherlands 2010).

Table 4. Summary table of the Dutch recommended OEL

Type of OEL		**HBROEL[10] for both chronic and short-term (8h-TWA and 15min-STEL) exposure to inhalable endotoxins**
RV	Organism	DECOS
	Year	2010
	Value	90 EU/m^3 (= 9 ng/m^3)
Target population		Workers
Critical Effect		FEV1 diminution
Key study	Reference	Castellan *et al*. (1987)
	Population of the study or species	Healthy volunteers
	Exposure (time, route)	108 sessions of 6 hours, inhalation
Supported studies	Reference	Smid *et al.* (1992) ; Post *et al.* (1998)
	Population of the study or species	Animal feed industry workers
	Exposure (time, route)	8 hours, inhalation
Point of departure (POD)*		90 EU/m^3 (= 9 ng/m^3)*
Time Adjustement		No
Dosimetric Adjustement		No
Uncertainty Factors (UF)		No
Notations (skin, noise)**		No

* According to the dose-response relationships calculated by linear regression in the Smid *et al*. (1992) and Post *et al*. (1998) studies, exposure to 90 EU/m^3 (9 ng/m^3) of endotoxins for 40 years would lead to a 120 mL reduction in FEV1 (in addition to the physiological decrease of FEV1 with age), which is not considered an adverse effect by DECOS.

6 Construction of the OELs

6.1 Choice of the critical effect

Available human studies show acute and chronic toxic effects after exposure to endotoxins. Studies on the association with the occurrence of cancer presented no significant relationship with non-Hodgkin's lymphoma (Wang et al. 2013), esophageal cancer (Gallagher et al. 2015) or pancreatic cancer (Reul et al. 2016). A small, borderline-significant association was identified for stomach cancer (Gallagher et al. 2015). The studies showed no significant neurotoxic effect associated with endotoxins (van der Mark et al. 2014; Checkoway et al. 2018; Visser et al. 2019). Regarding

[10] Health-based recommended exposure limit

cardiovascular effects, no significant associations were found with cardiovascular mortality (Gallagher et al. 2017), microvascular function (Karottki et al. 2014) or blood markers of platelet activation (Straumfors et al. 2018).

Therefore, the lung appears to be the main target organ after endotoxins exposure. Studies showed pulmonary function impairment such as:

- decrease in FEV1 (Haglind and Rylander 1984; Rylander et al. 1985; Castellan et al. 1987; Smid et al. 1992; Post et al. 1998; Kirychuk et al. 1998; Vogelzang et al. 1998; Zock et al. 1998; Kline et al. 1999; Donham et al. 2000; Wang, et al. 2005; Shi et al. 2010; Cyprowski et al. 2015);
- decrease in FVC (Milton et al. 1995; 1996; Vogelzang et al. 1998; Mitchell et al. 2015; Ghani et al. 2016);
- onset of respiratory symptoms reported by questionnaires such as chronic bronchitis (Shi et al. 2010; Basinas et al. 2012), chronic cough (Shi et al. 2010), wheezing (Lim et al. 2019) or rhinoconjonctivis (Lim et al. 2019);
- Organic dust toxic syndrome (ODTS), a non-infectious influenza-like illness that occurs after exposure by inhalation of organic dust contaminated with microorganisms (e.g., Gram-negative bacteria and fungi) (Boehmer et al. 2009; Smit et al. 2005; Smit et al. 2006; Basinas et al. 2012).

The only well-documented effects associated with acute and chronic exposure to endotoxins are altered respiratory flow and ODTS. Numerous studies have been carried out to characterize the relationship between endotoxins exposure and reduced respiratory parameters. With regard to ODTS, symptoms were reported by self-questionnaire and showed great inter-individual (and intra-individual) variability in subject sensitivity. According to published articles, specifically their methodology and the variability of ODTS occurrence, HRV Committee assumed that ODTS can not be used as the critical effect for the derivation of OEL for endotoxins. On the other hand, studies of FVC show no strong associations between endotoxins exposure and reduced FVC. Based on all available studies, the effect observed at the lowest concentrations is the decrease of FEV1.

As a decrease of at least 1% in FEV1 on a collective scale can be considered as an adverse effect, the HRV Committee retained the 1% decrease of FEV1 as critical effect for the derivation of 15min-STEL and 8h-OEL.

For most non-carcinogenic effects, it is considered by default and in the current state of knowledge that toxicity is only expressed above a dose threshold (Anses, to be published). Therefore, this critical effect is considered to be the result of a threshold mechanism.

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

6.2.1 Choice of the key study

After acute exposure to endotoxins, some studies suggest dose-response relationships between the decrease in FEV1 and exposure to endotoxins (Haglind and Rylander 1984; Rylander et al. 1985; Donham et al. 1989; Donham et al. 2000). However, these studies have limitations, such as poorly controlled endotoxins exposure conditions, particularly in cross-sectional studies (Donham et al. 1989; Donham et al. 2000), high exposure to endotoxins (Haglind and Rylander 1984; Rylander et

al. 1985), non-significant dose-response relationships (Haglind and Rylander 1984) and the non consideration of co-exposure to other contaminants that may cause respiratory effects (Rylander et al. 1985; Donham et al. 1989).

Among all the studies establishing dose response relationships on the 1% decrease of FEV1 after acute exposure to endotoxins, the study of Castellan *et al.* was identified as the most relevant and robust study for the derivation of the 15min-STEL (Castellan et al. 1987), as:

- volunteers (24-35 subjects) were exposed to cotton dust during 6 hr, with airborne endotoxins concentrations ranging from 60 – 7 790 EU/m^3 (6 to 779 ng/m^3) in 108 different exposure sessions;
- the concentration of endotoxins exposure was very well controlled: constant dust concentrations were maintained during the exposure sessions by adjusting the proportion of dust-contaminated exhaust air from the cardroom that entered the exposure room. Airborne dust concentrations were monitored with a continuous aerosol monitoring system and with approximately hourly mean gravimetric measurements of the inhalable fraction of airborne dust, as collected by four vertical elutriators, one in each quadrant in the room. All facilities were cleaned after each exposure;
- the population of volunteers excluded subjects suffering from asthma, chronic bronchitis and exertional breathlessness but included a sensitive population with response of FEV1 decrease of at least 5% and not more than 30% to pre-test exposure to 1 000 EU/m^3 (100 ng/m^3) LPS.

The 1987 study of Castellan *et al*. is selected by the HRV Committee as key study for the establishment of a 15min-STEL.

6.2.2 Identification of the point of departure (PoD)

In the study of Castellan *et al*., the authors calculated the zero percentage change in FEV1 during exposure to endotoxins to be 90 EU/m^3 (9 ng/m^3) using linear regression modeling (Castellan et al. 1987).

The HRV Committee retained the 90 EU/m^3 (9 ng/m^3) dose as a NOAEL for the 15min-STEL construction.

6.2.3 Application of uncertainty factors

The calculation of the 15min-STEL from the selected NOAEL was performed using the following uncertainty factors (FI) (Anses, to be published):

- Inter-species variability (UF_A)

Application of an UF_A of 1 as the key study used to establish the 15min-STEL is based on a human population.

- Inter-individual variability (UF_H)

Even though, key study selected sensitive subjects, subjects suffering from asthma, chronic bronchitis and exertional breathlessness, as well as volunteers with a more than 30% FEV1 decrease in the screening test were excluded. As a result, an UF_H of 5 is retained in accordance with the methodology.

- PoD used (BMDL, LOAEL or NOAEL) (UF_L)

Application of an UF_L of 1 since the PoD retained for the derivation of the 15min-STEL is a NOAEL.

- Quality of the database (UF_D)

Application of an UF_D of 1 given that the effects on FEV1, FVC and Organic Dust Toxic Syndrome (ODTS) reduction are well documented in the scientific literature.

The HRV Committee applies an overall uncertainty factor of 5 leading to a calculated 15min-STEL of 90 /5 EU/m^3, ie 18 EU/m^3 (1.8 ng/m^3) rounded up to 20 EU/m^3 (2 ng/m^3).

Therefore, the HRV Committee recommends a 15min-STEL of 20 EU/m^3 (2 ng/m^3)[11] to prevent a 1% decrease in FEV1. However, compliance with the recommended limit value does not guarantee that the toxic syndrome of organic dusts will not occur.

Insofar as monitoring a 15min-STEL involves measuring exposure peaks, and this can sometimes prove difficult to control over the duration of an 8 hr work shift, the experts expressed the need to recommend, in addition to the 15 min-STEL, a limit value not to be exceeded over an 8 hr duration. This need is reinforced in the case of endotoxins by the fact that a reduction in FEV1 during a single shift is predictive of a long-term FEV1 reduction. Indeed, Kirychuk *et al.* showed that the baseline across-shift change in FEV1 was a significant predictor of annual rate change in FEV1 (Kirychuk et al. 1998). In addition, Christiani *et al.* concluded about a significant correlation between chronic loss of pulmonary function and length of exposure (years worked in cotton mills) (Christiani et al. 2001). These functional losses were significantly associated with cumulative endotoxins exposure. The workers with a higher level of cumulative endotoxins exposure had significantly greater loss of FEV1 than did those with a low level (Christiani et al. 2001).

6.3 Establishment of an 8 hours occupational exposure level (8-hour OEL)

6.3.1 Choice of the key studies and identification of the point of departure (PoD)

Whatever the type (cross-sectional or longitudinal) of the epidemiological studies published, the characterization of a dose-response association between endotoxins exposure and FEV1 decrease is made difficult by co-exposures to other dust components (particulate matter, fungi, mites) and sometimes other toxic agents (ammonia, H_2S, aldehydes, etc.), these confounding factors being generally not taken into account in the analyses.

Furthermore, none of the available longitudinal studies can be used to derive an 8h-OEL because significant dose-response relationship or association between chronic endotoxins exposure level and decrease in FEV1 were lacking.

[11] Insofar as the 1987 study by Castellan was based on measurements of the inhalable fraction, the measurement of the inhalable fraction is considered for the recommendation of the 15min-STEL.

Available cross-sectional studies reporting respiratory effects after long-term exposure present additional limitations for the derivation of an 8h-OEL as they do not allow to differentiate between chronic and acute effects.

Therefore, the HRV Committee decided to retained 90 EU/m^3 (9 ng/m^3) corresponding to the NOAEL identified by Castellan *et al*. after 6 hr of exposure for the derivation of an 8h-OEL as PoD insofar as:

- two studies involving the same cohort of animal feed workers exposed to grain dust in the Netherlands (Smid *et al.* 1992; Post *et al.* 1998) enable to assess how the PoD of 90 EU/m^3 (9 ng/m^3) identified by the Castellan study would affect lung function over a 40-year working life. The two consistent regression equations identified in these studies showed that 40 years of endotoxins exposure at 90 EU/m^3 (9 ng/m^3) would result in an additional 120 mL loss of FEV1, on top of the physiological decrease in FEV1 with age. Compared with an average natural loss of FEV1 of 30 mL/year from the age of 30 in healthy subjects (Guénard and Rouatbi 2004; Lowery et al. 2013), an additional loss of 120 mL after 40 years of exposure does not appear to cause individual health effects. Therefore, the HRV Committee in accordance with DECOS considers that an additional 120 mL reduction in FEV1 after 40 years of endotoxins exposure should not be regarded as an adverse health effect (Health Council of the Netherlands 2010);
- pooled analysis of 4 cohort studies in several countries and several industries identifies a LOAEL of 100 EU/m^3 (10 ng/m^3) for the risk of chronic bronchitis (self-reported as the persistence of cough and sputum for at least 3 months, during the 12 months preceding the administration of a questionnaire) (Basinas et al. 2012). Despite the subjective statement of health effect, this LOAEL close to the NOAEL (90 EU/m^3 (9 ng/m^3)) is a further argument for retaining the same value as a NOAEL for chronic effects;
- the study of Kennedy *et al*. reported that even when there was a decrease of FEV1 over the course of a shift, there was no association of FEV1 decrease with duration of exposure or cumulative exposure. Acute effects on respiratory flow would therefore be detectable before the onset of chronic effects (Kennedy et al. 1987).

Based on all these elements, the HRV Committee retained the 90 EU/m^3 (9 ng/m^3) concentration as a NOAEL for the 8h-OEL derivation.

6.3.2 Application of uncertainty factors

The calculation of the 8h-OEL from the selected NOAEL of 90 EU/m^3 (9 ng/m^3) was performed using the following uncertainty factors (FI) (Anses, to be published):

- Inter-species variability (UF_A)

Application of an UF_A of 1 as the key study used to establish the 8h-OEL is based on a human population.

- Inter-individual variability (UF_H)

Even though, key study selected sensitive subjects, subjects suffering from asthma, chronic bronchitis and exertional breathlessness, as well as volunteers with a more than 30% FEV1 decrease in the screening test were excluded. As a result, an UF_H of 5 is retained in accordance with the methodology.

- PoD used (BMDL, LOAEL or NOAEL) (UF_L)

Application of an UF_L of 1 since the PoD retained for the derivation of the 8h-OEL is a NOAEL.

- Transposition for medium-term to long-term(UF_S)

Some studies showed that reduction in FEV1 during a single shift is predictive of long-term FEV1 reduction (Kirychuk et al. 1998; Christiani et al. 2001) and that acute effects on respiratory flow were detectable before the onset of effects (Kennedy et al. 1987). Moreover, the PoD in Castellan's study is close to the LOAEL identified for ODTS (Basinas et al. 2012) and has been studied for the effect of long-term exposure (Smid et al. 1992; Post et al. 1998).

Insofar as the key study of Castellan *et al.* was also supported by chronic studies (Smid et al. 1992; Post et al. 1998; Kennedy et al. 1987; Kirychuk et al. 1998; Christiani et al. 2001), an UF_S of 1 is retained.

- Quality of the database (UF_D)

Application of an UFD of 1 given that the effects on FEV1, FVC and Organic Dust Toxic Syndrome (ODTS) reduction are well documented in the scientific literature.

The HRV Committee applies an overall uncertainty factor of 5 leading to a calculated 8h-OEL of 90 /5 EU/m^3, ie 18 EU/m^3 (1.8 ng/m^3) rounded up to 20 EU/m^3 (2 ng/m^3).

Therefore, the HRV Committee recommends an 8h-OEL of 20 EU/m^3 (2 ng/m^3)[12] to prevent a 1% decrease in FEV1 for long term exposure to endotoxins. However, compliance with the recommended limit value does not guarantee that the toxic syndrome of organic dusts will not occur.

"Skin" notation

In the absence of quantitative data on dermal absorption, **no "skin" notation is recommended for endotoxins.**

"Noise" notation

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

[12] Insofar as the 1987 study by Castellan was based on measurements of the inhalable fraction, the measurement of the inhalable fraction is considered for the recommendation of the 8h-OEL.

7 Conclusions of the collective expert appraisal

Table 5. Summary table of recommended occupational threshold OELs

Type of OEL		**15min-STEL**	**8h-OEL**
RV	Organism	Anses	Anses
	Year	2024	2024
	Value	20 EU/m^3 (= 2 ng/m^3) (inhalable fraction)	20 EU/m^3 (= 2 ng/m^3) (inhalable fraction)
Target population		Workers	Workers
Critical Effect		1% FEV1 diminution	1% FEV1 diminution
Key study	Reference	Castellan *et al*. (1987)	Castellan *et al*. (1987)
	Population of the study or species	Healthy volunteers	Healthy volunteers
	Exposure (time, route)	108 sessions of 6 hours, inhalation	108 sessions of 6 hours, inhalation
Support studies	Reference	/	Smid *et al*. (1992) ; Post *et al*. (1998) ; Basinas *et al*. (2012) ; Kennedy *et al*. (1987) ; Kirychuk *et al*. (1998) ; Christiani *et al*. (2001)
Point of departure (POD)		NOAEL = 90 EU/m^3 (= 9 ng/m^3)	NOAEL = 90 EU/m^3 (= 9 ng/m^3)
Time Adjustement		No	No
Dosimetric Adjustement		No	No
Uncertainty Factors (UF)		UF_H = 5	UF_H = 5
Notations (skin, noise)		No	No

Insofar as the recommended value does not guarantee protection against the occurrence of ODTS, the HRV Committee recommends lowering endotoxins exposure levels to the lowest levels technically achievable.

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Annex 1: Literature search

To identify relevant references, the lexical query begins with the identification of keywords and the formalisation of a search strategy (e.g. period, scope, search equation, database, etc.).

Table 6. Keywords used for literature request

Thematic	MeSH Category	Key words		
Population	"Occupational Groups"	work* farmer	employee personnel	
Exposition	"Inhalation Exposure" "Occupational Exposure" "Air Pollutants, Occupational"	inhal* respirable airborne	aerosol dust mist	
Comparator		endotoxin lps lipopolysaccharide	“lipid A” “o antigen”	
Outcome	"Respiratory Tract Diseases" "Occupational Diseases" "Pathological Conditions, Signs and Symptoms"	disease toxic* “health effect*”	“adverse effect*” symptom ODTS	fever syndrome
Temporality		> 2010		

Study question: Identification of the toxicological studies performed with endotoxins following inhalation

The goal of this review was to derive occupational exposure limit values for endotoxins by inhalation.

Description of the review method

The literature search was conducted in the Pubmed and SCOPUS databases.

PUBMED

(("Respiratory Tract Diseases"[MeSH Terms] OR "Occupational Diseases"[MeSH Terms] OR "pathological conditions, signs and symptoms"[MeSH Terms] OR "disease"[Title/Abstract] OR "toxic*"[Title/Abstract] OR "health effect*"[Title/Abstract] OR "adverse effect*"[Title/Abstract] OR "symptom"[Title/Abstract] OR "ODTS"[Title/Abstract] OR "syndrome"[Title/Abstract] OR "fever"[Title/Abstract] OR "disorder"[Title/Abstract]) AND ("Inhalation Exposure"[MeSH Terms] OR "Occupational Exposure"[MeSH Terms] OR "inhal*"[Title/Abstract] OR "respirable"[Title/Abstract] OR "aerosol"[Title/Abstract] OR "airborne"[Title/Abstract] OR "dust"[Title/Abstract] OR "mist"[Title/Abstract]) AND ("endotoxins"[MeSH Terms] OR "endotoxin"[Title/Abstract] OR "lps"[Title/Abstract] OR "lipopolysaccharide"[Title/Abstract] OR "lipid A"[Title/Abstract] OR "o antigen"[Title/Abstract]) AND ("occupational groups"[MeSH Terms] OR "work*"[Title/Abstract] OR "farmers"[Title/Abstract] OR "employee"[Title/Abstract] OR "personnel"[Title/Abstract])) AND ((english[Filter] OR french[Filter] OR german[Filter]) AND (2010:2023[pdat]))

SCOPUS

(TITLE-ABS-KEY ("work*" OR farmers OR "employee" OR personnel)) AND (TITLE-ABS-KEY ("Occupational exposure" OR "respiratory exposure" OR inhal* OR respirable OR *aerosol OR airborne OR dust OR mist)) AND (TITLE-ABS-KEY (endotoxin OR lps OR lipopolysaccharide OR "lipid A" OR "o antigen")) AND (TITLE-ABS-KEY ("Respiratory" OR "disease" OR "toxic*" OR "health effect*" OR "adverse effect*" OR "symptom*" OR "ODTS" OR "syndrome" OR "fever" OR "disorder" OR "lung" OR "pulmonary" OR "inflammat*" OR "immun*")) AND (LIMIT-TO (LANGUAGE , "English") OR LIMIT-TO (LANGUAGE , "German") OR LIMIT-TO (LANGUAGE , "French")) AND (LIMIT-TO (PUBYEAR , 2021) OR LIMIT-TO (PUBYEAR , 2020) OR LIMIT-TO (PUBYEAR , 2019) OR LIMIT-TO (PUBYEAR , 2018) OR LIMIT-TO (PUBYEAR , 2017) OR LIMIT-TO (PUBYEAR , 2016) OR LIMIT-TO (PUBYEAR , 2015) OR LIMIT-TO (PUBYEAR , 2014) OR LIMIT-TO (PUBYEAR , 2013) OR LIMIT-TO (PUBYEAR , 2012) OR LIMIT-TO (PUBYEAR , 2011) OR LIMIT-TO (PUBYEAR , 2010)) AND (LIMIT-TO (EXACTKEYWORD , "Human") OR LIMIT-TO (EXACTKEYWORD , "Humans"))

In total, 359 publications (after duplicate removal) were identified based on a combined search in PubMed and Scopus databases. 110 references (toxicological / research reports, journal / articles, book section) were retained based on titles and abstracts. See the following diagram (Figure 3)

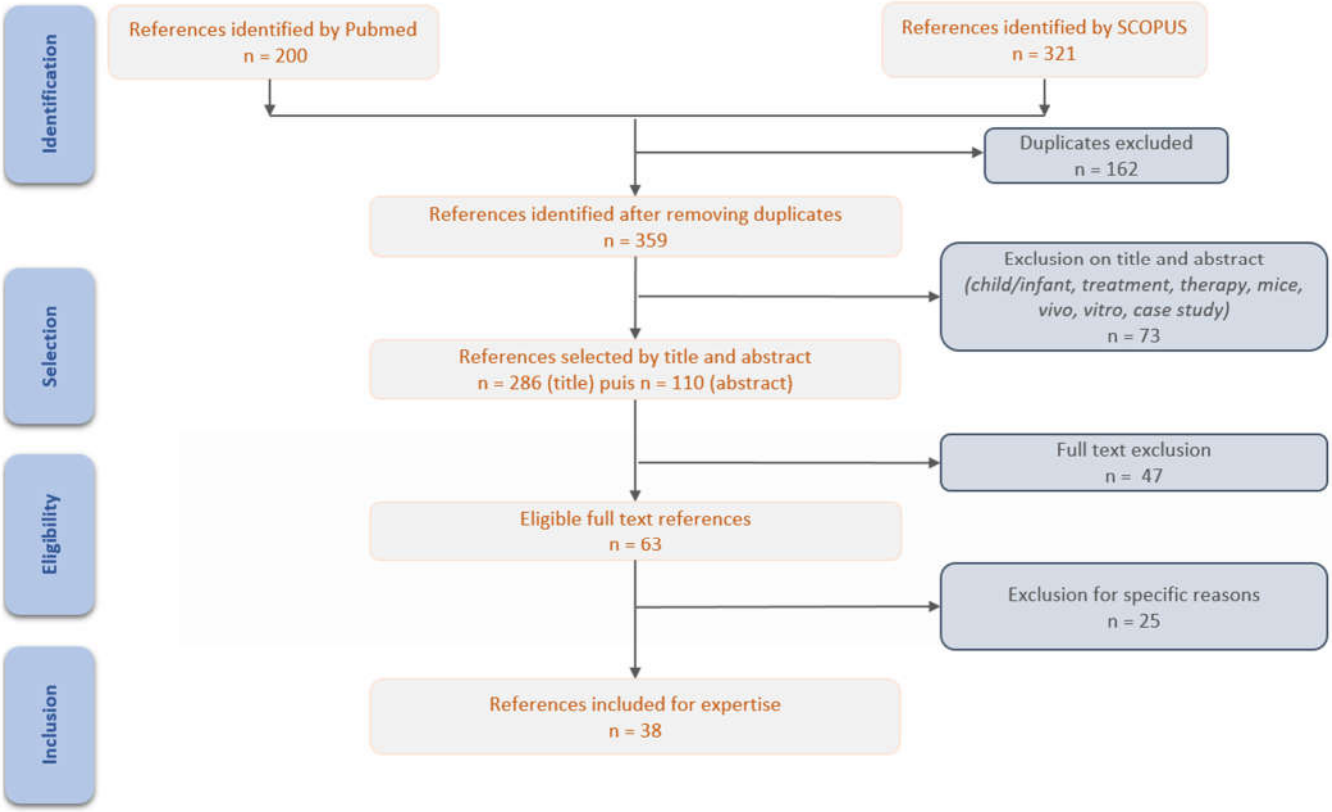

Figure 3 Diagram PRISM

Annex 2: Studies reporting biological responses described in the Health Council of the Netherlands report of 2010

Table 7. Biological effects caused by single-dose endotoxins in healthy volunteers (Health Council of the Netherlands 2010).

Exposure		Effects	Measured after	N	NOEL	Ref.
mg/m³	EU/m³					
100	1,000,000	PMN ↑	3 h	8	No	Thorn 2001
40	400,000	sputum: PMN ↑, ECP ↑, MP0 ↑ blood: PMN ↑, MP0 ↑, FEV1 2% ↓	24 h	21	No	Thorn and Rylander 1998
0.5	5,000	PMN ↓	6 h	9	No	Michel et al. 1997
5	50,000	blood: PMN ↑, CRP ↑ sputum: PMN ↑, MPO ↑, monocytes ↑				
50	500,000	sputum: lymphocytes ↑, TNFα ↑, ECP ↑				
0.1	1,000	-	4-24 h	16[a]	No	Peden et al. 1999
0.3	3,000	-				
1.0	10,000	NAL: eosinophils ↑ (only in atopics)				
5.4	54,000	BAL: total cells, TNF-α, IL-1β, IL-6 and IL-8				Jagielo et al. 1996
36	360,000	FEV1 ↓	4 h	14	No	

CRP = C-reactive protein (acute phase protein); ECP = eosinophilic cationic protein; IL = interleukin; MPO = myeloperoxidase; NAL = nasal lavage; PMN = neutrophils (polymorphonuclear leukocytes); TNFα= tumor necrosis factor alpha.

[a] of which 10 atopic subjects

Table 8. One-week epidemiological studies of biological effects (Health Council of the Netherlands 2010).

Ref	Study design	Control	Work history	Endotoxin exposure; mean (range)	Parameters measured	Effects measured
Fishwick et al. 2002	4 days follow-up of cotton workers, n=25	scientists n=9	>8 years	1-400 EU/m³ (0.1-40 ng/m³)	CD14 on monocytes in blood	CD14 ↑ at the end of first day of the week, but back to normal at the end of the week
Wouters et al. 2002	one-week follow-up of domestic waste collectors n=47	office workers n=15	5 years	GM = 39 (4-7,182) EU/m³ (3.9 ng/m³, range 0.4-718 ng/m³)	cells, IL-6, IL-8, IL-1β, TNFα in NAL; IgE in serum	IL-8 ↑ (1.8x) and cells ↑ (3.3) in NAL at the end of the week
Heldal et al. 2003	4 days follow-up of waste handlers n=31	No	1.5 years	MD = 13 (4-183) EU/m³ (1.3 ng/m³, range 0.4-18.3 ng/m³)	MPO, ECP, IL-8 and cell diff. in NAL	ECP ↑ (1.8x) and %PMN ↑ (1.6x) in NAL at the end of the week

CD14 = CD14 receptor; ECP = eosinophilic cationic protein; IL = interleukin; MPO = myeloperoxidase; NAL = nasal lavage fluid; %PMN = % neutrophils (polymorphonuclear leukocytes) of total cells; TNFα= tumor necrosis factor alpha.

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