Expert appraisal on recommending occupational exposure limits for chemical agents

Assessment of health effects for
titanium dioxide under nanoform ($nTiO_2$)
CAS N° 13463-67-7

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

Evaluation des effets sur la santé
Dioxyde de titane sous forme nanométrique ($nTiO_2$)
CAS N° 13463-67-7

OEL Permanent Mission/Mission permanente VLEP
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Collective expert appraisal
RAPPORT d'expertise collective

Expert Committee on « health reference values »

Comité d'experts spécialisé « Valeurs Sanitaires de Référence »

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Mots clés

VLEP, valeurs limites, niveaux d'exposition, milieu professionnel, agents chimiques, effets sur la santé, lieux de travail, valeur de référence, dioxyde de titane, TiO_2, nanométrique, nanoparticule, nanomatériau.

Key words

OEL, limit values, exposure levels, occupational, chemical agents, health effects, workplaces, reference value, titanium dioxide, TiO_2, nanometric, nanoparticle, nanomaterial.

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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portant sur l'évaluation des effets sur la santé sur le lieu de travail pour le dioxyde de titane sous forme nanométrique (CAS n°13463-67-7)

Ce document synthétise les travaux du comité d'experts spécialisé « Valeurs Sanitaires de Référence » (CES VSR)

Présentation de la question posée

En 2015, l'Anses a porté auprès de l'Agence européenne des produits chimiques (ECHA) une proposition de classification de la cancérogénicité par inhalation du TiO_2 (cancérogène de catégorie 1B) dans le cadre du règlement européen (CLP) n° 1272/2008 relatif à la classification, l'étiquetage et l'emballage des substances et des mélanges dangereux. En 2017, le comité d'évaluation des risques (RAC) de l'ECHA a conclu que le TiO_2 sous toutes ses formes devrait être classé comme cancérogène suspecté pour l'Homme (catégorie 2) par inhalation.

L'Anses a ainsi été saisie par la DGS, la DGPR et la DGT le 4 juillet 2017 pour définir une VTR chronique par inhalation pour le dioxyde de titane sous forme nanométrique. Cette demande résulte selon les termes de la saisine de « *l'analyse de la base de données R-Nano indiquant que de nombreux sites industriels en France utilisent du dioxyde de titane sous forme nanométrique. Ces manipulations peuvent être à l'origine d'exposition des travailleurs mais également d'exposition des populations via des émissions à l'extérieur des sites* ». La saisine relève que « *le Centre international de recherche sur le cancer (CIRC) a classé le dioxyde de titane sous forme de particules respirables en cancérogène possible par inhalation* ».

Un avis a été publié par l'Anses en avril 2019 définissant une VTR de 0,12 µg/m^3 applicable uniquement à la forme P25[1] du TiO_2. Le niveau de confiance de cette VTR a été qualifié de « modéré ». Suite à cet avis, et conformément au protocole d'accord relatif aux valeurs limites d'exposition professionnelle et valeurs limites biologiques (VLEP et VLB) établi entre le ministère du travail et l'Anses, l'Anses a lancé les travaux pour l'élaboration de VLEP.

Par ailleurs, dans le cadre du règlement REACh, l'Anses instruit actuellement un dossier d'évaluation des dangers et des risques du TiO_2 pour la santé humaine et pour l'environnement. Dans le cadre de l'instruction de ce dossier, des données supplémentaires sur les dangers, les usages du TiO_2 pourront être requises par l'Anses auprès des industriels.

[1] P25 : Anatase (80%) and rutile (20%); Taille de particules primaires : 21 nm; Aire de surface spécifique : 48.08 m^2/g

Contexte scientifique

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

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

L'organisation de la phase d'expertise scientifique nécessaire à la fixation des valeurs limites d'exposition professionnelle (VLEP) a été confiée à l'Afsset dans le cadre du plan santé au travail 2005-2009 (PST), puis à l'Anses suite à la fusion de l'Afsset et de l'Afssa en 2010.

Les VLEP telles que recommandées par le CES « expertise en vue de la fixation de valeurs limites à des agents chimiques en milieu professionnel », sont des niveaux de concentration en polluants dans l'atmosphère des lieux de travail à ne pas dépasser sur une période de référence déterminée et en deçà desquels le risque d'altération de la santé est négligeable. Même si des modifications physiologiques réversibles sont parfois tolérées, aucune atteinte organique ou fonctionnelle de caractère irréversible ou prolongée n'est admise à ce niveau d'exposition pour la grande majorité des travailleurs. Ces niveaux de concentration sont déterminés en considérant que la population exposée (les travailleurs) est une population qui ne comprend ni enfants ni personnes âgées.

Ces niveaux de concentrations sont déterminés par les experts du CES à partir des informations disponibles dans des études épidémiologiques, cliniques, de toxicologie animale, etc. L'identification de ces concentrations sécuritaires pour la santé humaine nécessitent généralement d'appliquer des facteurs d'ajustement aux valeurs identifiées directement par les études. Ces facteurs permettent de prendre en compte un certain nombre d'éléments d'incertitude inhérents à la démarche d'extrapolation conduite dans le cadre d'une évaluation des effets sanitaires des substances chimiques sur l'Homme.

Trois types de valeurs sont recommandées par le CES :

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

Ces trois types de valeurs sont exprimés :

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

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

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

En plus des VLEP, le CES évalue la nécessité d'attribuer ou non une mention « peau », lorsqu'une pénétration cutanée significative a été identifiée (Anses, 2017). Cette mention indique la nécessité de prendre en compte la voie d'exposition cutanée dans l'évaluation de l'exposition et, le cas échéant, de mettre en œuvre des mesures de prévention appropriées (telles que le port de gants de protection). En effet, la pénétration cutanée des substances n'est pas prise en compte pour la détermination des niveaux de valeurs limites atmosphériques et peut donc potentiellement entraîner des effets sanitaires indépendamment du respect de ces dernières.

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

Le CES évalue également les méthodes de référence applicables pour la mesure des niveaux d'exposition sur le lieu de travail. La qualité de ces méthodes et leur applicabilité à la mesure des expositions aux fins de comparaison à une VLEP ont été évaluées notamment sur leur conformité aux exigences de performance de la NF-EN 482 et de leur niveau de validation.

Organisation de l'expertise

L'Anses a confié au comité d'experts spécialisé (CES) « Valeurs Sanitaires de Référence » (CES « VSR ») l'instruction de cette saisine.

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

Le rapport produit tient compte des observations et éléments complémentaires transmis par les membres du CES.

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

Le rapport ainsi que la synthèse et les conclusions de l'expertise collective concernant les effets sur la santé ont été adoptées par le CES « Valeurs Sanitaires de Référence » le 28/11/2019. Trois experts ont exprimé un avis divergent et un s'est abstenu. Leur position est détaillée en français en annexe de cette note d'expertise.

Prévention des risques de conflits d'intérêts

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

Les déclarations d'intérêts des experts sont rendues publiques *via* le site internet de l'Anses (www.anses.fr).

Description de la méthode

Pour l'évaluation des effets sur la santé :

Un profil toxicologique a été élaboré par l'Anses et soumis au CES VSR qui l'a commenté et complété.

Le profil toxicologique est issu d'éléments bibliographiques prenant en compte la littérature scientifique parue sur cette substance jusqu'en janvier 2018. La recherche bibliographique a été effectuée dans les deux bases de données : PubMed et Scopus®. La littérature secondaire du CIRC, de l'OCDE, du NIOSH, de l'ECHA, de l'EFSA, du SCCS ainsi que la proposition de classification et d'étiquetage harmonisé de l'Anses (Anses, 2016) ont également été prises en compte.

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

Introduction

Le TiO_2 existe sous forme micro ou nanométrique. Le présent document concerne exclusivement le TiO_2 sous forme nanométrique (ci-dessous TiO_2-NP).

Selon la définition de la Commission européenne, « *on entend par « nanomatériau » un matériau naturel, formé accidentellement ou manufacturé contenant des particules libres, sous forme d'agrégat ou sous forme d'agglomérat, dont au moins 50 % des particules, dans la répartition numérique par taille, présentent une ou plusieurs dimensions externes se situant entre 1 nm et 100 nm. Dans des cas spécifiques, lorsque cela se justifie pour des raisons tenant à la protection de l'environnement, à la santé publique, à la sécurité ou à la compétitivité, le seuil de 50 % fixé pour la répartition numérique par taille peut être remplacé par un seuil compris entre 1 % et 50 %.*[2]» (recommandation de la CE 2011/696/UE). Cette définition est celle utilisée dans le présent avis pour définir le TiO_2-NP.

En plus de la taille, d'autres propriétés physico-chimiques intrinsèques au TiO_2 peuvent également varier et sont supposées influencer sa réactivité, dont (NIOSH, 2011 ; IARC, 2010) :

- la forme : sphérique, allongée, fibreuse etc.
- la nature de la surface (revêtement, fonctionnalisation) avec recouvrement par des substances inorganiques (silice, alumine…) ou organiques (siloxane, triméthylolpropane…)
- la cristallinité : 3 polymorphes naturels principaux existent : rutile, anatase et brookite. Cependant, au niveau industriel, seules la rutile et l'anatase sont utilisées.

[2] Le seuil de 50 % précisé dans la définition de la Commission européenne n'a pas été retenu pour exclure les études qui ne mentionnaient pas cette information.

Parmi les études identifiées dans la littérature et retenues pour cette expertise, plusieurs ont été réalisées avec du TiO_2 P25. Le P25 est une forme de référence du TiO_2-NP caractérisée de façon complète par l'OCDE (sous le nom de NM105). Il s'agit d'un mélange 80 % / 20 % anatase/rutile avec une taille primaire d'environ 20-25 nm.

Données de toxicocinétique

Les études de cinétique relatives à une exposition respiratoire au TiO_2-NP chez le rat se sont majoritairement intéressées à sa distribution et sa biopersistance au niveau pulmonaire. Les particules de TiO_2-NP sont principalement retrouvées dans les macrophages alvéolaires mais aussi, à un niveau moindre, au niveau des pneumocytes (Eydner et al. (2012)). Le temps de demi-vie estimé est approximativement de 2 mois chez le rat (Oyabu et al. (2017)). En absence de surcharge pulmonaire, la distribution pulmonaire ainsi que le temps de demi-vie ne semblent pas influencés par la durée d'exposition (Zhang et al. (2015); Bermudez et al. (2004)).

Une translocation[3] vers d'autres organes, tels que le foie, le cœur, les reins, le pancréas, la rate ou encore le cerveau a été rapportée par différents auteurs (Kreyling et al. (2017c) ; Pujalte et al. (2017) ; Husain et al. (2015) ; Eydner et al. (2012) ; Gate et al. (2017)), même si celle-ci ne semble pas prédominante. En effet, la vitesse de translocation est plus lente que celle de la clairance pulmonaire (Shinohara et al. (2014)).

Le TiO_2-NP est principalement excrété dans les fèces (Pujalte et al. (2017)), ce qui serait majoritairement consécutif à une déglutition des particules lors de la clairance mucociliaire au niveau du tractus respiratoire. En ce sens, cette excrétion n'est pas représentative d'une élimination de TiO_2-NP préalablement absorbé au niveau systémique.

Données de toxicité

Toxicité aiguë

La plupart des études de toxicité aiguë par voie respiratoire disponibles avec le TiO_2-NP se sont focalisées sur l'étude des effets pulmonaires. Les effets rapportés, que ce soit par inhalation ou par instillation intra-trachéale, consistent principalement en une inflammation associée ou non à des modifications histo-pathologiques (inhalation : Grassian et al. (2007a & b); Noel et al. (2012) ; Leppänen et al. (2011); Leppänen et al. (2015); Oyabu et al. (2016) – instillation : Oberdörster et al. (2000), Renwick et al. (2004), Chen et al. (2006), Nemmar et al. (2008), Nemmar et al. (2011), Liang et al. (2009), Sager and Castranova (2009), Cho et al. (2010), Roberts et al. (2011), Tang et al. (2011), Hurbankova et al. (2013), Husain et al. (2013), Husain et al. (2015), Lee et al. (2014), Choi et al. (2014), Yoshiura et al. (2015), Kobayashi et al. (2016), Wallin et al. (2017); Saber et al. (2013) ; Oyabu et al. (2013)).

Une série d'études réalisée par une même équipe s'est également intéressée aux effets du TiO_2-NP sur le système cardiovasculaire (Nurkiewicz et al. (2008), Nurkiewicz et al. (2009), LeBlanc et al. (2009), LeBlanc et al. (2010), Knuckles et al. (2012), Stapleton et al. (2015b)). Les auteurs ont observé qu'une exposition aiguë par inhalation corps entier au TiO_2 P25 (6 mg/m^3 ; 4 heures) entrainait une altération de la vasodilatation. Ils ont conclu que cette altération serait due à un dysfonctionnement endothélial médié par la production de radicaux libres réduisant ainsi la

[3] Translocation : déplacement des particules hors du site de dépôt pulmonaire initial (Handbook on the toxicology of metals, 2015).

biodisponibilité du monoxyde d'azote. Ces effets semblent apparaitre à des concentrations pouvant également induire une inflammation pulmonaire.

Toxicité subchronique et chronique

Données chez l'Homme

Huit études ont analysé les effets du TiO_2 chez les travailleurs. Trois études ont été réalisées en Chine (Zhen et al. (2012); Ichiara et al. (2016) and Zhao et al. (2018)) et cinq en République tchèque (Pelclova et al. (2015, 2016a, b, c et 2017)). Ces études, majoritairement transversales, suggèrent un effet possible du TiO_2-NP sur les fonctions respiratoire et cardiovasculaire. Cependant, aucune de ces études n'a permis de mettre en évidence une relation causale entre une exposition à du TiO_2 sous forme micro- ou nanométrique et l'apparition de ces effets. La possibilité de biais de sélection et de classification, notamment sur l'exposition, ainsi que de biais de confusion ne permettent pas de considérer ces études adéquates pour conclure sur la toxicité du TiO_2-NP chez l'Homme.

Données chez l'animal

- Effets pulmonaires

Comme pour les études de toxicité aiguë, la majorité des études de toxicité répétée par voie respiratoire se sont focalisées sur les effets pulmonaires du TiO_2-NP. Cinq études de toxicité répétée par inhalation utilisant plusieurs concentrations ont été identifiées dans la littérature.

Dans une étude de toxicité subchronique (Bermudez et al. (2004)), des femelles de trois espèces (rats, souris et hamsters) ont été exposées nez seul au P25 pendant 90 jours aux concentrations nominales de 0,5 ; 2,0 ou 10 mg/m^3. Alors que les hamsters ne présentaient aucun effet pulmonaire, une inflammation pulmonaire a été observée chez les souris à la plus forte concentration testée, ainsi que des effets histopathologiques au niveau du poumon chez les rats dès la concentration de 2,0 mg/m^3. Le rat est l'espèce la plus sensible dans cette étude avec l'observation d'effets pré-néoplasiques, tels que des métaplasies, à la plus forte concentration testée. Ainsi, une NOAEC[4] de 10 mg/m^3 peut être dérivée chez le hamster et une NOAEC de 2 mg/m^3 chez la souris. Chez le rat, une NOAEC de 0,5 mg/m^3 peut être dérivée sur la base d'hypertrophies et d'hyperplasies des cellules alvéolaires de type II, de sévérité minimale à la concentration de 2 mg/m^3 (LOAEC).

Après une exposition de 5 jours au TiO_2-NP (86%/14% anatase/rutile ; 25 nm) chez le rat mâle, Ma-Hock et al. (2009) ont mis en évidence des changements histopathologiques pulmonaires incluant une inflammation à toutes les concentrations testées (2, 10, 50 mg/m^3) et des hypertrophies / hyperplasies des bronches et bronchioles à la plus forte concentration de 50 mg/m^3. Une LOAEC[5] de 2 mg/m^3 a été identifiée par les auteurs. Une inflammation pulmonaire, mise en évidence par des modifications de paramètres dans le liquide de lavage broncho-alvéolaire, a été rapportée chez le rat mâle à cette même concentration par Landsiedel et al. (2014) également après une exposition de 5 jours au TiO_2-NP (rutile revêtu en surface par du diméthicone/méthicone). Dans cette dernière étude, la première dose testée, 0,5 mg/m^3, a donc été identifiée comme une NOAEC.

[4] NOAEC = No Observed Adverse Effect Concentration (= Concentration maximale n'entraînant pas d'effet néfaste observé)

[5] LOAEC = Lowest Observed Adverse Effect Concentration (= Concentration minimale entraînant un effet néfaste observé)

Oyabu et al. (2017) ne rapportent pas d'inflammation pulmonaire chez le rat mâle à des concentrations allant jusqu'à 1,84 mg/m^3 après une exposition de 4 semaines à du TiO$_2$-NP (rutile, 12x55 nm), mais les paramètres testés sont limités. Cependant, de nombreux effets pulmonaires ont été notés chez la souris à toutes les doses testées (LOAEC = 2,5 mg/m^3) par Yu et al. (2015) avec une forme non caractérisée de TiO$_2$-NP, et ce, pour une même durée d'exposition. Du fait de l'absence de caractérisation du matériel testé, cette dernière étude ne peut être utilisée pour la construction d'une valeur de référence.

Malgré des durées d'exposition plus courtes et la diversité des protocoles mis en œuvre (TiO$_2$-NP différent, espèces différentes…), toutes les études par inhalation décrites ci-dessus sont cohérentes avec celle de Bermudez et al. (2004).

D'autres études ont été identifiées dans la littérature, mais elles ont été réalisées par inhalation avec une seule concentration ou par instillation. Elles confirment néanmoins les résultats précédemment décrits, qualitativement ou quantitativement (Grassian et al. (2007b), Eydner et al. (2012), Jackson et al. (2013), Halappanavar et al. (2011) ; Leppänen et al. (2011 & 2015); Sun et al. (2012a, b), Li et al. (2013), Hong et al. (2017)).

- Effets sur le système cardiovasculaire

Cinq études réalisées par inhalation ou par instillation ont analysé les effets du TiO$_2$-NP sur le système cardiovasculaire après une exposition répétée. Divers effets, incluant des dysfonctionnements micro-vasculaires ou de l'athérosclérose, ont été rapportés chez le rat ou la souris dans quatre de ces études (Stapleton et al. (2013) ; Yu et al. (2014) ; Saber et al. (2013), Chen et al. (2013)). Les concentrations utilisées dans les études par inhalation (10 et 42 mg/m^3) sont cependant bien plus élevées que celles des études évaluant les effets pulmonaires (dès 0,5 mg/m^3), et ne permettent donc pas de comparer quantitativement ces effets avec ceux observés au niveau pulmonaire.

- Effets sur le système immunitaire

De nombreuses études évaluant les effets du TiO$_2$-NP sur le système immunitaire sont disponibles. Elles utilisent différents protocoles et différentes voies d'exposition (instillation, inhalation).

Deux études ont montré une diminution des cellules CD4+ et CD8+ avec un ratio CD4+/CD8+ augmenté, indiquant une perturbation du système immunitaire chez le rat (Chang et al. (2015), Gustafsson et al. (2011)). Une augmentation des cellules NK a également été observée suite à une exposition au TiO$_2$-NP chez le rat (Fu et al. (2014), Gustafsson et al. (2011)). Il semble néanmoins difficile de conclure quant à l'immunotoxicité du TiO$_2$-NP au regard des protocoles et des résultats hétérogènes.

- Effets sur le système nerveux central

Onze études relatives à la neurotoxicité du TiO$_2$-NP ont été identifiées dans la littérature.

Des altérations histologiques de l'hippocampe et du cortex cérébral ont été observées chez la souris (Zhang et al. (2011), Wang et al. (2008a, b)) suite à une administration intranasale avec différentes formes de TiO$_2$-NP. Zhang et al. (2011) rapportent une toxicité supérieure du TiO$_2$-NP rutile revêtu en surface par de la silice en comparaison d'une forme rutile non revêtue en surface. Wang et al. (2008 a & b) ont noté des effets plus sévères après une exposition à de l'anatase par rapport au rutile.

Une autre équipe a montré une accumulation du TiO$_2$-NP (anatase ; 6 nm) dans le cerveau de souris avec une prolifération des cellules gliales, une nécrose et des signes de dégénérescence cellulaire,

ainsi que des dérégulations de gènes liés au stress oxydatif, au développement du cerveau, à la mémoire et l'apprentissage.... Ces résultats suggèrent une toxicité dose dépendante du TiO_2-NP sur le cerveau, l'hippocampe étant identifié comme une région cérébrale plus particulièrement sensible (Ze et al. (2013), (2014a, b, c)).

- Hépatotoxicité

Alors qu'une analyse transcriptomique n'a pas rapporté d'effet hépatique du TiO_2-NP après une exposition gestationnelle de 10 jours par inhalation à la concentration de 42 mg/m^3 chez la souris (Halappanavar et al. (2011)), des œdèmes des cellules hépatiques ont été observés après une exposition par instillation pendant 4 semaines à du P25 chez le rat (Chang et al. (2015)).

- Effets sur les reins

Une seule étude, réalisée par instillation, portant sur les effets rénaux du TiO_2-NP a été identifiée dans la littérature. Des modifications histopathologiques, incluant une dilatation tubulaire et une nécrose, en présence d'une augmentation du stress oxydatif, ont été rapportées dès la dose de 0,5 mg/semaine pendant 4 semaines d'exposition au P25 chez la souris (Huang et al. (2015)).

Toxicité sur la reproduction et le développement

Plusieurs équipes ont analysé les effets sur le développement du TiO_2-NP suite à une exposition pré- ou péri-natale par inhalation ou par instillation. Ces travaux n'avaient pas pour but d'étudier la survenue de malformations mais plutôt d'effets mutagènes ou d'effets en lien avec une atteinte pulmonaire, cardiovasculaire ou neurocomportementale.

La première équipe (Hougaard et al. (2010), Boisen et al. (2012); Kyjovska et al. (2013), Jackson et al. (2013)) a exposé des souris femelles du 8ème au 18ème jour de gestation à du TiO_2-NP sous forme rutile avec un revêtement de surface organique (UV-Titan L181) à la concentration de 40 mg/m^3. A cette concentration, les mères présentaient une inflammation pulmonaire. Chez les petits, les effets rapportés incluaient des changements neurocomportementaux modérés (Hougaard et al. (2010)), ainsi qu'une altération de l'expression génique dans le foie des femelles (Jackson et al. (2013)). Une réduction non statistiquement significative du nombre de spermatozoïdes associée à un allongement du délai d'obtention de la première portée a également été rapportée par Kyjovska et al. (2013). *A contrario*, il n'a pas été observé d'augmentation des mutations, ni d'effet sur la viabilité ou le sexe-ratio des portées (Boisen et al. (2012)).

La deuxième équipe (Stapleton et al. (2013, 2015, 2018), Engler-Chiurazzi et al. (2016), Hathaway et al. (2017)) a testé une exposition des femelles gestantes à du P25 pendant environ 8 jours à partir de l'implantation, à la concentration de 10 mg/m^3 chez le rat. Lors de l'étude de l'impact de la durée d'exposition (<7j et >7j), Stapleton et al. (2013) ont observé une diminution de la taille et du poids des portées lors d'une exposition de 11 jours durant la période de gestation, contrairement à une durée d'exposition de 7 jours. Ces effets n'ont pas non plus été observés dans les études postérieures avec une durée d'exposition de 7-8 jours. Des altérations microvasculaires et cardiaques ont été observées chez les petits (Stapleton et al. (2013, 2015, 2018), Hathaway et al. (2017)) ainsi que des effets sur les fonctions cognitives et comportementales (Engler-Chiurazzi et al. (2016)).

Enfin, deux études par instillation se sont intéressées aux effets sur le développement pulmonaire chez la souris. Ambalavanan et al. (2013) suggèrent une augmentation du risque de survenue de maladies respiratoires après une administration unique d'anatase (6 nm) au 4ème, 7ème ou 10ème jour après la naissance. Une altération pulmonaire a également été notée par Paul et al. (2017), chez

des fœtus après une exposition au 2ème, 9ème et 16ème jour de gestation. Cet effet n'était pas associé à une réponse inflammatoire pulmonaire chez les mères ou les fœtus, ni au niveau placentaire.

Ainsi, les études décrites ci-dessus suggèrent un effet possible sur le développement après une exposition à du TiO_2-NP. Cependant, ces études, réalisées à une seule concentration, ne permettent pas d'identifier une NOAEC.

Génotoxicité

De nombreuses publications ont analysé les propriétés mutagènes du TiO_2-NP, principalement sous la forme anatase ou anatase/rutile.

Les études *in vitro* et *in vivo* rapportent des résultats contradictoires, avec des résultats positifs observés principalement à fortes doses dans des tests des comètes et des études de micronoyaux. Cette disparité des résultats pourrait s'expliquer par des différences dans les protocoles et/ou dans les formes de TiO_2-NP testées. Cependant, à ce jour, et malgré la quantité de données disponibles, il n'est pas possible d'identifier un paramètre clé relié aux effets génotoxiques identifiés (Magdolenova et al. (2014); Chen et al. (2014); ANSES (2016); Charles et al. (2018)).

D'un point de vue mécanistique, les données suggèrent que les effets génotoxiques seraient liés à un mécanisme secondaire, *via* la production de radicaux libres. (Charles et al. (2018)).

En conclusion, sur la base de ces résultats et considérant que les effets cancérogènes apparaissent uniquement à de fortes concentrations induisant une réponse inflammatoire et une altération de la clairance, le TiO_2-NP présente une faible génotoxicité. Ces conclusions sont similaires à celles du CIRC (2010), du NIOSH[6] (2011), de l'Anses (2016) et de l'OCDE[7] (2018).

Cancérogénicité

Données chez l'Homme

Sept études épidémiologiques, dont cinq études de cohorte rétrospectives (Chen & Fayerweather (1988); Fryzek et al. (2003) ; Boffetta et al. (2004) ; Ellis et al. (2010 & 2013)) et deux études cas-témoin (Boffetta et al. (2001), Ramanakumar et al. (2008)) ont analysé le lien entre une exposition au TiO_2 et l'apparition de cancers. Le TiO_2 n'étant pas caractérisé dans les publications, il ne peut pas être exclu que les populations analysées soient exposées, au moins en partie, à du TiO_2 sous forme nanoparticulaire.

La plupart de ces études rapportent une augmentation (significative ou non) de la mortalité par cancer pulmonaire. Cependant, aucune n'a permis de mettre en évidence une relation causale entre une exposition à du TiO_2 et l'apparition de cet effet. L'identification de biais de sélection, de classification, notamment sur l'exposition ainsi que de biais de confusion, ne permettent pas de considérer ces études adéquates pour conclure à l'absence ou l'existence d'effet cancérogène chez l'Homme.

Données chez l'animal

Les effets cancérogènes du TiO_2 (sous toutes ses formes) ont été analysés par différentes instances nationales ou internationales d'experts, incluant l'Anses en 2015.

[6] National Institute for Occupational Safety and Health

[7] Organisation de Coopération et de Développement Economiques

Concernant le TiO_2-NP, une seule étude de cancérogénicité par inhalation est disponible (Heinrich et al. (1995)). Dans cette étude, une augmentation de l'incidence de tumeurs pulmonaires bénignes et malignes (tumeurs cystiques des cellules squameuses, carcinomes des cellules squameuses et adénomes/adénocarcinomes bronchioalvéolaires) a été observée chez des rats exposés par inhalation corps entier à du P25 (7,2 mg/m^3 pendant 4 mois, puis 14,8 mg/m^3 pendant 4 mois et enfin 9,4 mg/m^3 pendant 16 mois). Des tumeurs similaires ont également été rapportées chez des rats après une instillation répétée de P25 (Pott et al. (2005)).

A contrario, aucun effet promoteur n'a été identifié dans deux études réalisées par instillation (Xu et al. (2010); Yokohira et al. (2009)). Cependant, les protocoles de ces différentes études présentaient des biais méthodologiques (pas d'information sur la cristallinité du TiO_2-NP, peu d'expérience avec le modèle utilisé, pas de justification du choix des marqueurs et des critères d'évaluation des tumeurs etc…) ne permettant pas d'utiliser ces études pour la construction d'une valeur de référence.

En conclusion, le TiO_2-NP est considéré comme un agent cancérogène chez le rat à des concentrations induisant une inflammation pulmonaire et une altération de la clairance pulmonaire. Les données épidémiologiques sont inadéquates pour conclure à la pertinence de cet effet chez l'Homme. Ces conclusions sont en accord avec celle du CIRC (2010), qui a classé le TiO_2 comme cancérogène possible pour l'Homme (groupe 2B) et du RAC[8] (2017) concluant que le TiO_2 doit être classé comme cancérogène suspecté (catégorie 2) selon le règlement CLP n°1272/2008.

Construction des VLEP

VLEP-8h

Choix de l'effet critique

Sur la base des données disponibles chez l'animal, le TiO_2-NP induit des effets au niveau pulmonaire (à la fois néoplasiques et non-néoplasiques), du système cardiovasculaire, du cerveau, du foie et des reins. Des effets sur le développement ont également été rapportés après une exposition gestationnelle chez le rongeur.

L'analyse de l'ensemble des études de toxicité répétée réalisées par inhalation identifie l'inflammation pulmonaire comme effet critique, c'est-à-dire l'effet apparaissant aux concentrations les plus faibles. L'inflammation pulmonaire est rapportée à des concentrations supérieures ou égales à 2 mg/m^3 chez le rat. Des atteintes pulmonaires plus sévères, incluant une tumorigenèse, apparaissent chez le rat à des concentrations plus élevées (≥ 10 mg/m^3).

Les études visant à l'identification d'autres organes cibles n'ont été réalisées qu'à une seule concentration, souvent bien supérieure à 2 mg/m^3. Ainsi, les effets sur le système cardiovasculaire ont été rapportés à la concentration de 6 mg/m^3, les effets sur le cerveau et sur le développement à la concentration de 10 mg/m^3 et les effets sur le foie à la concentration de 42 mg/m^3. Concernant les effets sur les reins, la seule étude identifiée a été réalisée par instillation.

- Extrapolation de l'animal à l'Homme

[8] Risk Assessment Committee (ou CER : Comité d'évaluation des risques) de l'ECHA (European Chemicals Agency)

Les données expérimentales suggèrent que le rat est particulièrement sensible à la toxicité pulmonaire du TiO_2-NP en comparaison à d'autres rongeurs. En effet, des différences inter-espèces claires ont été observées dans l'étude de Bermudez et al. (2004) réalisée avec du P25 pendant 13 semaines chez des femelles de trois espèces : rats, souris et hamsters. Les lésions pulmonaires étaient plus sévères et apparaissaient à des concentrations plus faibles chez le rat, qui était la seule espèce à développer des lésions fibro-prolifératives. Ces différences inter-espèces pourraient être expliquées, au moins en partie, par des différences dans leur système de détoxification. En effet, une augmentation des niveaux de concentration de certains antioxydants a été observée dans les tissus pulmonaires chez les souris par rapport aux rats, après une exposition particulaire (Oberdörster, 1995). Par ailleurs, il est reconnu que les hamsters ont un système de clairance pulmonaire très efficace, ceci étant démontré par un temps de demi-vie du TiO_2 P25 au niveau pulmonaire très inférieur dans cette espèce par rapport aux rats ou souris (Bermudez et al. (2004)).

Il existe des différences de distribution/dépôt entre les poumons des rats et de l'Homme, qui résultent d'importantes différences anatomiques au niveau des bifurcations bronchiques. Ainsi, chez l'Homme, les particules se déposent massivement dans le tissu interstitiel au niveau des zones proches des bifurcations bronchiques. Chez le rat, un dépôt plus intense et plus uniforme au niveau des alvéoles est observé dans la périphérie pulmonaire, au niveau des bronchioles terminales et des zones alvéolaires immédiatement adjacentes, avec une clairance pulmonaire plus rapide que chez l'Homme. Malgré ces différences, l'Homme et le rat présentent, après une exposition particulaire, des réactions physiopathologiques comparables incluant une fibrose interstitielle diffuse, une lipoprotéinose, une fibrose et une hyperplasie des alvéoles et des bronchioles. Ainsi, les effets pulmonaires rapportés chez le rat sont considérés extrapolables à l'Homme (NIOSH, 2011).

Le CES retient donc l'inflammation pulmonaire comme effet critique.

Choix de l'étude clé

Les données humaines ont toutes été considérées comme inadéquates pour l'établissement de la VLEP-8h.

Parmi les études expérimentales de toxicité répétée, la majorité a été réalisée par instillation intra-trachéale, ce qui ne permet pas de les utiliser pour l'élaboration de la VLEP-8h. En effet, en induisant un effet *bolus*, et en passant outre le passage des voies aériennes supérieures, ce mode d'administration n'est pas jugé représentatif d'une exposition par inhalation.

Parmi les quelques études de toxicité par inhalation disponibles (Ma-Hock et al. (2009); Landsiedel et al. (2014); Yu et al. (2015); Oyabu et al. (2017), Bermudez et al. (2004)), l'étude de Bermudez et al. (2004) a été retenue comme étude clé. En effet, il s'agit d'une étude de toxicité sub-chronique réalisée avec plusieurs concentrations et sur une forme de TiO_2-NP bien caractérisée par l'OCDE (P25). La caractérisation du matériel testé est jugée essentielle vu la diversité des formes de TiO_2-NP disponibles sur le marché, présentant des propriétés physicochimiques différentes pouvant impacter sa réactivité et sa cinétique. Néanmoins, certaines limites méthodologiques ont été identifiées dans cette étude :

- comme seule la toxicité pulmonaire a été analysée, il n'est pas possible de savoir si l'inflammation pulmonaire est réellement l'effet le plus sensible. C'est cependant le cas dans la majorité des études par inhalation disponibles ;
- seules des femelles ont été utilisées. Ce point n'est pas jugé critique, car il n'est pas attendu de forte variabilité inter-sexe quant à la réponse inflammatoire ;

- les rats ont été exposés corps entier, alors qu'actuellement l'exposition par nez seul est privilégiée par l'OCDE. Cependant, au regard de l'effet critique, il n'est pas attendu d'impact majeur de ce mode d'administration. Ceci a été confirmé par Oyabu et al. (2016) qui ont comparé les réponses inflammatoires pulmonaires après une exposition au TiO$_2$-NP par ces deux modes d'administration ;
- étant donné que le P25 n'a pas été dispersé avant exposition, les animaux ont été davantage exposés à de grands agglomérats plutôt qu'à des particules libres ou à des petits agrégats. Même si cela n'est pas protecteur, considérant que les particules de plus petites tailles présentent une plus forte réactivité, cette exposition semble plus proche de la réalité de l'exposition chez l'Homme.

Au vu de ces données, et au regard des autres études disponibles, l'étude de Bermudez et al. (2004) reste l'étude la plus pertinente pour l'établissement de la VLEP-8h, et est donc retenue comme étude clé. Il est également à noter que les autres études de toxicité répétée par inhalation, même si elles sont réalisées avec d'autres formes de TiO$_2$-NP et avec des durées d'exposition inférieures (Ma-Hock et al. (2009); Landsiedel et al. (2014); Yu et al. (2015); Oyabu et al. (2017)) confortent les résultats de Bermudez et al. (2004).

Choix de la dose critique

A ce jour, la plupart des études réalisées avec du TiO$_2$-NP expriment les expositions en mg/m^3. De nombreuses discussions sont en cours sur la façon d'exprimer les concentrations pour les particules faiblement solubles et en particulier celles sous forme nanométriques. En effet, les concentrations peuvent également être exprimées en aire de surface, en nombre de particules ou en volume. Certaines études suggèrent que la réponse biologique dépend davantage de l'aire de surface que de la masse (Oberdorster (2002), NIOSH (2011)). L'expression de la concentration en masse reste cependant toujours pertinente et a le mérite d'être communément utilisée (Sager and Castranova (2009), Noel et al. (2017), NIOSH (2011)). Ainsi, en l'absence de consensus, l'expression de la concentration en mg/m^3 est retenue pour la dérivation de la VLEP-8h.

D'après l'étude de Bermudez et al. (2004), les effets rapportés chez le rat à la concentration de 0,5 mg/m^3 sont une diminution réversible du poids corporel, la présence de particules dans les macrophages alvéolaires et une faible accumulation de macrophages dans les poumons. A la concentration de 2 mg/m^3, des hypertrophies et hyperplasies des cellules alvéolaires de type II et une augmentation réversible de la réplication des cellules alvéolaires et bronchiolaires sont également observées, ainsi qu'une accumulation des macrophages alvéolaires. Les effets deviennent plus sévères à la concentration de 10 mg/m^3 avec des changements métaplasiques dans la région centro-acinaire.

Sur la base de l'effet d'augmentation de la prolifération cellulaire, dans un premier temps, une modélisation BMD a été réalisée, considérant l'existence d'une relation dose réponse. Cependant, cette approche a été écartée aux motifs suivants : faible nombre d'animaux analysés par dose pour le paramètre considéré (n=5) et forte variabilité interindividuelle. Certains critères d'acceptation d'une BMD n'étaient en effet pas remplis (US EPA, 2012) :

- Le ratio BMD/BMDL est d'environ 10, ce qui démontre une forte incertitude ;
- La BMDL est 10 fois plus faible que la plus faible dose testée;
- La valeur de la BMD se situe entre le groupe contrôle et la plus faible dose.

Une BMD ne pouvant pas être établie, un couple NOAEL/LOAEC est proposé.

Sur la base des effets précédemment décrits, la LOAEC retenue est donc de 2 mg/m^3 et la NOAEC de 0,5 mg/m^3.

Choix de l'hypothèse de construction

Les substances cancérogènes sont traditionnellement divisées en deux catégories selon le mode d'action: génotoxique ou non génotoxique.

Comme indiqué ci-dessus, le TiO$_2$-NP est un génotoxique faible, dont l'effet n'apparaît qu'à des doses élevées et avec une relation dose-réponse identifiée dans de nombreuses études expérimentales. Les données disponibles indiquent qu'une génotoxicité secondaire, consécutive à un stress oxydatif, serait le principal mécanisme d'action. Les effets cancérogènes apparaissent également à des concentrations élevées, associées à une altération de la clairance pulmonaire et à une réponse inflammatoire.

La qualité des études est importante pour évaluer la génotoxicité d'une substance et choisir entre la construction d'une VLEP-8h à seuil ou sans seuil. Pour le TiO$_2$-NP, la majorité des résultats positifs sont obtenus à partir de tests des comètes. Bon nombre des tests des comètes disponibles ont été réalisés *in vitro*. Ce test des comètes *in vitro* n'est pas un protocole faisant l'objet d'une ligne directrice de l'OCDE, qui sont considérées comme des protocoles standards pour évaluer la mutagénicité des substances chimiques. De plus, ces tests mesurent les lésions précoces de l'ADN qui peuvent être réparées par la suite (Charles et al. (2018)).

Pour chercher à évaluer la génotoxicité d'une substance chimique, Brusick *et al.* (2016), dans une approche fondée sur le poids de la preuve, ont attribué un faible poids de preuve à ce type de tests. L'OCDE précise que « lors de l'évaluation du potentiel mutagène d'un produit chimique à l'essai, il faudrait accorder plus de poids à la mesure des changements permanents de l'ADN (c'est-à-dire les mutations) qu'aux événements réversibles » (OCDE, 2015). Par conséquent, conformément à la méthodologie Anses (2017), les réponses positives obtenues avec les tests « indicateurs » (mesure des cassures de l'ADN, échanges de chromatides sœurs, etc.) sont certainement associées à l'exposition mais doivent être considérées comme insuffisantes pour caractériser un effet mutagène.

En conclusion : considérant la faible génotoxicité du TiO$_2$-NP, associé à un mécanisme d'action génotoxique décrit dans les études comme majoritairement secondaire et du faible poids des tests positifs disponibles pour parvenir à cette conclusion, la construction d'une VLEP-8h à seuil est considérée comme le choix le plus pertinent pour le TiO$_2$-NP.

Ajustements allométrique et temporel

Le calcul de la concentration équivalente humaine (CEH) pour le TiO$_2$-NP est basé principalement sur la méthodologie utilisée par la « Maximale Arbeitsplatz-Konzentration » (MAK) pour le calcul de la valeur limite de la fraction alvéolaire des poussières granulaires biopersistantes (MAK, 2012).

Cette méthodologie est fondée sur l'hypothèse d'une même sensibilité du rat et de l'Homme au TiO$_2$-NP, pour une même dose de particules par unité de surface pulmonaire. Elle suit les étapes suivantes :

1. Evaluation de la **fraction de dépôt** dans le poumon.

La fraction de dépôt pulmonaire est le ratio du nombre de particules déposées dans les poumons sur le nombre de particules entrant dans le tractus respiratoire.

Pour estimer cette fraction, le modèle MPPD (Multiple Path Particle Dosimetry) (version 3.04, 2016) a été utilisé. Ce modèle a été développé par le Chemical Industry Institute of Toxicology (CIIT), NC (Caroline du Nord), USA, et l'Institut néerlandais de santé publique et de l'environnement (Rijksinstituut voor Volksgezondheid en Milieu (RIVM)). Les valeurs physiologiques (volumes courant, fréquence respiratoire…) utilisées dans les calculs sont celles rentrées par défaut dans le modèle MPPD (cf. ci-dessous). Les demi-vies d'élimination sont issues des publications de Brown et al. (2005) pour le rat et Kreyling and Scheuch (2000) pour l'Homme.

Rat : 0,056 (sans unité)

Homme : 0,1032 (sans unité)

2. Calcul du **volume de dépôt**, en m^3/jour :

Volume de dépôt = fraction de dépôt × volume courant × fréquence respiratoire × temps d'exposition

Rat : volume de dépôt = 0,056 x (2,1/1 000 000) x 102 x 60 x 6 x 5/7 = 0,003084 m^3/jour

2,1 mL = volume courant du rat

102/min = fréquence respiratoire du rat

60 min x 6 h x 5/7 j = temps d'exposition de l'étude, exprimée en jours

Homme : volume de dépôt = 0,1032 x (1040/1 000 000) x 20 x 60 x 8 x 240/365 = 0,677 m^3/jour

1040 mL = volume courant de l'Homme

20/min = fréquence respiratoire de l'Homme

60 min x 8 h x 240/365 j = temps d'exposition, exprimé en jours

3. Calcul de la **constante d'élimination**, en jours :

Constante d'élimination = -ln(0,5)/Demi-vie d'élimination

Rat : Constante d'élimination = -(ln0,5)/60[9] = 0,0116/jour

Homme : Constante d'élimination = -(ln0,5)/400[10] = 0,00173/jour

4. Calcul de la **charge pulmonaire** à l'état d'équilibre, en m^3 :

Charge pulmonaire à l'état d'équilibre = volume de dépôt /constante d'élimination

A noter que la charge pulmonaire à l'état d'équilibre exprimée en mg est obtenue en multipliant cette valeur par la concentration de poussières dans l'air en mg/m^3, c'est-à-dire la NOAEC.

Rat : Charge pulmonaire à l'état d'équilibre = 0,003084/0,0116 = 0,2659 m^3

Homme : Charge pulmonaire à l'état d'équilibre = 0,677/0,00173 = 391,61 m^3

5. Enfin, la charge pulmonaire ramenée à la surface des poumons est calculée pour le rat et l'Homme et le rapport de ces valeurs est utilisé pour le calcul de la **concentration équivalente humaine** en multipliant par la NOAEC :

NOAEC$_{CEH}$ = NOAEC x (charge pulmonaire à l'état d'équilibre/surface spécifique pulmonaire)$_{rat}$ /(charge pulmonaire à l'état d'équilibre /surface spécifique pulmonaire)$_{humain}$

NOAEC$_{CEH}$ = 0,5 x (0,2659/0,297[11])/(391,61/57,22[11]) = 0,5 x (0,8953/6,84) = 0,5 x 0,1309

NOAEC$_{CEH}$ = 0,065 mg/m^3

Choix des facteurs d'ajustement

Le calcul de la VLEP-8h à partir de la NOAEC$_{CEH}$ a été effectué à l'aide des facteurs d'ajustement suivants (Anses, 2017) :

[9] Brown et al. 2005, confirmé par les résultats de Bermudez et al. 2004

[10] Kreyling and Scheuch 2000

[11] U.S. EPA, 2009, en m²

- Variabilité inter-espèces (FA_A) : l'ajustement allométrique réalisé par modélisation a permis le calcul d'une concentration équivalente humaine. Tel que prévu dans le guide méthodologique, une valeur de **3** a été retenue pour prendre en compte la variabilité toxicodynamique et les incertitudes résiduelles.
- Variabilité inter-individuelle (FA_H) : en l'absence de données permettant de réduire le facteur par défaut, une valeur de **3** a été retenue.
- Transposition subchronique – chronique (FA_S) : L'étude clé pour la construction de la VLEP (Bermudez et al., 2004) est une étude de toxicité subchronique. En l'absence de données permettant d'exclure que des concentrations plus faibles seraient suffisantes pour induire un effet suite à de plus longues expositions, la valeur par défaut de **3** a été retenue.
- Utilisation d'une BMDL, LOAEC ou NOAEC (FA_L) : une valeur de 1 a été retenue, le point de départ étant une NOAEC.
- Incertitudes dues aux lacunes de la base de données (FA_D) : La plupart des études réalisées sur le TiO$_2$-P25 ne sont pas jugées fiables pour l'évaluation des risques chroniques (administration intratrachéale, une seule concentration élevée testée, aucune étude chronique). De plus, plusieurs études de toxicité à doses répétées ont montré des effets sur d'autres organes que les poumons (système cardiovasculaire, foie, reins...). Cependant, comme la majorité des études de toxicité par inhalation à doses répétées n'ont investigué qu'un seul paramètre à la fois, on ne peut exclure que les autres effets nocifs puissent survenir à des concentrations infra-inflammatoires. Dans ce contexte, la valeur **3** a été retenue.

Le facteur d'incertitude global pour la dérivation de la VLEP-8h est donc de **81.**

Proposition de VLEP-8h

Une VLEP-8h de **0,80 µg/m^3** a été dérivée.

Cette valeur est directement applicable au P25 qui est la forme de TiO$_2$ testée dans l'étude de Bermudez et al. (2004).

La pertinence de cette valeur pour les autres formes de TiO$_2$-NP n'a pu être évaluée considérant l'existence de plus de 350 formes différentes de TiO$_2$ ayant des propriétés physicochimiques variées. En effet, sur la base de la littérature disponible, les propriétés intrinsèques d'un nanomatériau semblent influencer sa cinétique et sa réactivité.

Concernant la **taille** du TiO$_2$, il est attendu une plus forte réactivité des nanoparticules en comparaison des particules sous forme micrométrique, du fait d'une augmentation de la réponse pulmonaire consécutive à une altération de la clairance, d'une plus longue biopersistance et d'une pénétration plus en profondeur dans les régions interstitielles des alvéoles. Ainsi, de nombreuses publications rapportent une inflammation pulmonaire plus sévère avec des formes plus petites de TiO$_2$-NP (Drew et al. (2017), Halappanavar et al. (2015), Hashizume et al. (2016), Kobayashi et al. (2009), Rahman et al. (2017), Noel et al. (2013)). Cependant, *a contrario*, d'autres auteurs n'ont pas identifié de lien direct entre inflammation et taille des particules de TiO$_2$ (Li et al. (2007), Rossi et al. (2009), Roursgaard et al. (2011)).

L'importance de la **phase cristalline** sur la toxicité a été confirmée par de nombreux auteurs (Okada et al. (2016), Warheit et al. (2007), Rushton et al. (2010), Rahman et al. (2017) and Numano et al. (2014), Roursgaard et al. (2011), Park et al. (2014)), même si l'ensemble des données disponibles à ce jour ne permet pas d'identifier la forme cristalline la plus toxique.

La présence d'un **revêtement de surface** peut également influer sur la cinétique, la production d'espèces réactives et les interactions du TiO_2-NP avec les macromolécules mais aussi potentiellement libérer des substances toxiques issues de ce revêtement. Bien que cette question ait été peu étudiée, il ressort de la littérature que les formes revêtues avec de l'alumine, avec des groupes amino- chargés positivement, avec des substances hydrophiles ou avec de la silice, pourraient induire une plus forte inflammation pulmonaire, en comparaison avec les formes non revêtues en surface (Hashizume et al. (2016), Halappanavar et al. (2015), Rahman et al. (2017), Rossi et al. (2009)).

Enfin, l'influence des **différentes formes** de TiO_2-NP, telles que les nanosphères, les nanotubes, les nano-fibres etc…, sur la toxicité pulmonaire a été étudiée dans la littérature. Il est généralement montré que les formes fibreuses sont plus toxiques que les formes sphériques (Hamilton et al. (2009), Porter et al. (2013), Silva et al. (2013)). Cependant, ces études ont été réalisées avec du TiO_2-NP fabriqué en laboratoire et la présence des formes non sphériques sur le marché européen reste à ce jour à définir.

Il ne peut pas être établi à ce stade que les données disponibles sur le P25 soient représentatives de toutes les formes de TiO_2-NP. Il ne peut pas également être exclu, en l'état actuel des connaissances, que le P25 soit moins toxique que d'autres formes de TiO_2-NP.

VLCT-15min

Faute de données disponibles quant aux effets toxiques à court terme du TiO_2-NP, afin de limiter l'importance et le nombre de pics d'exposition, le CES VLEP recommande, conformément à sa méthodologie (Anses, 2017), de ne pas dépasser sur une période de 15 minutes la valeur de 5 fois la valeur de la VLEP-8h, soit 4 $\mu g.m^{-3}$.

Ainsi le CES VLEP recommande une VLCT-15 min pragmatique de **4 $\mu g.m^{-3}$**.

Mention « peau »

Au regard de l'absence de pénétration cutanée du TiO_2-NP, comme conclu par le Comité scientifique pour la sécurité des consommateurs (SCCS, 2014), l'attribution de la mention « peau » n'apparait pas nécessaire.

Mention « bruit »

Aucune étude disponible ne suggère d'effet ototoxique du TiO_2-NP. En conséquence, la mention « bruit » n'est pas attribuée.

Annexe

Trois experts du CES VSR ont exprimé une position divergente et un expert du CES VSR s'est abstenu durant la validation de l'avis.

Leur position est présentée ci-dessous.

« Le calcul de la concentration sans effet chez l'homme ($NOAEC_{CEH}$) de 65 µg.m^{-3} à partir de la NOAEC chez le rat (500 µg.m^{-3}), selon la méthodologie MAK décrite dans le rapport, ne nous paraît pas discutable ; en revanche, le choix ou les justifications apportées pour certains facteurs d'ajustement le sont.

En particulier :

- Un FA_S de 3 a été retenu alors que la $NOAEC_{CEH}$ prend en compte :
    1. le calcul du taux de dépôt pulmonaire humain pour une exposition 8h/j et 5j/ semaine, 240j/an, vie-entière ;
    2. la différence de demi-vie d'élimination (épuration) chez l'Homme (400 jours) par rapport à celle du rat (60 jours) ;
    3. le fait qu'il s'agit d'un composé à seuil d'effet dans l'espèce la plus sensible. Les données expérimentales suggèrent en effet que le rat est particulièrement sensible à la toxicité pulmonaire du TiO_2-NP en comparaison à d'autres rongeurs (souris et hamsters) ; mais aussi par rapport au singe et à l'homme (cf § 4.4 du Rapport d'expertise collective).
- Un FA_A de 3 a été retenu alors qu'à l'état d'équilibre de la charge pulmonaire, la sensibilité du rat, considéré comme l'espèce la plus sensible, et de l'Homme ne diffère pas pour une même dose par m^2 de surface pulmonaire. Par ailleurs, la composante toxicodynamique devrait être limitée en comparaison avec les agents solubles ou sous forme de vapeur car le TiO_2 est pratiquement insoluble.
- Enfin, un FA_D de 3 a aussi été retenu sur l'argument : « *it cannot be ruled out that other adverse effects could occur at sub-inflammatory concentrations.* ». Il y a toujours lieu de s'interroger sur la qualité de la base de données, mais l'ensemble du corpus de données scientifiques actuelles ne suggère pas d'effet pouvant survenir à des concentrations d'exposition plus faibles que celles sans effet observé. Aucun des dossiers VLEP traités jusqu'à présent ne fournissait des données absolument exhaustives sur tous les organes et toutes les fonctions biologiques. Ainsi, il nous semble qu'appliquer un FA_D de 3 dans le présent dossier sur la seule base « on ne peut exclure que » devrait impliquer d'appliquer pareil facteur systématiquement dans tous les dossiers VLEP et VTR. Cela nous semble inapproprié. »

Collective Expert Appraisal Report

Acronyms and abbreviations

Ach	Acethylcholine
AF	Adjustment Factors
ANSES	*Agence Nationale de Sécurité Sanitaire de l'alimentation, de l'environnement et du travail* [French Agency for Food, Environmental and Occupational Health & Safety]
ALP	Alkaline Phosphatase
ALT	Alanine aminotransferase
AST	Aspartate Transaminase
BALF	Bronchoalveolar Fluid
BER	Base Excision Repair
BET	Brunauer–Emmett–Teller surface area analysis
BMD	Benchmark Dose
BUN	Blood Urea Nitrogen
BSA	Bovine Serum Albumin
BW	Body Weight
CAMKIV	Calcium/calmodulin-dependent protein kinase type IV
CES	ANSES Expert Committee
CIIT	Chemical Industry Institute of Toxicology
CINC-1	Cytokine-Induced Neutrophil Chemoattractant 1
CLP	*Classification Labelling and Packaging*
CMR	Carcinogenic, Mutagenic, Reprotoxic
COX	Cyclo-oxigenase
CRC	Chemical Rubber Company
DAF	Dosimetric Adjustment Factor
DGCCRF	*Direction Générale de la Concurrence, de la Consommation et de la Répression des Fraudes* [Directorate General for Competition Policy, Consumer Affairs and Fraud Control]
DFOSB	Truncated form of FosB missing the C-terminal 101 amino acids
DGS	*Direction Générale de la Santé* [Directorate General for Health]
DHPN	N-bis(2-hydroxypropyl)nitrosamine
DLS	Dynamic Light Scattering
DNA	Deoxyribonucleic acid
DNEL/DMEL	Derivative No Effect Level / Derivative Minimum Effect Level
DSP	Daily Sperm Production
ECHA	European Chemicals Agency
ESTR	Expanded Simple Tandem Repeat
FGF-18	Fibroblast Growth Factor-18
FIOH	Finnish Institute of Occupational Health
FOSB	FBJ murine osteosarcoma viral oncogene homolog B
GD	Gestational Day

GLP	Good Laboratory Practice
GSD	Geometric Standard Deviation
HCSP	High Council for Public Health
HDL-C	High-Density Lipoproteins Cholesterol
HEC	Human Equivalent Concentration
HIF-1α	Hypoxia-Inducible factor 1-alpha
HMPC	Hydroxypropylmethylcellulose
HO-1	Heme oxygenase 1
IARC	International Agency for Research on Cancer
IFN-γ	Interferon gamma
IL	Interleukine
INERIS	*Institut national de l'environnement industriel et des risques* [French National Institute For Industrial Environment And Risks]
LDH	Lactate Dehydrogenase
LY/LYP	Lymphocytes/Percentage of Lymphocytes
LOAEC	Lowest-Observed-Adverse-Effect Level
M-CSF	Macrophage Colony-Stimulating Factor
MAK	Maximale Arbeitsplatz-Konzentration
MCP	Monocyte Chimoattractant Protein
MCV	Mean Corpuscular Volume
MDA	Maleic Dialdehyde
MDC	Macrophage-Derived Chemokine
MIP-2	Macrophage Inflammatory Protein 2
MMAD	Mass Median Aerodynamic Diameter
MMP-9	Matrix Metalloproteinase 9
MPPD	Multiple Path Particle Dosimetry
NER	Nucleotide Excision Repair
NIOSH	National Institute for Occupational Safety and Health
NMDA	*N*-methyl-D-aspartate
NO	Nitric Oxid
NOAEC	No-Observed-Adverse-Effect Level
NP	Nanoparticle
NK	Natural Killer
OECD	Organisation for Economic Co-operation and Development
OEL	Occupational Exposure Limit
OVA	Ovalbumin
PBS	Phosphate-Buffered Saline
PBS-HEC	Phosphate-Buffered Saline - Hydroxyethyl cellulose
PCNA	Proliferating Cell Nuclear Antigen
PMN	Polymorphonuclear Neutrophils

1001	PND	Postnatal Day
1002	QSAR	Quantitative structure-activity relationship
1003	RAC	Risk Assessment Committee
1004 1005	REACh	Regulation (EC) No 1907/206 of 18/12/06 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACh)
1006	RDI	Relative Deposition Index
1007	RDW	Red Blood Cell Distribution Width
1008 1009	RIVM	*Rijksinstituut voor Volksgezondheid en Milieu* [Netherlands National Institute for Public Health and the Environment]
1010	RMOA	Risk Management Option Analysis
1011	RNA	Ribonucleic Acid
1012	RNS	Reactive Nitrogen Species
1013	ROS	Reactive Oxygen Species
1014	RT-PCR	Reverse Transcription Polymerase Chain Reaction
1015	SAA	Serum Amyloid A
1016	SMR	Standardized Mortality Ratio
1017	SNP	Sodium Nitroprusside
1018	TG	Triglycerides
1019	TEM	Transmission Electron Microscopy
1020	TiO_2	Titanium dioxide
1021	VEGF-α	Vascular Endothelial Growth Factor-alpha
1022	VT	Tidal Volume
1023	WBC	White Blood Cell

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Preamble

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

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

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

In 2015, Anses submitted a proposal of classification to the European Chemicals Agency (ECHA) for the carcinogenicity by inhalation of TiO_2 (carcinogenic category 1B) under European Regulation (CLP) N° 1272/2008 on the classification, labelling and packaging of dangerous substances and mixtures. In 2017, ECHA's Risk Assessment Committee (RAC) concluded that TiO_2 in all its forms should be classified as a suspected human carcinogen of category 2 by inhalation.

Anses was requested by the Directorate General for Health (DGS), Directorate General for Risk Prevention (DGPR) and Directorate General for Labour (DGT) on 4 July 2017 to estabilsh a chronic TRV by inhalation for TiO_2 under nanoform. This request under the terms of the referral is the result of “the analysis of the R-Nano database indicating that many industrial sites in France use titanium dioxide under nanoform. These uses can lead to exposure of workers but also to exposure of populations via off-site emissions”. The referral notes that “the International Agency for Research on Cancer (IARC) has classified titanium dioxide as respirable particles as a possible carcinogen by inhalation”. An opinion was published in April 2019 defining a TRV applicable only to TiO_2-P25 of 0.12 µg/m^3, with a confidence level moderate (Anses, 2019). Following this work, and in accordance with the corresponding protocol of agreement, Anses launched the work for the establishment of OELs.

In addition, under the REACh regulation, Anses is currently examining a dossier for assessing the hazards and risks of TiO_2 to human health and the environment. As part of the examination of this dossier, additional data on the hazards and uses of TiO_2 may be required by Anses from industry.

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

workers. These concentration levels are determined by considering that the exposed population (the workers) is one that excludes both children and the elderly.

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

The Committee recommends the use of three types of values:

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

These three types of values are expressed:

- in $mg.m^{-3}$, i.e. in milligrams of chemical per cubic metre of air, or in ppm (parts per million), i.e. in cubic centimetres of chemical per cubic metre of air, for gases and vapours;
- or in $mg.m^{-3}$ only for liquid (fog) and solid (fumes) aerosols;
- or in $f.cm^{-3}$, i.e. in fibres per cubic centimetre for fibrous materials.

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

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

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

The HRV Committee assesses the need to assign a “noise” notation indicating a risk of hearing impairment in the event of co-exposure to noise and the substance below the recommended OELs,

to enable preventionists to implement appropriate measures (collective, individual and/or medical) (Anses 2017).

The HRV Committee also assesses the applicable reference methods for the measurement of exposure levels in the workplace. The quality of these methods and their applicability to the measurement of exposure levels for comparison with an OEL are assessed, particularly with regards to their compliance with the performance requirements in the NF-EN 482 Standard and their level of validation[12]. Once they have been assessed, these methods can be classified into one of the following categories:

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

Organisation of the expert appraisal

ANSES entrusted examination of this request to the expert committee on health reference value (HRV Committee).
The methodological and scientific aspects of the work were regularly submitted to the Expert Committee.
The report produced takes account of observations and additional information provided by the Committee members.
This expert appraisal was therefore conducted by a group of experts with complementary skills. It was carried out in accordance with the French Standard NF X 50-110 “Quality in Expertise Activities”.

This collective expert appraisal work and its conclusions and recommendations concerning the health effects were adopted by the HRV Committee on 28 November 2019. Three experts expressed a minority opinion and one abstained. Their position is laid out in annex 3.

Preventing 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 made public on ANSES's website (www.anses.fr).

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

Description of the method

For the assessment of the health effects:

A toxicological profile was prepared by ANSES's officers and submitted to the HRV Committee, which commented on it and added to it.

The toxicological profile is mainly based on bibliographical information taking into account the scientific literature published on this substance until January 2018. The bibliographical research was conducted in the two following databases: Medline and Scopus®. The secondary literature of IARC, OECD, NIOSH, ECHA, EFSA and SCCS as well as the Anses‘s harmonized proposal for classification and labelling 5Anses, 2016) have also been taken into account.

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Part A – Report on assessment of health effects

1 General information

1.1 Substance identification

Table 1: Substance identity

Name	Titanium Dioxide
CAS number	13463-67-7 (All forms) 1317-70-0 (Anatase) 1317-80-2 (Rutile) 12188-41-9 (Brookite)
EC number	236-675-5
Synonyms	Dioxotitanium
Molecular formula	TiO_2
Structural formula	

TiO_2 exists under micro and nanosize. The present report refers specifically to TiO_2 under nanoform (namely TiO_2-NP in this report). According to European Commission (EC, 2011), a nanomaterial means:

"A natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm - 100 nm.

In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50 % may be replaced by a threshold between 1 and 50 %."

1.2 Discussion on different forms of TiO_2 under nanoform

Intrinsic physico-chemical properties of a nanomaterial, such as particle crystallinity, size, surface area and surface modification, are presumed to influence its reactivity and behaviour.

Regarding **crystallinity**, three main naturally titanium dioxide polymorphs exist: rutile, anatase and brookite, the most commonly studied and used being rutile and anatase (Carp, Huisman, and Reller 2004, NIOSH 2011, IARC 2010).

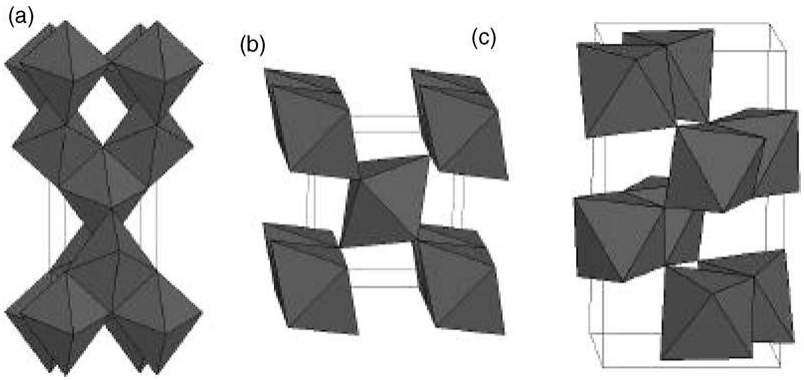

Figure 1 : Crystal structures of anatase (a), rutile (b), and brookite (c) from Carp, Huisman, and Reller (2004)

The importance of **crystal form** in assessing the pulmonary toxicity of TiO_2-NP was confirmed by different authors but with contradictory conclusions.

A distinct inflammatory potential was noted by Aragao-Santiago et al. (2016) between anatase TiO_2-NP and rutile TiO_2-NP, in which the latter did not induce inflammatory response. Okada et al. (2016) found that mixed-crystal phase and amorphous TiO_2-NP engender the most severe fibrosis compared to anatase and rutile forms. Warheit et al. (2007) and Rushton et al. (2010) also observed a more pronounced inflammation with mixed-crystal phase. In contrast, Rahman et al. (2017) and Numano et al. (2014) reported a higher overall biological response of the lung with rutile compared to anatase. A higher inflammatory response for rutile form compared to anatase and amorphous TiO_2-NP was also reported by Roursgaard et al. (2011); but in addition, they identified amorphous polymorph TiO_2-NP as the most potent in regard to acute tissue damage, based on the level of total protein in bronchoalveolar fluid (BALF). Park et al. (2014) studied differences in pulmonary toxicity of anatase and brookite TiO_2-NP nanorods prepared in laboratory. They found that the brookite form caused more severe and frequent lesions in the lung than the anatase form, along with higher cytokine levels in BALF.

Regarding **particle size**, nanoparticles are expected to be more reactive than bulk materials with an increase in the pulmonary response due to a delayed clearance, longer biopersistence and deeper penetration into interstitial regions of alveoli. In particular, Drew et al. (2017) found that metrics related to particle size (such as density, surface area and diameter) appeared to be the most predictive for estimating potency of a nanomaterial in eliciting pulmonary inflammation. A similar conclusion – the smaller the particle size, the greater the inflammatory response - is also reached by several authors when they compared the lung toxicity (gene expression response, examination of BALF, lung histopathology) after an intratracheal exposure to various forms of titanium dioxide (Halappanavar et al. 2015, Hashizume et al. 2016, Kobayashi et al. 2009, Rahman et al. 2017). Noel et al. (2013) hypothesized that the lower cytotoxicity observed for the larger TiO_2-NP could possibly be due to their less efficient penetration into cells (the smaller size of particles would facilitate their

possible and rapid translocation[13]). Contrasting with these findings, other authors did not find any evidence of a direct association between particle size and inflammatory potential of TiO_2-NP (Li et al. 2007, Rossi et al. 2009, Roursgaard et al. 2011).

Moreover, the behaviour of nanoparticles in the medium, the production of reactive oxygen and nitrogen species or the interaction with macromolecules, can also be influenced by th**e presence of a coating** which may itself also release toxic material (Charles et al. 2018). Even if coated forms of TiO_2-NP are not commonly tested in toxicological studies, some publications emphasize that it is essential to take into account surface coating in risk assessment. Hashizume et al. (2016) reported that $Al(OH)_3$-coated TiO_2-NP induced a greater pulmonary inflammatory response than non-coated particles. Halappanavar et al. (2015) also noted that changes in the surface characteristics, such as the addition of positively charged amino groups, can further enhance the inflammatory potential of TiO_2-NP. Similarly, Rahman et al. (2017) demonstrated the important role of coating with an exacerbation of the pulmonary response when animals were exposed to TiO_2-NP covered with an hydrophilic coating, compared with no or hydrophobic coating. Among different forms of TiO_2-NP tested, Rossi et al. (2009) found that only Si-coated rutile TiO_2-NP elicited clear pulmonary inflammation compared to uncoated TiO_2-NP.

Finally, the influence of different **shapes of TiO_2-NP** such as nanospheres, nanobelts, nanorods, nanodots, needles, tubes, fiber-like, on lung toxicity have been studied in the literature. For example, Hamilton et al. (2009) demonstrated that alteration of TiO_2-NP into a fibre structure greater than 15 µm creates a highly toxic particle which initiates an inflammatory response by alveolar macrophages. Similar conclusions were reached by Porter et al. (2013) or Silva et al. (2013) who reported more severe pulmonary responses with nanobelts compared to nanospheres. In contrast, Warheit et al. (2006) did not find significant differences in the pulmonary responses between anatase nanodots and anatase nanorods, despite a six-fold difference in the surface area. It is worth to note that these publications refer to self-synthesized TiO_2-NP, which actual relevance on the European market cannot be assessed.

1.3 Physicochemical properties

Table 2: Physico-chemical properties of TiO_2 under nanoform (CRC Handbook of Chemistry and Physics, Lide 2000, Weast 1991)

State of the substance at 20°C and 101,3 kPa	Solid, crystalline, white, odourless inorganic substance.
Molecular weight (g/mol)	79.87
Boiling point	ca. 3000 °C
Melting/freezing point	Anatase: 1560 °C, Rutile: 1843 °C,

[13] Translocation : movement away from the site of deposition either within or outside the lungs (Elder, Nordberg and Kleinman 2015)

	Brookite: 1825 °C
Relative density (g/cm^3, 20 °C)	Anatase = 3.9, Rutile = 4.26, Brookite = 4.17
Water solubility	Not soluble
Solubility in organic solvent	Not soluble

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2 Summary of scientific recommendations concerning OELs

2.1 SCOEL synthesis summary

No SCOEL document on TiO_2 under nanoform was available at the time of drafting this report.

2.2 Other scientific recommendations

2.2.1 National Institute for Occupational Safety and Health (NIOSH, 2011)

Concerning TiO_2 under nanoform, the NIOSH has established two values associated with pulmonary inflammation and lung tumors.

Pulmonary inflammation

- Key studies: Data from four different subchronic inhalation studies in rats were used to investigate the relationship between particle surface area dose and pulmonary inflammation response (Tran et al. (1999), Cullen et al. (2002), Bermudez et al. (2002) Bermudez et al. (2004)). Data from the two Bermudez et al. studies were combined and treated as a single study for dose-response analysis purposes.
- Critical effect: the critical dose or BMD was defined as the particle surface area per gram of lung tissue associated with a 4% inflammatory response of neutrophils in BALF.

Table 4–2. Benchmark dose estimates for particle surface area dose (m^2) per gram of lung associated with pulmonary inflammation in rats (as PMNs in BALF), based on a Hill model

Data modeled	MLE	95% LCL
TiO_2 [Tran et al. 1999]	0.0205	0.0191
TiO_2 [Cullen et al. 2002]	0.1054	0.0861
TiO_2 [Bermudez et al. 2002 and Bermudez et al. 2004, combined]	0.0159	0.0144

BALF = bronchoalveolar lavage fluid; LCL = lower confidence limit; MLE = maximum likelihood estimate; PMNs = polymorphonuclear leukocytes; TiO_2 = titanium dioxide.

The critical doses were then multiplied by 1.5 in order to normalize them to rats of the size used as a reference for lung surface area, as these were estimated to have lung weights of approximately 1.5 grams, based on the animal's body weights. The critical doses were then extrapolated to humans based on the ratio of rat lung to human lung surface areas, which were assumed to be 0.41 m^2 for Fischer 344 rats, 0.4 m^2 for Sprague-Dawley rats, and 102.2 m^2 for humans (Mercer et al. 1994). These critical particle surface area doses were then converted back to particle mass dose for humans.

The multiple-path particle dosimetry model (MPPD2) human lung dosimetry model (CIIT and RIVM 2002) was used to estimate the working lifetime airborne mass concentrations associated with the critical doses in human lungs.

Table 4–3. Estimated mean airborne mass concentrations of fine and ultrafine TiO_2 in humans and related human lung burdens (TiO_2 surface area dose) associated with pulmonary inflammation after a 45-year working lifetime

	Critical dose in human lungs					
	Particle surface area (m^2/lung)		Particle mass (g/lung)		MPPD (ICRP) lung model (mg/m^3)	
Particle size and study	MLE	95% LCL	MLE	95% LCL	MLE	95% LCL
Fine TiO_2 (2.1 µm, 2.2 GSD; 6.68 m^2/g):						
Tran et al. [1999]	7.86	7.32	1.18	1.10	1.11	1.03
Cullen et al. [2002]	40.39	33.00	6.30	5.15	5.94	4.86
Bermudez et al. [2002 and 2004]	6.09	5.52	0.91	0.83	0.86	0.78
Ultrafine TiO_2 (0.8 µm, 1.8 GSD; 48 m^2/g):						
Tran et al. [1999]	7.86	7.32	0.164	0.153	0.136	0.127
Cullen et al. [2002]	40.39	33.00	0.842	0.687	0.698	0.570
Bermudez et al. [2002 and 2004]	6.09	5.52	0.127	0.115	0.105	0.095

NIOSH considered, regarding results in the table, that "*a concentration of approximately 0.11 mg/m³ is appropriate as the starting point for developing recommended exposures to ultrafine TiO_2.*"

Since the rat BMDs were extrapolated to humans using a deposition/clearance model, NIOSH considered reasonable to assume that the animal-to-human toxicokinetic subfactor of 4 has already been accounted for; therefore, a total uncertainty factor of 25 (2.5 for animal-to-human toxicodynamics times ; 10 for interindividual variability) should be applied. This results in estimated exposure concentrations designed to prevent pulmonary inflammation of 0.004 mg/m^3 for ultrafine TiO_2.

Lung tumors

- Key studies: NIOSH used dose-response data from chronic inhalation studies in rats exposed to TiO_2 to estimate working lifetime exposures and lung cancer risks in humans. These studies include fine (pigment-grade) rutile TiO_2 (Lee et al. 1985; Muhle et al. 1991) and ultrafine anatase TiO_2 (Heinrich et al. 1995).
- Critical effect: risk estimates for TiO_2-induced lung tumors are based on the combined male and female rat lung tumors, excluding the squamous cell keratinizing cystic tumors. The estimated particle surface area dose associated with a 1/1000 excess risk of lung tumors was chosen for the derivation of critical dose.

The critical doses were then multiplied by 1.5 in order to normalize them to rats of the size used as a reference for lung surface area, as these were estimated to have lung weights of approximately 1.5 grams, based on the animal's body weights. The critical doses were then extrapolated to humans based on the ratio of rat lung to human lung surface areas, which were assumed to be 0.41 m^2 for Fischer 344 rats, 0.4 m^2 for Sprague-Dawley rats, and 102.2 m^2 for humans (Mercer et al. 1994). These critical particle surface area doses were then converted back to particle mass dose for humans.

The multiple-path particle dosimetry model (MPPD2) human lung dosimetry model (CIIT and RIVM 2002) was used to estimate the working lifetime airborne mass concentrations associated with the critical doses in human lungs.

Table 4–7. Model average estimates† of mean airborne mass concentrations of fine and ultrafine TiO_2 in humans and related human lung burdens (TiO_2* surface area dose) associated with various levels of excess risk of lung cancer after a 45-year working lifetime

Particle size and lifetime added risk estimated from rat dose-response data for lung tumors¶	Critical dose in human lungs‡				Mean airborne exposure§	
	Particle surface area (m^2/lung)		Particle mass (g/lung)		MPPD (ICRP) lung model (mg/m^3)	
	MLE	95% LCL	MLE	95% LCL	MLE	95% LCL
Fine TiO_2 (2.1 µm, 2.2 GSD; 6.68 m^2/g):						
1 in 500	114.2	24.9	17.1	3.7	16.1	3.5
1 in 1000	93.5	17.0	14.0	2.5	13.2	**2.4††**
1 in 2000	76.3	11.1	11.4	1.7	10.8	1.6
1 in 5000	57.5	6.2	8.6	0.9	8.1	0.9
1 in 10,000	46.4	3.8	6.9	0.6	6.5	0.5
1 in 100,000	21.4	0.5	3.2	0.1	3.0	0.1
Ultrafine TiO_2 (0.8 µm, 1.8 GSD; 48 m^2/g)**:						
1 in 500	114.2	24.9	2.38	0.52	1.97	0.43
1 in 1000	93.5	17.0	1.95	0.35	1.62	**0.29††**
1 in 2000	76.3	11.1	1.59	0.23	1.32	0.19
1 in 5000	57.5	6.2	1.20	0.13	0.99	0.11
1 in 10,000	46.4	3.8	0.97	0.08	0.80	0.07
1 in 100,000	21.4	0.5	0.45	0.01	0.37	0.01

*Abbreviations: MA = model average; BMD = benchmark dose; GSD = geometric standard deviation, LCL = lower confidence limit; MLE = maximum likelihood estimate; TiO_2 = titanium dioxide, MPPD = multiple-path particle dosimetry [CIIT and RIVM 2002] model.

†Model averaging combined estimates from the multistage, Weibull, and log-probit models [Wheeler and Bailer 2007].

‡MLE and 95% LCL were determined in rats (Table 4–5) and extrapolated to humans based on species differences in lung surface area, as described in Section 4.2.3.

§Mean concentration estimates were derived from the CIIT and RIVM [2002] lung model.

¶Without keratinizing cystic lesions.

**Mass median aerodynamic diameter (MMAD). Agglomerated particle size for ultrafine TiO_2 was used in the deposition model [CIIT and RIVM 2002]. Specific surface area was used to convert from particle surface area dose to mass dose; thus airborne particles with different specific surface areas would result in different mass concentration estimates from those shown here.

††The exposure levels shown in boldface are the 95% LCL estimates of the concentrations of fine and ultrafine TiO_2 considered appropriate for establishment of a REL. The ultrafine exposure level of 0.29 mg/m^3 was rounded to 0.3 for the REL.

The concentrations shown in bold for fine and ultrafine TiO_2 represent 1 per 1000 risk levels, which NIOSH has used as the basis for establishing RELs. The REL for ultrafine TiO_2 was rounded from 0.29 mg/m^3 to 0.3.

NIOSH discussed the relevance of the two values derived.

"*occupational exposure concentrations designed to prevent pulmonary inflammation, and thus prevent the development of secondary toxicity (including lung tumors), are 0.04 mg/m^3 for fine TiO_2 and 0.004 mg/m^3 for ultrafine TiO_2. In comparison, modeling of the dose-response relationship for lung tumors indicates that occupational exposure concentrations of 2.4 mg/m^3 for fine TiO_2 and 0.3*

mg/m^3 for ultrafine TiO$_2$ would be sufficient to reduce the risk of lung tumors to a 1/1000 lifetime excess risk level. The discrepancy between the occupational exposure concentrations estimated from modeling either pulmonary inflammation or lung tumors raises serious questions concerning the optimal basis for a TiO$_2$ REL. However, it must be acknowledged that the two sets of possible RELs are not based on entirely comparable endpoints. The pulmonary inflammation-based exposure concentrations are expected to entirely prevent the development of toxicity secondary to pulmonary inflammation, resulting in zero excess risk of lung tumors due to exposure to TiO$_2$. In contrast, the lung tumor-based exposure concentrations are designed to allow a small, but nonzero, excess risk of lung tumors due to occupational exposure to TiO$_2$.

As discussed in Section 3.4.1, particle-induced pulmonary inflammation may act as a precursor for lung tumor development; however, pulmonary inflammation itself is not a specific biomarker for lung cancer. As noted in Section 3.5.2.2, the precise level of sustained inflammation necessary to initiate a tumorigenic response is currently unknown. It is possible that the 4% PMN response used in this analysis as the benchmark response level for pulmonary inflammation is overly protective and that a somewhat greater inflammatory response is required for tumor initiation. It is also possible that the 25-fold uncertainty factor applied to the critical dose estimate for pulmonary inflammation may be overly conservative, since pulmonary inflammation is an early event in the sequence of events leading to lung tumors. However, NIOSH has not previously used early events or secondary toxicity as a rationale for applying smaller than normal uncertainty factors. Given that in this case the primary objective of preventing pulmonary inflammation is to prevent the development of lung tumors, and given that lung tumors can be adequately controlled by exposures many-fold higher than the inflammation-based exposure concentrations, NIOSH has concluded that it is appropriate to base RELs for TiO$_2$ on lung tumors rather than pulmonary inflammation. However, NIOSH notes that extremely low-level exposures to TiO$_2$—i.e., at concentrations less than the pulmonary inflammation-based RELs—may pose no excess risk of lung tumors."

2.2.2 Scaffold project (Scaffold, 2014)

The EU-funded SCAFFOLD (Innovative strategies, methods and tools for occupational risks management of manufactured nanomaterials (MNMs) in the construction industry) project sought to help manage occupational exposure to MNMs by developing, testing, validating and disseminating strategies, methods and software tools. In the framework of this project, occupational exposure limits were formulated for several nanomaterials, including titanium dioxide.

The critical effects are related to pulmonary inflammation, which has been observed in animal inhalation studies at various exposure durations. The study of Bermudez et al. (2004) was identified as the key study for pulmonary effects after repeated dose exposure. In rats responses were observed in animals exposed to 2 mg/m^3. Based on this, a NOAEC of 0.5 mg/m^3 was identified.

The limit value was calculated as follows: First, the starting point, i.e. NOAEC, was corrected in order to consider differences in exposure time (6 h versus 8 h / day and in breathing volume for rest versus light work) (ECHA 2012):

Corrected starting point = 0.5 mg/m^3 x (6 h/day / 8 h/day) x (6.7 m^3 / 10 m^3) = 0.251 mg/m^3

In order to cover the potential differences related to the sensitivity of different individuals, it was decided to use the assessment factor of 2.5.

By applying the above mentioned factor, the calculations for an OEL for TiO$_2$ are as follows:

OEL = 0.251 mg/m^3 / 2.5 = 0.1005 mg/m^3 ≈ 0.1 mg/m^3.

3 Toxicokinetics and metabolism

As an introduction, it has to be noted that kinetics of TiO_2-NP after inhalation, as an inorganic particle, depends only on the extent of lung deposition and clearance. This section will therefore mainly deal with respiratory tract kinetics.

3.1 Lung kinetics

Lung is the portal of entry for inhalation exposure to TiO_2-NP and many studies have focused on local pulmonary fate of TiO_2-NP.

Oyabu et al. (2017) assessed the biopersistence of TiO_2-NP (spindle-shaped; 12x55 nm) in the lung after instillation or inhalation for 4 weeks. With the two conditions of exposure, the biological half-life was approximately 2 months. The authors suggest the biopersistence to be a good indicator of TiO_2-NP hazard, as a good correlation was found between biopersistence and effects observed. Similar retention half-times in the lung (≥ 60 days) were reported for TiO_2-NP (P25; anatase/rutile; 21 nm) after whole-body inhalation for 13 weeks in rats (Bermudez et al. 2004) or after 3 instillations at a 4-day interval (Relier et al. 2017). This biological half-life value was obtained at concentrations inducing lung inflammation but without generating an overload situation.

Eydner et al. (2012) nose-only exposed rats to 10 mg/m^3 TiO_2 P25, 6h/day for 21 consecutive days, with the aim to assess the Relative Deposition Index (RDI). Particle deposition took place mainly in alveolar macrophages and to a lesser extent in type-I pneumocytes and no particles were found in cell organelles such as mitochondria or nuclei.

Shinohara et al. (2014) reported a dose-dependent accumulation following acute intratracheal exposure in rat and proposed a simple model to describe the clearance of TiO_2 P25 in lung. They concluded that the translocation is a slower process than the lung clearance and, in addition, lung clearance is more influenced by the dose, the higher the dose, the lower the elimination.

Zhang et al. (2015) compared pulmonary TiO_2-NP (P25) microdistribution in rats administered intratracheally with one or multiple dose at the same total dosage. The results suggested that multiple-dose administrations do not offer more advantages over single-dose administration in the study of pulmonary NP microdistribution: there are no prominent differences in the pattern of the pulmonary microdistribution of TiO_2. However, the multiple-dose administration reduced variations in the TiO_2 content in each lung lobe (Zhang et al. 2016).

3.2 Distribution to other organs

It is generally recognized that an estimated 1% or less of TiO_2-NP deposited in the lungs translocates to systemic circulation and enters other organs. To investigate this postulate, a few recent studies investigated the translocation from lung and distribution of TiO_2-NP in the organism.

Based on the detection of nanoparticles in granulocytes located inside a capillary, Eydner et al. (2012) suggested that distribution to other organs via the blood circulation is possible, although only to a minimal extent.

Kreyling and colleagues analysed the tissue distribution of anatase TiO_2-NP following a single intratracheal instillation exposure (Kreyling, Holzwarth, Haberl, Kozempel, Wenk, et al. 2017, Kreyling, Holzwarth, Schleh, et al. 2017). They observed a translocation of TiO_2-NP across the air-blood barrier into the circulation, leading to small but persistent TiO_2-NP accumulation in almost all studied organs and tissues. The largest fraction of translocated TiO_2-NP was found in soft tissue followed by skeleton while the highest concentrations per organ weight were found in kidneys, liver and spleen. This resulted in a similar distribution pattern compared to the gavage exposure (Kreyling et al. 2017b) but very different from intravenous exposure (Kreyling, Holzwarth, Haberl, Kozempel, Hirn, et al. 2017). Moreover, the authors confirmed that the TiO_2-NP cleared from the lungs after instillation can be absorbed in the gastrointestinal tract.

Another interesting study explored the distribution of TiO_2-NP (20 nm anatase) in rat tissues following a 6h nose only inhalation of 15 mg/m^3 TiO_2-NP (Pujalte, Serventi, et al. 2017). The authors confirmed translocation of particles to blood and distribution in others tissues (liver, kidneys or pancreas). They also found detectable amount of TiO_2-NP in brain.

Husain et al. (2015) demonstrated the presence of TiO_2-NP in liver and heart after acute instillation exposure to TiO_2 UV-Titan L181.

Gate et al. (2017) focused on the differences between young and elderly rats exposed 6 h/day, 5 days/week for 4 weeks by nose-only inhalation to 10 mg/m^3 TiO_2 P25. They confirmed translocation to liver and spleen of TiO_2-NP but never found difference with control group for kidneys or brain. They observed that the amount recovered in spleen and liver was higher in old than in young adults. According to the same authors, the elimination from lung was slower in old rats.

3.3 Excretion

Pujalte, Dieme, et al. (2017) showed that TiO_2-NP is mainly excreted in feces following inhalation compared to urine. The authors stated that these data combined with the observed time courses of TiO_2-NP in lung, blood and lymph nodes are compatible with a mucociliary clearance from the respiratory tract and ingestion of particles, as concluded by Kreyling et al. (2017c).

4 Toxicity data

4.1 Acute toxicity

4.1.1 Pulmonary effects

The following acute toxicity studies focused on pulmonary effects. All studies reported pulmonary inflammation to various extent depending on the protocol used.

Grassian and colleagues investigated the pulmonary effects of two different TiO_2-NP (5 nm anatase and 21 nm anatase/rutile) with a comparable protocol of exposure (about 0.7 and 7 mg/m^3 by inhalation for 4 hours). Similar effects were reported with both forms at high concentration, including inflammation in the BALF without histopathological changes in the lung (Grassian, Adamcakova-Dodd, et al. 2007, Grassian, O'Shaughnessy P, et al. 2007).

Macrophage-engulfed pigment-like components were reported in the lung after 6 hour-exposure to rutile TiO_2 (10x50 nm) at 4 mg/m^3 by whole body or nose-only protocol, suggesting no difference between these two types of administrations (Oyabu et al. 2016).

Noel et al. (2012) also showed that an acute (6 hours) inhalation of 5 nm TiO_2 (anatase) with two distinct agglomeration states, smaller or larger than 100 nm, induced mild pulmonary effects at 7 mg/m^3.

In a study of Leppänen et al. (2011), mice were exposed by nose-only inhalation to anatase:brookite (3:1) 20 nm TiO_2 for 30 min to 0, 8, 20 and 30 mg/m^3. The main effect was an airflow reduction, which occurred at each studied concentration. Thereafter, the same authors investigated respiratory effects in mice following 30 min exposure to nano silica-coated rutile TiO_2 (10 x 40 nm) at 0, 5, 10, 20 and 30 mg/m^3. The exposure induced first phase of pulmonary irritation (rapid and shallow breathing), starting at 10 mg/m^3 exposure, but did not induce inflammation (Leppanen et al. 2015)

Numerous acute toxicity studies by intratracheal instillation are also available. Although not transposable quantitatively, as they are not representative of normal exposure (the upper respiratory tract is bypassed), they can bring additional information for hazard identification. Most of those studies showed similar pulmonary effects as studies by inhalation (Oberdörster et al. 2000, Renwick et al. 2004, Chen et al. 2006, Nemmar, Melghit, and Ali 2008, Nemmar et al. 2011, Liang et al. 2009, Sager and Castranova 2009, Cho et al. 2010, Roberts et al. 2011, Tang et al. 2011, Hurbankova et al. 2013, Husain et al. 2013, Husain et al. 2015, Lee et al. 2014, Choi et al. 2014, Oyabu et al. 2013, Yoshiura et al. 2015, Kobayashi et al. 2016, Wallin et al. 2017, Saber et al. 2013). In contrast, another study did not show any effect of anatase TiO_2-NP 7 nm at 0.5 mg/mL (0.2 mg/0.4 mL) (Horie et al. 2012).

Unfortunately, the doses used in instillation studies are difficult to compare with each other due to different metrics used by the authors and even more so with inhalation studies.

4.1.2 Cardiovascular effects

A series of publications from the same team studied the effect of TiO_2 P25 (anatase/rutile; 21 nm) exposure on microvascular function (Nurkiewicz et al. 2008, Nurkiewicz et al. 2009, LeBlanc et al. 2009, LeBlanc et al. 2010, Knuckles et al. 2012, Stapleton, McBride, et al. 2015). Animals were exposed to different time-concentration combinations in the first study chronologically and then to 6 mg/m^3 for 4 hours in the other ones.

Those studies showed that:

- inhalation of P25 caused an impaired vasodilatation capacity in the systemic microcirculation;
- this reduced vasoreactivity was observed after co-exposure to ACh (activation of endothelial NO synthase and prostaglandin production), A23187 (interaction with endothelial cells to increase intracellular Ca^{2+} concentration and subsequently stimulation of nitric oxide production) or an active hyperemia (through the stimulation of muscular contraction), while smooth muscle responsiveness to NO remained unaltered (co-exposure to sodium nitroprusside (SNP), an NO “donor”, causes no differences between control and exposed group);
- those changes are consistent with an endothelial dysfunction (microvascular NO bioavailability compromised after nanoparticle exposure);
- COX inhibition significantly decreased arteriolar-induced dilation in exposed animals ;
- this observation is consistent with a COX mediated compensation for the reduced NO bioavailability;
- exposure to P25 increased ROS (reactive oxygen species) and RNS (reactive nitrogen species) production in the microvascular wall;
- the impairment of vasodilation was restored by incubation with ROS scavengers.

These studies show that acute exposure to P25 induces vascular dysfunction via ROS generation, which leads to reduce NO bioavailability and ultimately impairs vasodilation. These results suggest that COX pathways would mediate a compensation mechanism for the reduced NO bioavailability. Moreover, considering the observations described above, it can be estimated that the cardiovascular effects observed are concomitant with a weak pulmonary inflammatory effect.

The effects on cardiovascular function after acute exposure were also observed in other studies performed by instillation:

In the study of Savi et al. (2014), male rats were exposed to TiO_2-NP (25-35 nm; anatase/rutile) at a single dose of 2 mg/kg by instillation. The authors observed that TiO_2-NP enhanced the susceptibility to cardiac arrhythmias, via shortening of repolarization time and increase of cardiac excitability. The authors also demonstrated the presence of TiO_2-NP into cardiomyocytes via Transmission Electron Microscopy (TEM). In the study of Saber et al. (2013), female mice were exposed intra-tracheally once to 18, 54 and 162 µg of TiO_2-NP (UV Titan L181, rutile surface coated, 17 nm). Exposure to

UV Titan L181 increased pulmonary Serum Amyloid A (SAA, a risk factor for cardiovascular disease in mice) mRNA expression in a time- and dose-dependent manner. The strongest response was seen at the early time points (400-fold increase in Saa3 mRNA expression in lung at day 1 at the highest dose). Saa3 expression remained significantly increased 28 days after exposure in all mice exposed to the highest dose. According to the authors, this result indicates a long-lasting induction of acute phase response.

4.2 Repeated dose toxicity

4.2.1 Human data

Nine human studies have been identified on toxicological effects following inhalation exposure of TiO_2 in workers. Among them, three studies dealt with Chinese workers (Zhen et al. 2012, Zhao et al. 2018, Ichihara et al. 2016) and 5 investigated a sample of workers in the Czech Republic (Pelclova et al. 2017, Pelclova, Zdimal, Kacer, Vlckova, et al. 2016, Pelclova, Zdimal, Kacer, Fenclova, et al. 2016, Pelclova, Zdimal, Fenclova, et al. 2016, Pelclova et al. 2015). All these studies have been considered inadequate, due to selection bias, classification bias for exposure to TiO_2 and confounding factors.

The study by Zhen et al. (2012) focused on short-term cardiopulmonary effects after exposure to inhalable TiO_2 in an (unspecified) finished-product production workshop. Zhao et al. (2018), who belonged to the same team as Zhen et al., re-analysed short-term cardiopulmonary effects in a sample of workers in a nano-TiO_2 manufacturing plant using a cross-sectional design. Ichihara et al. (2016) undertook a pilot study, with a cross-sectional design, in a plant handling TiO_2 in Shanghai, China. The number of exposed workers included in these studies was between 7 and 83. Finally, the five publications by Pelclova and colleagues were part of the same research project, sub-divided into several studies depending on the type of effect studied and the analysis time. All of the studies had a cross-sectional design, but some were repeated, with differences in exposure measurement and biological sampling protocols. The authors distinguished between several sub-groups of workers considered as exposed, all from the same production plant for paints and pigments containing TiO_2 and other compounds (iron oxide). The sizes of the sub-groups varied depending on the publication, except for researchers (n=4) and exposed office workers (n=22). The authors studied several types of effects including respiratory function with markers of oxidative stress and lipid peroxidation.

On the whole, none of the nine studies considered enable a causal relationship to be established between exposure to nano- or micro-metric (both corresponding to the respirable fraction) size ranges of TiO_2 and the occurrence of biological or health effects in workers. These studies suggest possible effects on respiratory and cardiovascular function whose mechanisms may include oxidative stress reactions, inflammation and the regulation of the parasympathetic nervous system. However, none of them include dose response analyses based on the concentrations of TiO_2

measured at the workstations or individually. The lack of a validated method for assessing individual exposure is a common limitation of all of the studies with on the one hand, the inability to compare nanometric TiO_2 concentrations with the background noise formed by other indoor air particles, and on the other hand, the inability to assess the internal dose or effective dose (TiO_2-NP deposited in the airways).

4.2.2 Animal data

Table 3 presents the repeated studies conducted by inhalation, with several or single concentrations. The studies conducted by instillation route are discussed in the text below the table.

Table 3 : Repeated toxicity studies by inhalation route

Method	Results	Remarks	Reference
Studies with several concentrations			
13-week study by whole-body exposure Females B3C3F1/CrlBR mice, CDF(F344)/CrlBR rats, Lak:LVG(SYR)BR hamsters 25/species/time point Uf-TiO$_2$ (P25, average primary particle size of 21 nm) 0.5, 2.0, or 10 mg/m^3 Corresponding to actual concentrations: - mice: 0.54 ± 0.06, 2.2 ± 0.1 and 10.8 ± 1.0 mg/m^3 - rats: 0.52 ± 0.03, 2.1 ± 0.1, and 10.5 ± 0.7 mg/m^3 - hamsters: 0.53 ±0.03, 2.1 ± 0.1, and 10.7 ± 0.6 mg/m^3 6 h/day, 5 days/week, for 13 weeks, whole body Additional recovery groups for post-exposure periods of 4, 13, 26, or 52 (49 for hamster) weeks in clean air. MMAD = 1.37 µm (1.29-1.44µm) Parameters: mortality, clinical observations, body weight, BALF, lung cell proliferation and lung histopathology	**Mice:** Treatment-related mortalities during exposure phase (4). Post-exposure: 4 deaths not treatment related. Reversible depression of BW gain in all groups. ↑ TiO$_2$ burden in lung and lymph nodes at 10 mg/m^3: retention half-times in lung: 48, 40, and 319d at each concentration. • 0.5 and 2 mg/m^3: Particles free, within alveolar macrophages and in alveolar septal regions. • 10 mg/m^3: ↑ total number of cells and total proteins (still significant at 52w post-exposure) and Lactate Dehydrogenase (LDH) (normal at 26w) in BALF. Reversible ↑ in terminal bronchiolar cell replication Aggregations of heavily particle laden macrophages in central lobar centriacinar sites, concentrating over time and moved to interstitial areas. Perivascular lymphoid proliferation. **Rats:** Post-exposure: 7 deaths not treatment related. Reversible depression of BW gain in all groups. ↑ TiO$_2$ burdens in lung and lymph nodes (mid and high doses): retention half-times in lung: 63, 132, and 395d, at each concentration. • 0.5 mg/m^3: Particles within alveolar macrophages and very minimal changes in the patterns of alveolar macrophage accumulation in lungs. • 2.0 mg/m^3: Particle laden macrophage accumulation, minimal hypertrophy and hyperplasia of type II alveolar epithelial cells. Significant ↑ in terminal bronchiolar cell and in alveolar cell replication (mid and high dose) reversible. • 10 mg/m^3: ↑ total number of cells (normal by 26w post-exposure), total protein (normal by 4w) and LDH (normal by 26w) in BALF. Metaplastic changes in the centriacinar region (bronchiolization of alveolar epithelium) associated with particle and particle-laden macrophage accumulation – not fully reversible at 52w.	NOAEC = 2.0 mg/m^3 in mice NOAEC = 0.5 mg/m^3 in rats NOAEC = 10 mg/m^3 in hamsters No sonication performed before administration. Only females tested. Only examination of lung response. No full characterization of the tested material but P25 is a well-characterized form of TiO$_2$. No detailed results on histopathology. Reliability = 1[14] **Key study**	Bermudez et al. (2004)

[14] Reliability was assessed via the software ToxRTool

	Hamsters: ↑ morbidity and mortality (35 animals during postexposure), not treatment related BW loss at the end of the exposure (9–15%), slow recovery. ↑ TiO_2 lung burdens; retention half-times in lung: 33, 37, and 39d at each concentration. BALF: significant ↑ neutrophils at the end of exposure. Significant terminal bronchiolar cell replication at 10 mg/m^3 (normal at 4w post-exposure). Alveolar and interstitial macrophages containing particles and occasional aggregation of particle-laden macrophages in high dose group. No pathology.		
Repeated-dose toxicity study by nose-only inhalation Male Wistar rats 6 rats/time point/dose Uncoated TiO_2 with hydrophobic surface (14% rutile, 86% anatase). Average primary particle size : 25.1±8.2 nm (13-71 nm) 2.0, 10 or 50 mg/m^3 Nose-only exposure 6h/day for 5 days followed by a recovery period of 3 or 16 days Parameters: lung burden analysis, BALF, cell mediators in BALF and serum, haematology and serum troponin I, histopathology (lung, nasal cavity and larynx), cell proliferation and apoptosis	No significant effect on BW. Lung weights ↑ immediately after exposure at 50 mg/m^3. Concentration related ↑ in total cell counts (↑ numbers of PMN (Polymorphonuclear Neutrophils)), total protein and enzyme activities. After exposure: • 10 mg/m^3: ↑ PMN counts and ↑ γGT activity, • 50 mg/m^3: ↑ total protein content and activities of all 4 enzymes examined. 3d post-exposure, minimal effects on total protein and some enzyme activities at 2 mg/m^3: most prominent changes. 16d post-exposure most of these parameters returned to control level. Immediately after exposure: • 10 mg/m^3: ↑ MCP-1, MCP-3, M-CSF, MDC, myeloperoxidase MIP-2, and osteopontin • 50 mg/m^3: same parameters + clusterin and haptoglobin. 3d post-exposure: • 2 mg/m^3: ↑ clusterin and haptoglobin. • Except osteopontin, all mediators ↑ at 10 and 50 mg/m^3. No significant changes in haematological parameters in all groups. No evidence for any heart muscle damage. Minimal and minimal to mild diffuse alveolar infiltration with histiocytes at 10 and 50 mg/m^3. Hypertrophy/hyperplasia of bronchioles and bronchi at 50 mg/m^3. ↑ labelling indices in large/medium bronchi and terminal bronchioles in all groups after the end of exposure.	LOAEC = 2 mg/m^3 Probably TiO_2 P25 but not specifically named in the publication. Only males. Too high concentrations tested as no NOAEC was identified Reliability = 2 Supportive study	Ma-Hock et al. (2009)
Repeated-dose toxicity study by nose-only exposure Male Wistar rats (8/group)	No effects observed at 0.5 mg/m^3 At 2 mg/m^3 and 10 mg/m^3:	LOAEC= 2 mg/m^3 NOAEC = 0.5 mg/m^3 Only males tested.	Landsiedel et al. (2014)

nano-TiO_2 (T-Lite SF, 15x50 nm) rutile with minimal anatase, coated with dimethicone/methicone copolymer Concentration: 0.5, 2 and 10 mg/m^3 mg/m3 Exposure: 6 h/day for 5 days, and 3 week post-exposure for the group exposed to 10 mg/m^3 Parameters: measurement of cells and marker of inflammation in the BALF, and lung histopathology	• concentration-dependent ↑ in PMN and monocytes in the BALF • ↑ in LDH and Alkaline Phosphatase (ALP) release At 10 mg/m^3: numerous pigment-loaded alveolar macrophages within the alveoli and slight diffuse histiocytosis not fully reversible after 3w of recovery.	Good characterization of TiO_2-NP and exposure Reliability = 1 Supportive study: only 5 days of exposure	
Repeated-dose toxicity study by nose-only inhalation Five-week-old A/J Jms Slc mice 10 mice/time point/dose Average primary particle size : 19.3±5.4 nm 2.5, 5, 10 mg/m^3 whole body, 4 weeks (5d/w, 6h/d) Protocol according to OECD 412 (2009) guideline Parameters assessed: Biochemical analysis of serum, Whole blood analysis, Haematoxylin & eosin staining and immunofluorescence (IF) assay, Western blot analysis, lung histology	No adverse effects on growth or food intake Significantly higher levels of ALT, AST, blood urea nitrogen (BUN), and triglycerides (TG) in exposed groups. Levels of MCV, RDW, LYP, and LY significantly higher in TiO_2-inhaled blood samples. • 2.5 mg/m^3: hyperplasia and haemorrhage. • 5 mg/m^3: hyperaemia, bronchial atelectasis, and brown particle-laden alveolar macrophages. • 10 mg/m^3: bronchial atelectasis and multifocal lymphoid tissue hyperplasia. Dose-related ↑ expressions of CD31 and PCNA. At 5 and 10 mg/m^3, dose-dependent ↑ of phospho-p38, NF-kB, and VCAM-1. Dose-dependent ER swelling and mitochondrial disruption in exposed lungs. Dose dependent ↑ expression levels of proteins Grp78/Bip, CHOP, inositol-requiring enzyme 1 alpha (IRE-1a), LC3, p62, and Beclin 1 in exposed mouse lungs. Autophagosomes in the lungs.	LOAEC = 2.5 mg/m^3 No information on crystallinity of nano-TiO_2 used. Too high concentrations tested as no NOAEC was identified This study has to be disregarded due to the lack of sufficient characterization of the tested material. Even if it is stated that the study was performed according to OECD 412 guideline, only blood, serum and protein analysis, IF and lung histology were evaluated which is not in line with the guideline. Reliability = 3 Disregarded study	Yu et al. (2015)
Repeated-dose toxicity study by whole-body inhalation Male Fisher rats (10/group/time point) TiO_2 (MT-150AW); spindle-shaped; 12x55 nm; average agglomerated particle size:	Biological half-time were 2.0 months for the low tested concentration and 1.8 months for the high tested concentration. Nanoparticles were phagocytized by macrophages, and each particle seemed to exist individually inside the macrophage. Cells with TiO_2 particles were almost normal.	NOAEC = 1.84 mg/m^3 The aim of this study was to determine whether biopersistence is a useful indicator for evaluating the toxicity of nanoparticles.	Oyabu et al. (2017)

44.9 nm; purity = 99.5%; surface area = 111 m^2/g Concentration: 0.50 ± 0.26 and 1.84 ± 0.74 mg/m^3 Exposure: 4 weeks (6 h/day, 5 days/week); sacrifice after 3 days, 1 or 3 month post-exposure Measurement of TiO_2 amount in the whole lung and BALF; observation of cells in the BALF, lung histopathology (only at 3 days after exposure for the highest concentration)	Some alveolar macrophages with a pigment-like material deposition were observed in the alveoli at 3 days after exposure.	Therefore, there is only limited information on the toxicity effects reported. Only males tested. No information on crystallinity in this publication but information available from Morimoto et al., 2016 (rutile) In this study, groups of rats exposed by instillation to 0.2 mg, 0.36 mg or 1 mg were also included, showing similar results. Reliability = 2 Supportive study	
Studies with one concentration			
Repeated-dose toxicity study by whole body inhalation male C57Bl/6 mice (6/group) TiO_2 anatase, 2-5 nm, BET = 219 +/-3 m^2/g 8.88 ±1.98 mg/m^3, 4h/day for 10 days, sacrifice after last dose and after week 1, 2, 3 post-exposure Parameters: BALF (enumeration of cells, LDH, cytokines) and lung histopathology	Cumulative inhaled TiO_2 dose was 154 µg per mouse. Number of alveolar macrophages elevated in the groups of animals necropsied at weeks 0, 1, and 2 postexposure but not in mice necropsied at week 3 post-exposure. No other respiratory effect.	Only males treated.	Grassian, O'Shaughnessy P, et al. (2007) Grassian et al. (2007a)

Repeated-dose toxicity study by nose-only inhalation Female Wistar rats TiO_2 P25 10 mg/m^3 6h/day 21 consecutive days, followed by recovery periods of 3, 28 or 90 days Parameters: lung histopathology, haematology, BALF, electron microscopy of lungs, quantification of lung septum components and relative deposition index	In few lungs, multifocal, acute alveolar emphysema, accentuated in caudal and marginal lung parts observed. Moderate alveolar infiltration with particle-laden macrophages. Few particle-laden macrophages intraluminally and subepithelially in bronchi and bronchioles. Rare particle agglomerates in bronchiolar epithelium, in type-I pneumocytes, and as free particles in alveoli. Minimal interstitial infiltration with mononuclear cells and minimal alveolar infiltration with neutrophilic granulocytes. Minimal bronchiolo-alveolar hyperplasia in few animals. 3d recovery: no statistically significant changes evident in haematology. ↓ activity of β-glucuronidase. Randomly distributed, multifocal, white foci of 0.5–2 mm. 28d recovery: WBC and lymphocyte counts significantly ↓. Alterations in haematology parameters similar after a 90-day recovery period, with ↓ WBC counts, lymphocyte counts and number of segmented neutrophils. No significant changes in RBC count.		Eydner et al. (2012)
Repeated-dose toxicity study by nose-only inhalation male Crl:WI (Han) Wistar rats (6-7 per time point) TiO_2-NP, anatase being the major crystal phase; spherical; wide range of sizes with a few particles up to 100 nm with some agglomerates Concentration: 0 and 11.39 ± 0.31 mg/m^3 Exposure: 2 weeks (6 h/day, 5 days/week) followed by recovery periods of 1, 7 or 15 days. Parameters: biochemistry in BALF and serum, liver, spleen and lung weights, histopathology (lung, nasal cavity)	No significant clinical sign induced. No significant changes in BW. No significant change of liver and lung weights. ↑ relative spleen weight. No effect on cytology and biochemical parameters. No observation of significant differences in levels of IL-4, IL-6, or IL-10 between control and treated groups on 1, 7, and 15d post-exposure Numerous brown pigmented macrophages in alveoli until 15d post-exposure. At 1d post-exposure, olfactory epithelium degeneration/regeneration with inflammatory cell infiltration in the nasal septum and ethmoid turbinate of the treated rats. Basal cell proliferation in the ethmoid turbinate at 7 day post-exposure. Lesions not observed at 15d post-exposure.	This study has to be disregarded due to the lack of sufficient characterization of the tested material.	Kwon et al. (2012)
Repeated-dose toxicity study by nose-only inhalation 7 week-old male Crl:WI (Han) Wistar rats	Ti only detectable in lung and mediastinal lymph nodes of exposed animals. TiO_2 mainly in alveolar macrophages. Number of alveolar macrophages moderately ↑ and few numbers of neutrophils within alveolar space.	Relevance of the results questionable due to too high concentration used.	van Ravenzwaay et al. (2009)

Anatase/rutile (70/30) TiO_2 particles were in the size range 20–30nm Concentration : Target : 100 mg/m^3 (measured : 88 mg/m^3); 6 h/day, 5 days/week followed by 14 days of recovery Parameters: histopathology of the respiratory tract, electron microscopy and BALF	Particles mainly located extracellularly in lumen of alveoli and bronchi + in cytoplasm of alveolar macrophages. TiO_2 found in lung mostly agglomerates of about the same size as in atmosphere; no signs of disagglomeration. Significant ↑ in total cell count and PMN, slightly ↑ lymphocytes and monocytes in lavage fluid. Significantly ↑ of total protein and activities of LDH, ALP, γGT and N-acetyl-glucosaminidase. ↑ of BALF parameters partly reversible. Exposure resulted in >30% ↑ in lung weight. Diffuse histiocytosis and mild neutrophilic inflammation. Hyperplasia in Mediastinal lymph nodes. Declined inflammatory response after recovery, only focal infiltrates of alveolar macrophages, still containing particles in cytoplasm. Lung weight returned to control levels.		
Repeated-dose toxicity study by whole-body inhalation Male Kunming mice 15/animals/group Anatase TiO_2, 20 nm 6.34 +/- 0.22 mg/m^3 Every day for 3 weeks Parameters: distribution (brain, lung, liver, kidney, spleen), BALF and brain homogenate extract, differential blood count, prothrombin time and blood biochemical indexes, pathological examination of lungs, brains, livers and kidneys	TiO_2-NP mainly accumulated in the lungs. Concentration in liver blood and urine also increased. Significant ↑ of H_2O_2 and MDA concentrations in brain homogenate extracts observed. ↓ in WBC count and percentage of lymphocytes and ↑ in percentage of neutrophilic granulocytes, PLT and reticulocytes count. Significant ↑ in ALT and AST observed. No obvious pathological lesions in the lung, brain, liver or kidneys.	Results not well detailed and almost not discussed.	Yin et al. (2014)
Repeated-dose toxicity study by whole-body inhalation Female C57BL/6BomTac mice 17 animals: 9 controls and 8 exposed UV-titan L181 42 mg/m^3	BALF: ↑ in the percentage of neutrophils; Transcriptomic analysis of the lungs: gene inductions for inflammation (cytokines and receptors), oxidative stress, chemotactism, complement; modulation of a few miRNAs; Transcriptomic analysis of the liver: no significant changes	Relevance of the results questionable due to too high concentration used.	Halappanavar et al. (2011)

Exposure: 1h/d for 11 days Parameters: BALF, Gene Expression Analysis, RT-PCR, Immunoassay			
Repeated-dose toxicity study by whole-body inhalation Outbred Crl:OF1 male mice 4-6/animals/group anatase + brookite (3:1)TiO_2, 20 nm 30 mg/m^3 1 h/day, 4 days/week for 4 weeks Parameters: respiratory rate, time of inspiration, time of expiration, time of pause after expiration, time of braking after inspiration, tidal volume, and airflow at midpoint of expiration	Airflow limitation stronger along the exposure period, Sensory irritation fairly minor, and observed also in the control group with the same intensity. Pulmonary irritation observed both in the exposure and control groups. The highest "time of pause" values ↑ along with the exposure days in the exposure group, whereas in the control group, such trend not observed.	Relevance of the results questionable due to high concentration used.	Leppänen et al. (2011)
Repeated-dose toxicity study by whole-body inhalation female BALB/c/Sca mice 8/animals/group Rutile Si-coated TiO_2, 10x40 nm 30 mg/m^3 1 h/day, 4 days/week for 4 weeks on days 1–4, 8–10, 12, 15–18 and 22–25 Parameters: BALF, pathological examination of lungs, immunohistochemical staining	Pulmonary irritation, stronger during the first days of the exposures (days 1–4), and after, the effect was not as intense. Airflow limitation in the conducting airways. Inflammation in the airways: infiltration of inflammatory cells in peribronchial and perivascular areas.	Relevance of the results questionable due to high concentration used.	Leppanen et al. (2015)

4.2.2.1 Pulmonary effects

Four reliable studies (reliability 1 or 2) by inhalation with several concentrations are available on TiO_2-NP and are described and discussed below. These studies mainly focus on pulmonary effect without considering other effects. The studies by van Ravenzwaay et al. (2009), Kwon et al. (2012), and Yin et al. (2015) reported in the table above are not described in the text as they have been disregarded.

In the study performed by Bermudez et al. (2004), female CDF(F344)/CrlBR rats, B3C3F1/CrlBR mice and Lak:LVG(SYR)BR hamsters were treated with target aerosol concentrations of 0.5, 2 or 10 mg/m^3 of TiO_2-NP (P25, average primary particle size of 21 nm) for 13 weeks. Groups of 25 animals for each species and time point were used. Following the exposure period, animals were held for recovery periods of 4, 13, 26 or 52 weeks (49 weeks for the nano-TiO_2-exposed hamsters). At each time point, burdens in the lung and lymph nodes and selected lung responses were examined. The responses studied were chosen to assess a variety of pulmonary parameters, including inflammation, cytotoxicity, lung cell proliferation and histopathological alterations.

Particle size analysis and chamber concentrations of P25 aerosol are given in table 4. It can be noted that the aerosol generated was made up of particle aggregates.

Table 4: Summary of exposure conditions in Bermudez et al. (2004)

Species	Chamber concentrations (mg/m^3)	Mass median aerodynamic diameter (µm)
Hamster	0.54 ± 0.06 2.2 ± 0.1 10.8 ± 1.0	1.29 ± 0.30
Mouse	0.52 ± 0.03 2.1 ± 0.1 10.5 ± 0.7	1.45 ± 0.49
Rat	0.53 ± 0.03 2.1 ± 0.1 10.7 ± 0.6	1.44 ± 0.57

Treatment-related deaths were noted in 4 mice during the exposure phase. During the post-exposure phase, unscheduled mortalities, distributed over the different treatment groups, were reported in all species. Hamsters presented the greatest morbidity/mortality (35 animals), presumably due to severe chronic renal disease.

Following the end of the exposure period, a decrease in body weight was noted in all groups and all species. A more marked body weight loss was noted in hamsters (9-15%). Recovery occurred over

the next three to four weeks in mice and rats but was slower in hamsters, with recovery within approximately 6 weeks.

Clear species differences in pulmonary clearance and lesions were observed, rats being the most sensitive.

Rats and mice exhibited equivalent TiO_2 lung burdens whereas lung burdens in hamsters were approximately 2 to 5 fold lower after 13 weeks of exposure. At the end of the recovery period, rats of the high-dose group retained approximately 57% of the initial burden compared to approximately 46% for mice and approximately 3% for hamsters. The calculated particle retention half-times for the three dose levels were 63, 132 and 395 days in rats, 48, 40 and 319 days in mice and 33, 37 and 39 days in hamsters. Therefore, under the conditions of this study, hamsters had better ability to clear TiO_2 nanoparticles than similarly exposed mice and rats.

Inflammation was noted in rats and mice at 10 mg/m^3, as evidenced by increases in macrophage and neutrophil numbers and in soluble indices of inflammation (LDH and protein) in BALF.

Significant terminal bronchiolar cell replication was observed at the end of the exposure period in mice and hamsters of the high-dose group and in rats of the mid- and high-dose groups. The indices returned to control levels at 4 weeks post-exposure. Alveolar cell replication was significantly increased at the end of the exposure in rats of the mid- and high-dose groups; and returned to control values by 4 weeks and 26 weeks in the mid- and the high–dose groups, respectively. In mice, a transient increase in alveolar cell replication was only noted at 13 and 26 weeks post-exposure. Hamster mitotic indices remained equivalent to controls throughout the study.

The histopathological evaluation showed that the pulmonary lesions were the most severe in rats compared to mice and hamsters. Only appearance of particles within alveolar macrophages and very minimal changes in the patterns of alveolar macrophage accumulation in the lung were noted in the rats exposed to the low concentration. At the mid- and high concentrations, epithelial and fibroproliferative lesions, which were progressive even following cessation of particle exposure and diminution of pulmonary inflammation, were reported. These effects consisted of alveolar hypertrophy and hyperplasia of type II epithelial cells surrounding aggregations of particle-laden macrophages of minimal to mild severity, which became more severe at the highest concentration of 10 mg/m^3. Alveolar metaplasia (bronchiolization) and septal fibrosis were also noted in rats of the high dose group by 52 weeks post-exposure. In contrast, no epithelial, metaplastic or fibroproliferative changes were observed in mice and hamsters. In mice, the findings were limited to the presence of particles free and within alveolar macrophages and in alveolar septal regions at the low and middle concentrations. Minor epithelial changes primarily consisted of aggregations of heavily particle-laden macrophages concentrated in central lobar centriacinar sites, with perivascular lymphoid proliferation. Over the post-exposure period, there was evidence of concentration of these cell aggregates and movement to interstitial areas, primarily around blood vessels and peribronchiolar interstitium. No pathologies associated with treatment exposure were noted in hamsters, except particle-laden alveolar and interstitial macrophages and occasional aggregation of these particle-laden macrophages in the high concentration-exposed group.

The NOAEC for rats was established at 0.5 mg/m^3, based on inflammation evidenced in the BALF and pulmonary lesions (minimal hypertrophy and hyperplasia of type II alveolar epithelial cells) at 2 mg/m^3. The NOAEC for mice was set at 2 mg/m^3 based on inflammation evidenced in the BALF. The NOAEC for hamster was set at the highest tested concentration of 10 mg/m^3.

Some limitations associated with the testing protocol can be noted. First, the study was only performed on females and the analysis only focused on lung response, with limited level of details for the histopathological findings. Therefore, it cannot be completely ruled out that other findings occurred at non-inflammatory concentrations in other organs. In addition, even if P25 is a well-known form of TiO$_2$ (80%/20% anatase/rutile, 21 nm), the nanoparticles were not fully characterised in the study. Finally, P25 was not dispersed (by sonication for example) before exposure in order to generate the largest amount of free and/or aggregate particles.

Male Wistar rats were treated by head-nose inhalation with concentrations of 2, 10 or 50 mg/m^3 of TiO$_2$-NP (uncoated TiO$_2$ with hydrophobic surface, 14% rutile and 86% anatase forms, average primary particle size: 25.1±8.2 nm) for 5 days (Ma-Hock et al. 2009). Groups of 6 animals for each time point were used in the study. Following the exposure period, animals were held for recovery periods of 3 or 16 days. Changes in BALF parameters (increased of total cells and neutrophils) were observed, more pronounced 3 days after the end of the exposure than immediately after and decreasing 16 days post exposure. There were also minimal and minimal to mild diffuse alveolar infiltration with histiocytes at 10 mg/m^3 and 50 mg/m^3 and hypertrophy/hyperplasia of bronchioles and bronchi at 50 mg/m^3. In addition, the authors observed an increase in labelling indices in both large/medium bronchi and terminal bronchioli in all treatment groups after the end of the exposure period. Based on this last observation, they establish the LOAEC at the minimal concentration of 2 mg/m^3. The results and conclusions of the current study are very consistent with those of Bermudez et al. (2004), although the duration of exposure in this study is shorter.

Male Wistar rats (8 animals/group) were exposed for 6h/day on 5 consecutive days by head-nose exposure to 0.5, 2 and 10 mg/m^3 nano-TiO$_2$ (T-Lite SF, 15x50 nm, rutile with minimal anatase, coated with dimethicone/methicone copolymer). An additional group exposed to 10 mg/m^3 was held for a recovery period of 3 weeks (Landsiedel et al. 2014). Exposure to T-Lite SF induced a concentration-dependent increase in PMN and monocytes in the BALF at 2 mg/m^3 and 10 mg/m^3. The inflammatory response was associated with an increase in LDH and ALP release at the same concentrations. The BALF parameters remained elevated at the end of the recovery period for the group exposed to 10 mg/m^3. In addition, numerous pigment-loaded alveolar macrophages were observed within the alveoli along with slight diffuse histiocytosis at this concentration, not fully reversible after 3 weeks of recovery. The authors report a NOAEC of 0.5 mg/m^3 based on the pulmonary inflammation evidenced in the BALF parameters. The concentration-dependent increase in pulmonary inflammation observed is this study is consistent with the findings of Bermudez et al. (2004).

Male Fischer rats were exposed whole body to 0.50 ± 0.26 and 1.84 ± 0.74 mg/m^3 of TiO_2 (MT-150AW; rutile; spindle-shaped; 12 x 55 nm; purity = 99.5%) for 4 weeks, 6h/day, 5 days/week (Oyabu et al. 2017). Following the exposure period, animals were held for recovery periods of 3 days, 1 or 3 months. The study primarily focused on biopersistence, but some information on lung histopathology was also reported. After exposure to MT-150AW for 4 weeks, the biological half-time was estimated to be approximately 2 months for both tested concentrations. Histopathologically, only alveolar macrophages containing nanoparticles were noted at 3 days after exposure to 1.84 mg/m^3. For comparison, other groups of animals were exposed by instillation to 0.2, 0.36 or 1 mg of TiO_2. Similar results in term of biological half-life and histopathological findings were reported. Based on these results, the authors conclude to a comparatively good correlation between biological half-life and total cell counts, PMN, LDH, cytokine-induced neutrophil chemoattractant 1 (CINC-1), and HO-1 in the BALF.

Additional studies were performed by inhalation, but with only one concentration, which does not allow to establish a dose-response relationship.

Male C57Bl/6 mice exposed to 5 nm anatase TiO_2 for 10 days at 8.88 +/- 1.98 mg/m^3 showed modest but significant inflammatory response (number of alveolar macrophages elevated) among animals necropsied at week 0, 1, 2 and 3 postexposure. Mice fully recovered from the inflammatory response at week 3 post-exposure (Grassian, O'Shaughnessy P, et al. 2007)

Female Wistar rats were nose-only exposed to 10 mg/m^3 P25 TiO_2, 6h/day for 21 consecutive days. Following the exposure period, animals were held for recovery periods of 3, 28 or 90 days. Toxicological investigations, limited to the description of lung toxicity, were not the primarily aim of this study, which was to assess the RDI of TiO_2-NP as described in section 3.1. The authors reported, in a few lungs, multifocal, acute alveolar emphysema, accentuated in caudal and marginal lung parts. Minimal interstitial infiltration with mononuclear cells and minimal alveolar infiltration with neutrophilic granulocytes were also observed. A few animals showed minimal bronchiolo-alveolar hyperplasia. The leucopenia observed after 28 and 90 days recovery (reduced white blood cell and lymphocytes count) could be explained by the infiltration of the lungs with these cells (Eydner et al. 2012). This leucopenia was also observed after a daily whole body exposure for 3 weeks to 6 mg/m^3 of anatase TiO_2 (20 nm) (Yin et al. 2014)

Persistent inflammation was also reported in female mice when TiO_2-NP (UV Titan L181; rutile surface coated, 17 nm, Chemical composition: Na_2O (0.6%), SiO_2 (12.01%), Al_2O_3 (4.58%), ZrO_2 (1.17%), TiO_2 (70.81%), polyalcohol adding to the remaining wt %) was administered whole-body 10 days during gestation at 42 mg/m^3, 1 hour per day (Jackson et al. (2013) - see section 3.4.4 for details). At the same dose and with similar exposure scenario (11 days, 1h/day), Halappanavar et al. (2011) exposed mice by whole body inhalation and reported slight changes in pulmonary inflammation biomarkers (induction of genes associated with inflammation and increased in neutrophils proportion in BALF). However, the relevance of the results from these studies is questionable considering the relatively high concentration used.

In the study of Leppänen et al. (2011), mice were exposed by whole body inhalation to anatase:brookite (3:1) 20 nm TiO_2, 30 min, 4 days/weeks for 4 weeks to 0 and 30 mg/m^3. In this study, the authors observed airflow limitation, sensory irritation and pulmonary irritation. However, sensory and pulmonary irritations were both reported in the exposure and control groups.

In the study of Leppanen et al. (2015), mice were exposed by whole body inhalation to silica-coated rutile TiO_2 (10x40 nm) 30 min, 4 days/weeks for 4 weeks to 0 and 30 mg/m^3. An inflammation was observed in the murine airways as evidenced by the infiltration of inflammatory cells in peribronchial and perivascular areas. This inflammatory response was not likely due to radical formation since silica-coated nano TiO_2 did not significantly produce °OH radicals under UV radiation.

All the studies described above are in line and confirm the results from Bermudez et al. (2004), showing inflammatory effects at doses above 0.5 mg/m^3.

In line with acute toxicity, repeated dose toxicity studies are also available by intratracheal instillation, with doses varying from 1.25 to 10 mg/kg. These studies cannot be used in risk assessments because they are not representative of normal inhalation (the upper respiratory tract is bypassed) and they do not provide external exposure concentrations. However, even if they are not transposable quantitatively, they can provide additional useful information for hazard identification. Overall, those studies showed similar effects on the pulmonary tract as studies by inhalation (Sun, Tan, Ze, et al. 2012, Sun, Tan, Zhou, et al. 2012, Li et al. 2013, Hong et al. 2017).

4.2.2.2 Cardiovascular effects

Five studies identified from the literature evaluated cardiovascular effects of TiO_2-NP after repeated exposure.

Pregnant Sprague-Dawley female were exposed to 10 mg/m^3 P25 TiO_2 5h/d during approximately 8 days. The exposure induced a significant uterine microvascular dysfunction, with an alteration of reactivity after pharmacologic stimulation (ACh, NO donor...) or shear stress (Stapleton et al. (2013) – see section 4.5 for details).

Saber et al. (2013) analysed mRNA response of SAA, after an inhalation exposure to UV-Titan L181 at 42 mg/m^3 10 days during pregnancy (gestation days 8-18) in mice. The authors showed an increased in pulmonary Saa3 mRNA 5 days and 26–27 days after exposure compared to controls exposed to filtered air, but those results have to be taken with reservation because of the high dose used.

In the study performed by Yu et al. (2014), atherosclerosis occurred in mice after a daily exposure for 9-month by instillation treatment (1.25; 2.5 and 5 mg/kg) with anatase 5 nm, with effects seen at the low dose, increasing dose-dependently. The authors hypothesized that inhaled particles exert cardiovascular effects indirectly through the passage of inflammatory mediators from the lung to the systemic circulation, as the increased atherogenesis was concomitant with pulmonary inflammation and oxidative stress.

ApoE male knockout mice were exposed to 5-10 nm anatase TiO_2 twice a week during 6 weeks by instillation to 10, 50 or 100 µg/week. In accordance with studies described above, endothelial dysfunction, evidenced by dose dependant decrease in NO concentration and eNOS activity, and progression of atherosclerosis were observed. Lipid metabolism was also impacted with increased serum total cholesterol and decreased high density lipoprotein cholesterol (HDL-C) (Chen et al. 2013).

In contrast to the above-mentioned studies (acute and repeated exposures) reporting effects of TiO_2-NP on cardiovascular system with impaired vasodilation, there was no alteration of vasodilatory function in aorta segment in mice exposed by instillation of UV-Titan L181, and even an increased NO production in ex vivo experiment on human cells. This study also shows a modest increased atherosclerotic plaque progression (Mikkelsen et al. 2011).

4.2.2.3 Immunotoxicity

Several studies have evaluated immune effects of TiO_2: five of them used intratracheal instillation at doses up to 32 mg/kg for 4 or 5 weeks; six of them used an allergen sensitization and challenge to ovalbumin (OVA) with various routes of exposure (aerosolized, inhalation, intranasal exposure, nose-only application) at doses up to 15.7 mg/m^3 or 200 µg/mouse for single or repeated exposures (6 hours, 3 days, 4 weeks).

Rossi et al. (2010) observed that whole-body exposure to 10 ± 2 mg/m^3 of TiO_2-NP (silica coated, needle-like; 10 x 40 nm) for 4 weeks (3 times a week for 2 hours) induced Th1 type inflammation in healthy mice, with an increase in neutrophil attracting chemokine CXCL5 mRNA expression and in neutrophil influx in the lungs. Fu et al. (2014) observed a slight congestion and brown particulate accumulation in spleen along with increased T and B cells and an enhanced NK cell activity after instillation of P25 twice a week for 4 consecutive weeks in rats. In a similar protocol, Chang et al. (2015) observed that rat immunologically competent cells CD3+, CD4+ and CD8+ were significantly lower after exposure to P25 than in control group. Also, the ratio of CD4+ to CD8+ was significantly increased showing a disturbance of cellular immune function. However, no significant changes in IFN-γ and IL-4 were observed.

During OVA sensitization and challenge studies, exposure to TiO_2-NP can display an adjuvant activity on allergic airway inflammation depending on the timing of exposure. Indeed, this timing may account for differences in effects induced by TiO_2 nanoparticles by either promoting inflammation when TiO_2 exposure occurs prior to challenge (De Haar et al. 2006, Kim et al. 2017) or suppressing such effect when exposure is subsequent to the challenge (Scarino et al. 2012, Rossi et al. 2010). All these studies have used only one dose, except the study by Scarino et al. where rats were exposed by nose-only inhalation to 9.4 or 15.7 mg/m^3 of anatase 5 nm for 6 hours. Interestingly, two studies (Kim et al. 2017, Rossi et al. 2010) have evaluated a functional parameter i.e. airway hyperresponsiveness in addition to biochemical markers of inflammation. In the study by Rossi et al. (2010), TiO_2-NPs (silica coated, needle-like; 10 x 40 nm), administered at 10 mg/m^3 3 times a week for 2 hours for 4 weeks, downregulated Th2 type inflammation (i.e. infiltration of eosinophils and

lymphocytes in the lungs and expression of Th2 cytokines) and reduced the OVA-induced air hyperresponsiveness to the control levels of non-sensitized mice. In another study with sensitization and challenge with TDI, the authors showed that acute instillation of TiO_2-NPs (anatase; 15 nm) at 0.8 mg/kg significantly increased the inflammatory response in TDI-sensitised animals (significantly higher neutrophils, macrophages infiltration) (Hussain et al. 2011). In rats primed with endotoxin, anatase (20 nm) TiO_2-NPs caused a significant amplification of the inflammatory response induced by endotoxin or the particles alone after acute instillation (Oberdörster et al. 2000).

Liu et al. (2010) observed a reduced chemotactic ability and decreased expression of both Fc receptors and MHC-class II molecules on the alveolar macrophage cell surface after acute instillation exposure to anatase 5 nm. The mechanism responsible for this effect appears to involve increased nitric oxide and tumour necrosis factor-α.

Gustafsson et al. (2011) demonstrated that intra-tracheal exposure to P25 at 5 mg/kg in rats induced a transient influx in eosinophils and a more sustained neutrophilic response, followed by a recruitment of dendritic cells and lymphocytes expressing NK receptors. In line with the study of Chang et al. (2015) described above, a late-phase influx of lymphocytes to the rat lungs was dominated by CD4+ T-cells with smaller fractions of CD8+ T-cells and B-cells.

Scuri et al. (2010) whole-body exposed newborn, young and adult rats for 3 days to 12 mg/m^3 of P25. Although no differences were observed in BALF analysis and in lung histopathology, they showed that exposure of newborn and weanling rats to P25 influenced the expression of lung neurotrophins (NGF and BDNF), which play a critical role in the pathophysiology of childhood asthma.

4.2.2.4 Neurotoxicity

Eleven studies related to the neurotoxicity of TiO_2-NP have been evaluated: eight of them were from the same laboratory in China and investigated in mice:

1) the brain toxicity of the daily intranasal administration (500 µg in 10 µL Milli-Q water/mouse/day) for 30 days of 4 preparations of TiO_2-NP that differed by size and surface coating (Zhang et al. 2011);

2) differences in brain toxicity of TiO_2-NP according to the crystalline form of the preparation (anatase vs rutile), 500 µg in 10 µL Milli-Q water/mouse/day for 30 days (Wang, Liu, et al. 2008, Wang, Chen, et al. 2008);

3) the effect on brain and behaviour of the daily intranasal administration of TiO_2-NPs for 90 days at 3 doses (2.5, 5 and 10 mg/kg/day) (Ze et al. 2013, Ze, Sheng, Zhao, Ze, et al. 2014, Ze, Sheng, Zhao, Hong, et al. 2014, Ze, Hu, et al. 2014);

4) the neurotoxicity of the chronic intranasal administration (9 months) at doses of 1.25, 2.5 and 5 mg/kg/day (Ze et al. 2016).

The results obtained from Zhang et al. (2011) showed that the most deleterious effects on the brain (histological lesions of hippocampus and cortex tissue, and disturbances of extracellular dopamine, serotonin and noradrenergic levels measured in the same regions) were observed with rutile Si-coated TiO_2-NP (diameter = 10 or 50 nm) intranasally instilled (500 µg in 10 µL Milli-Q water/mouse/day) for 30 days compared to rutile uncoated TiO_2-NP (1 µm or 10 nm in diameter according to the preparation studied) administered using the same protocol.

The two studies by Wang and colleagues aimed to compare the neurotoxicity of a once every two days intranasal administration of 10 µL of two suspensions of non-coated TiO_2-NP for 30 days in female mice, one preparation containing rutile TiO_2 (diameter = 80 nm) and the other one anatase TiO_2 (diameter = 155 nm) (Wang, Liu, et al. 2008, Wang, Chen, et al. 2008). The results indicated the same histological and neurochemical alterations with both forms after 30 days of exposure, which were more pronounced with the anatase form compared to the rutile one. Results also showed a higher susceptibility of hippocampus to TiO_2-NP compared to the other brain regions studied that was correlated with the greater accumulation of TiO_2 observed in this part of the brain.

Studies from Ze and colleagues assessed the neurotoxicity in hippocampus of a repeated intranasal instillation of TiO_2-NP for 90 days at doses of 2.5, 5.0 or 10 mg/kg/day in mice. TiO_2-NP (anatase 6 nm, surface area 175 m^2/g form) was suspended in HMPC 0.5% for administration (Ze et al. 2013, Ze, Sheng, Zhao, Ze, et al. 2014, Ze, Sheng, Zhao, Hong, et al. 2014, Ze, Hu, et al. 2014). The findings of the four studies showed a translocation and accumulation of TiO_2-NP in brain with an overall proliferation of glial cells, tissue necrosis and signs of cellular degeneration observed in the hippocampus considered as a brain region of interest. Changes in hippocampal cell ultrastructure were observed in both TiO_2-NP exposed groups and were indicative of cell apoptosis (Ze, Hu, et al. 2014) possibly related to oxidative stress (Ze et al. 2013, Ze, Hu, et al. 2014) through activation of the p38-Nrf-2 signalling pathway (Ze et al. 2013), neuroinflammation and alterations of cytokine expression (Ze, Sheng, Zhao, Hong, et al. 2014). Down- or up-regulations of brain gene expression in genes associated with oxidative stress, immune response, apoptosis, memory and learning, brain development, signal transduction, metabolic process, DNA repair, response to stimulus and cellular process were observed with the dose of 10 mg/kg TiO_2-NP in the same region (Ze, Hu, et al. 2014). Finally, subchronic TiO_2-NP exposure induced significant long-term potentiation reduction and down-regulation of glutamate NMDA receptor subunits (NR2A and NR2B) expression associated with the simultaneous inhibition of CaMKIV, cyclic-AMP responsive element binding proteins (CREB-1, CREB-2) and FosB/DFosB in mouse hippocampal tissues, with a spatial memory recognition impairment (Ze, Sheng, Zhao, Ze, et al. 2014). Taken together, all these results suggest dose-dependent neurotoxicity of TiO_2-NP in anatase form, with hippocampus as a brain region of higher susceptibility, leading to impairments of synaptic plasticity and learning performances possibly related to neuroinflammation and oxidative stress.

Three other studies using intratracheal instillation (Horvath et al. 2017) or inhalation (Disdier et al. 2017, Yin et al. 2014) for TiO_2-NP exposure were also considered. In the study from Horvath et al. (2017), adult rats were dosed intratracheally (1 mL/kg b.w., 5days/week) daily for 28 days with a

suspension of TiO_2-NP (diameter = 10 nm) in PBS-HEC 1% at doses of 1, 3 or 10 mg/kg/day and were studied for various electrophysiological activities including spontaneous cortical activity, sensory evoked potentials and tail nerve action potential. Results showed the slow-down of the sensory evoked potentials and tail nerve action potential which were moderately correlated with brain Ti level and oxidative stress parameters. In the study of Disdier et al. (2017), young and aged rats (12-13 weeks and 19 months of age, respectively) were exposed to TiO_2-NP (75% anatase and 25% rutile, Aeroxide P25, diameter = 21 nm) nose-only 6 hours/day, 5 days/week for 4 weeks. Results showed increasing blood-brain barrier permeability in aged rats associated with neuroinflammation and decreased synaptophysin, a marker of neuronal activity. Yin et al. (2014) observed significant increases of H_2O_2 and MDA concentrations in mice brain homogenate extracts after whole body inhalation exposure to 6 mg/m^3 of 20 nm anatase TiO_2-NP for 3 weeks, suggesting that the brain was injured after inhalation of TiO_2-NP. No histological lesions were observed in this study.

4.2.2.5 Liver toxicity

Few studies have investigated liver toxicity induced by TiO_2-NPs. Halappanavar et al. (2011), in a study described above (cf. 3.4.1.1), observed no changes in the liver in a transcriptomic analysis.

A 4-week instillation study performed with TiO_2-NP (80% anatase/20% rutile, 21 nm) at concentrations of 0.5, 4 and 32 mg/kg twice a week in male Sprague-Dawley rats showed statistically significant increases in the AST level and oedema and loose cytoplasm on liver cells (Chang et al. 2015).

4.2.2.6 Kidney toxicity

Huang et al. (2015) studied effects of P25 TiO_2-NP exposure by instillation for 4 weeks at concentrations of 0.1, 0.25 or 0.5 mg/week on kidneys of ICR mice.

TiO_2 contents in the kidneys of P25-treated mice were significantly increased as compared with controls. Incidences of histological changes such as tubular dilation, necrosis and loss of the brush border were statistically significant at 0.5 mg/week and dose dependent. Alterations in oxidative stress markers (HO-1, nitrotyrosine, HIF-1α...) and renal function markers (BUN and creatinine) were also observed.

4.3 Genotoxicity

Publications related to mutagenicity of TiO_2-NP have been summarized in several reviews (Chen, Yan, and Li 2014, Magdolenova et al. 2012, Charles et al. 2018, ANSES 2016).

In vitro:

Many in vitro genotoxicity studies are available for TiO_2-NP. A review of in vitro data published between 2010 and 2016 was performed by Charles et al. (2018). A summary of the studies with the higher level of confidence is presented in the table below.

Most of the published results refer to the anatase form as well as mixture of anatase and rutile (generally P25). Very few studies assessed the genotoxicity of coated TiO_2-NP or rutile forms.

Table 5: Summary of genotoxicity studies on TiO_2-NP (from Charles et al. (2018))

Form of the TiO_2-NPs tested	MN assay	Comet assay	Chromosomal aberrations assay	Total
Anatase	14/25 (56%)	46/77 (58%)	1/3 (33%)	61/105 (58%)
Rutile	3/3 (100%)	12/24 (50%)	0/0 (0%)	15/27 (55%)
Mixture anatase/rutile	7/15 (47%)	25/37 (68%)	0/2 (0%)	32/54 (59%)
Coated-rutile	2/3 (67%)	8/13 (61%)	0/0 (0%)	10/16 (62%)
Rutile-brookite-anatase	0/1 (0%)	0/0 (0%)	0/0 (0%)	0/1 (0%)
Coated-anatase	1/2 (50%)	1/4 (25%)	0/0 (0%)	2/6 (33%)
Anatase-brookite	0/1 (0%)	4/6 (67%)	0/0 (0%)	4/7 (57%)
Coated-anatase-brookite	1/2 (50%)	15/16 (94%)	0/0 (0%)	16/18 (89%)
Total	18/52 (35%)	111/177 (63%)	1/5 (20%)	140/234 (60%)

[a]Since specific protocol parameters (e.g. cells, media, exposure-duration, standard or modified protocol, etc.) and forms of TiO_2-NPs varied among the 88 assays, the number of total testing conditions ended up to 234.

According to Charles et al. (2018), both negative and positive results are reported in the *in vitro* mutagenic assays. Most of the positive results were found at high doses in micronucleus and Comet assays with a dose-response relationship. Inconsistencies observed in the results of the studies may be the result of differences in test materials (e.g. size, crystallinity, coating). However, it currently remains difficult to highlight which parameter(s) could drive these differences. Those inconsistencies could also be explained by the various test conditions used, including dispersion of the material, concentrations and exposure duration, cell/organ examined and parameter assessed. Moreover, numerous interferences with TiO_2 can occur due to fluorescence and absorbance interaction. Other interactions with the proteins or enzymes used during the assay are likely to occur; unfortunately, these interferences were not properly tested in most of the publications. All these elements did not permit an easy comparison of the studies.

In vivo:

A review of the available *in vivo* studies has been performed by ANSES (2016) with the following statements: *"Several in vivo studies with different protocols, tested materials, routes of exposure are available with TiO_2-NP. Most of the studies referred to the anatase form. Thirty-eight experiments over the 125 identified reported positive results. Most of the positive results were found in Comet assays, 8-oxodG tests and H2Ax phosphorylation assays. Several limitations can be noted from almost all studies including the lack of positive control, the absence of evidence of uptake, insufficient characterization of the tested material etc".*

Table 6 : Summary of positive responses in function of crystalline phase of TiO_2-NP according to the authors – all routes of exposure (extracted from ANSES (2016))

Assays	Micronucleus assay	Comet assay	Mutation assay	DNA oxidative lesions	DNA adducts	H2Ax phosphorylation assay	Total
Nanoforms							
Anatase	2/9	5/22	0/7	5/6	0/0	1/1	13/45
Rutile	0/2	0/0	0/0	1/3	0/0	0/0	1/5
Anatase/rutile	3/7	8/20	2/2	1/2	0/1	1/1	15/33
Anatase coated	0/1	1/5	0/0	0/0	0/0	0/0	1/6
Rutile coated	0/6	5/22	0/1	0/0	0/0	0/0	5/29
Anatase/rutile coated	0/0	0/0	0/0	0/1	0/0	0/0	0/1
Brookite/anatase	0/1	0/1	0/0	0/0	0/0	0/0	0/2
Unspecified	1/1	1/1	0/0	1/2	0/0	0/0	3/4
Total	6/27	20/71	2/10	8/14	0/1	2/2	38/125

Some studies include several experiments with different NM and some NM can show negative and positive results within a study, depending on the organ examined. Each result was counted in all the relevant sections. An experiment is defined by a tested material and a specific protocol (ex. organ examined, duration…).

Based on the ANSES (2016) review, an update of the literature search for TiO$_2$-NP has been performed up to December 2017, focusing on *in vivo* studies carried out by the respiratory route (see annex 2). The following new *in vivo* studies by respiratory route have been identified in the literature:

Tableau 7 : Summary of the *in vivo* mutagenicity studies performed with TiO$_2$-NP by the respiratory route, identified during the update of the literature search

Method	Results	Remarks	Reference
INHALATION ROUTE			
BALB/CcJ female mice TiO$_2$ anatase, 10 nm, BET: 173 m^2/g, geometric mean diameter in dispersion: 504 nm (mostly aggregates/agglomerate) - 1.1x10^5 particles/cm^3 271 mg/m^3 for 1 hour, inhalation head-only (as dry powder)	Comet: Positive in the lung Airway irritation, lung inflammation	No positive control presented Only one high concentration.	Larsen et al. (2016)

Estimated deposited dose in airways: 90.5 µg Comet assay on BAL and lung 24h after inhalation			
INSTILLATION ROUTE			
Male Sprague-Dawley rats (12/group) P25 in PBS (phosphate-buffered saline): primary particles (25 +/- 15 nm) and 100 nm agglomerates 3 endotracheal instillation at a 4-days interval. Sacrifice after 2h and 35 days. 0.17, 0.83, and 3.33 mg/ml, respectively, by instillation --> total: 0.5, 2.5, and 10 mg/kg or 87, 437, and 1700 cm^2/lung. Comet assay on lung cells, blood cells and liver cells ɣ-H2AX assay on lung, blood and liver cells Pig A in blood cells 35 days after exposure.	**Comet assay:** Positive (lung, blood and liver after 2h and/or 35 days). No increase of hedgehog. **ɣ-H2AX assay:** Positive in lung (immediately after exposure at highest dose) and Negative in liver and blood **Pig A:** Negative Acute lung inflammation (only 2h after administration) but no oxidative stress. Kinetics part of the study: measured lung burdens after 3 months (20%, 63%, and 83% of initial lung burden for low, mid, and high doses, respectively) --> half-retention time > 60 days for the 2 highest doses.	No negative or positive control presented Instillation route, worst case exposure (bolus administration)	Relier et al. (2017)
Female mice (8/dose/time point) NRCWE-001: TiO$_2$ unmodified rutile with endogenous negative surface charge; 10 nm; BET = 99 m^2/g NRCWE-002: TiO$_2$ rutile with positive surface charge by coating with 3-aminopropyltriethoxysilane (purity 99%); 10 nm; BET = 84 m^2/g Agglomerates smaller than 100 nm in water 18, 54 or 162 µg/mouse by instillation, mice were killed after 1, 3 or 28 days. Comet assay on BAL cells, lung and liver tissues.	**NRCWE-001:** Positive in the lung (at 1 and 28 days) and in the BAL (at 18 and 54 µg on day 3) but Negative in the liver **NRCWE-002:** Positive in the lung (at 1 and 28 days) and in the liver and the BAL (only on day 1; not consistent finding) Inflammation, time and dose-dependent which persisted at the highest dose 28 days after exposure.	No significant effect of the charge on the result of the comet assay Instillation route, worst case exposure (bolus administration)	Wallin et al. (2017)

gpt delta transgenic mice (4-8/groups; both sexes) TiO_2; 28 nm ; 10-60 nm by TEM; BET = 45 m^2/g; 90% anatase/10% rutile In physiological saline = 280 nm (DLS). 50 µg administrated by instillation once. Lung collected 4 months after exposure. Determination of gp mutant frequency (genomic DNA from lung) 8 oxodG measured in the lung by ELISA ɣ-H2AX assay	**gp mutant frequency**: negative **8 oxodG**: negative **ɣ-H2AX assay**: negative (slight but not significant increase) None or a mild inflammatory response, no obvious fibrotic response in the lungs at 4 months after TiO_2 exposure	Negative control included but no positive control. Instillation route, worst case exposure (bolus administration) Only one high concentration.	Wan et al. (2017)

Similarly to *in vitro* data, results from *in vivo* studies are inconsistent and positive results are provided mainly by Comet tests at high doses. It was again not possible to identify the causes of the inconsistencies (e.g. different protocols, different forms of TiO_2-NP). It should also be noted that most of the *in vivo* studies are associated with several methodological limitations (lack of positive control, no proof of target organ exposure, insufficient characterization of the tested material, non-physiological route of administration, etc…).

Hypothesized genotoxic mode of action of TiO_2-NP:

Primary genotoxicity could be the result of direct interaction with DNA or indirect mechanisms with molecules interacting with the genetic material. Secondary genotoxicity could result from ROS generated by the catalytic potential of the particles, activation by UV light or during particle-elicited inflammation.

Theoretically, TiO_2-NPs may **interact with DNA**, since particles were detected (by TEM and/or Raman imaging) inside the nucleus in a few *in vitro* and *in vivo* studies. However, the possible mechanism of penetration into the nucleus is unclear.

DNA damage can also arise through **indirect mechanisms** where the NPs do not physically interact with the DNA molecule. In particular, decreased nucleotide excision repair (NER) and base excision repair (BER) activities reported in A549 cells exposed to TiO_2-NPs (Jugan et al. 2012, Armand et al. 2016) suggest an effect on repair mechanism. In addition, some publications reported disturbances of mitosis and abnormal multipolar spindle formation, chromosomal alignment and segregation during anaphase and telophase, as well as disturbance of the cell cycle checkpoint function (Magdolenova et al. 2012). It is difficult to judge the relevance of these results since there is no harmonized tool to investigate these types of mechanisms.

Secondary genotoxicity can be induced by ROS or reactive nitrogen species (RNS) generated at the surface of NPs or produced by inflammatory cells. Positive results reported both *in vitro* and *in vivo* were often associated with oxidative stress (evidenced by increase of ROS production, glutathione content, lipid peroxidation, malondialdehyde level, antioxidant enzyme level or suggested in the *in vitro* comet assay where specific DNA repair enzymes had been added) and/or with inflammation (up-regulation of pro-inflammatory cytokines and increased cells such as neutrophils in the BALF) (Charles et al. 2018).

In summary, the production of ROS seems to be the major mode of action explaining these effects, as evidenced by oxidative stress/ inflammation reported in many positive studies. However, ROS production may not be the sole mechanism explaining the genotoxicity found with TiO_2-NPs. At present, other mechanisms of action cannot be formally ruled out but there is no validated tool to investigate other possible genotoxic modes of action.

Conclusion on genotoxicity of TiO_2-NP:

Data from published reviews on the genotoxic potency of TiO_2-NP are numerous resulting from a large panel of tests carried out both *in vitro* and *in vivo*, of variable quality. In addition, various forms of TiO_2-NP with different physico-chemical characteristics (shape, size, coating, surface reactivity, charge, crystallinity…) were tested in rodents by inhalation or intratracheal instillation and on several cell types and tissues under different conditions of exposure. Although an impressive data set is available, it is still difficult to distinguish the key physico-chemical parameter(s) of the nanoparticles that are related to the genotoxic effect.

Despite the inconsistency of the response among all the available data, it is noted that TiO_2-NP may induce genotoxicity in rodent lungs but also in organs such as liver and in *ex vivo* cells or cell lines. Most of dose-related positive results were obtained in the Comet and micronucleus assays and only at high concentrations. Such genotoxic effects are probably due to an increase in ROS/RNS secondary to an inflammatory process that is frequently involved in the toxicity of TiO_2-NP. On the other hand, it is still unclear whether other pathways than oxidative stress could also play a role in the induction of DNA damage.

In summary, considering that:

- **the results from *in vitro* and *in vivo* mutagenic assays are rather inconsistent;**
- **the positive effects were primarily reported at high concentrations;**
- **the identification of the underlying reasons for the differences of responses reported is not possible;**
- **the data point to a secondary genotoxic mode of action involving ROS/RNS production;**
- **the carcinogenic effects appear only at high concentrations, associated with altered clearance and inflammatory responses (see also section 3.4.3),**

it can be concluded that TiO_2-NP is a weak genotoxic agent. These conclusions are in line

with those from other organisations or reviews of TiO_2 genotoxicity (IARC 2010, NIOSH 2011, ANSES 2016, OECD 2018a, Charles et al. 2018).

4.4 Carcinogenicity

4.4.1 Human data

Seven epidemiological studies analyzed potential link between occupational exposure to TiO_2 and the occurrence of cancers, including 5 report historical industry-based cohorts (Chen and Fayerweather 1988, Fryzek et al. 2003, Boffetta et al. 2004, Ellis et al. 2010, Ellis et al. 2013) and 2 case-control studies (Boffetta et al. 2001, Ramanakumar et al. 2008). The characterization (size, crystallinity) of TiO_2 is not provided in the publications. In this context, it cannot be excluded that workers are exposed, at least partially, to TiO_2 under nanoform in these studies.

Despite the availability of five retrospective cohort studies, some of them share a large part of their population leading in fact to only three separate populations. All of these studies present biases for worker selection and possible misclassification of exposure and health status. Even with these limitations, two publications on two different populations (one US and one European) reported statistically increased mortality by lung cancer (Ellis et al. 2013, Boffetta et al. 2004). Boffetta et al. (2004) reported a statistically significant increased standardized mortality ratio (SMR) for mortality from lung cancer (23% increase considering all studied countries with a 51% increase in Germany). Ellis et al. (2013) reported a significant increase of mortality by lung cancer (68% increase) when comparing to Dupont referent workers. In addition, increased mortality by lung cancer of borderline significance was also consistently found in all publications, except Chen & Fayerweather (1988). The absence of statistical relationship between duration / level of exposure to TiO_2 and an excess of lung cancer can be hidden due to the methodological deficiencies (bias selection, misclassification of exposure and health status, worker health effect, confounding factors, categorization by level of exposure etc.).

Regarding case-control studies, they are judged of limited relevance to conclude on lung carcinogenicity of TiO_2 considering the mode of action expected to be primarily linked to its dust nature.

In conclusion, the epidemiological data are inadequate to conclude on the carcinogenicity of TiO_2-NP (Guseva Canu et al., 2019[15]).

4.4.2 Animal data

[15] This study, outside the time period of the bibliographic search, was exceptionally added as Anses was involved.

Carcinogenic potential of TiO_2 (under micro and nanoforms) was reviewed by IARC (IARC, 2006 & 2010), by NIOSH (NIOSH 2011) and also recently by RAC (Risk Assessment Committee) of ECHA (ECHA 2017) in the framework of CLP Regulation (EC n°1272/2008), based on a classification proposal made by France (ANSES 2016, ECHA 2017).

Experimental studies on TiO_2-NP are summarized in table 8 below.

Table 8 : Summary of animal carcinogenicity data on TiO_2-NP by respiratory route (extracted from ANSES, 2016)

Method	Results	Remarks	Reference
Inhalation route			
Female Wistar rats [Crl:(WI)BR] and NMRI mice TiO_2, 15-40 nm, P25 (≈ 80% anatase and ≈ 20% rutile) Whole body exposure by inhalation: 18h/d, 5 days/week: 7.2 mg/m3 for the first 4 months, then 14.8 mg/m3 for 4 months followed by 9.4 mg/m3 for 16 months for rats and 5.5 months for mice (cumulative particle exposure: 88.1 g/m3xh for rats and 51.5 g/m3xh for mice) Recovery period: 6 months for rats and 9.5 months for mice Not guideline, no GLP status	↑ benign keratinizing cystic squamous cell tumours, squamous-cell carcinomas, bronchioalveolar adenomas and adenocarcinomas in rats. Not carcinogenic in mice. ↑ mortality and ↓ body weight in both species. Impairment of clearance function, bronchioalveolar hyperplasia and interstitial fibrosis in rats.	Purity lacking. One concentration varying during the experiment, only females tested. High background tumour response in control mice. Non-neoplastic effects and lung clearance not reported in mice. R = 3	Heinrich et al. (1995)
Instillation route			
SPF Wistar female rats TiO_2 P25: 5x3mg, 5x6 mg or 10x6 mg by instillations TiO_2 P805: 15x0.5 mg or 30 x0.5 mg by instillation Animals sacrificed after 30 months. Not guideline, no GLP status	↑ benign tumours (adenomas and epitheliomas) and malignant tumours (adenocarcinomas and squamous cell carcinomas) at all tested doses.	TiO_2-NP P25, majority anatase, 25 nm TiO_2-NP P805 (P25 coated with trimethoxyoctyl-silane), 21 nm Purity lacking. Only females tested. Higher number of tumours with TiO_2-NP compared to fine TiO_2. R = 2	Pott and Roller (2005)
F344/DuCrl Crj male rats	No promotor potential by instillation.	Many parameters did not match with standard protocol for carcinogenesis assessment; no valid	Yokohira et al. (2009)

TiO_2-NP, 80 nm DHPN (initiation) for 2 weeks, then 0.5 mg/rat TiO_2 once in week 4 by instillation. Not guideline, no GLP status	No lung lesion without pre-treatment with DHPN.	positive control; only males tested; no clear information on crystallinity R = 3	
Hras 128 transgenic female rats TiO_2 non coated, rutile, 20 nm DHPN (initiation) for 2 weeks. Then, 250 µg/ml or 500 µg/ml TiO_2 once every 2 weeks from the end of the week 4 to week 16 by instillation. Not guideline, no GLP status	Promotor effect observed: ↑ multiplicity of DHPN-induced alveolar cell hyperplasia and adenomas in the lung at all doses, and the multiplicity of mammary adenocarcinomas at 500 µg/ml. Not carcinogenic without pre-treatment with DHPN.	Purity lacking. Little experience with this model. No positive control included. Only females tested. R = 3 Not reliable study.	Xu et al. (2010)

Only one carcinogenicity study by inhalation is available with adequately characterized TiO_2-NP (Heinrich et al. 1995). Female Wistar rats [Crl:(WI)BR] and NMRI mice were exposed whole body 18h/day, 5 days/week to aerosol of TiO_2 (P25, primary particle size 15-40 nm, ≈ 80% anatase and ≈ 20% rutile). The mean particle mass exposure concentration was 7.2 mg/m^3 for the first 4 months, followed by 14.8 mg/m^3 for 4 months and 9.4 mg/m^3 for 16 months for rats and 5.5 months for mice. Following the exposure period, the animals were kept under clean air conditions for an additional 6 months for rats and 9.5 months for mice. Rats developed lung tumours (benign keratinizing cystic squamous cell tumours, squamous-cell carcinomas, bronchioalveolar adenomas and adenocarcinomas) from 18 months of exposure. At the tested concentration, an increased mortality rate (60% versus 42% in the control group), a decreased body weight, an increase of lung wet weight, an alteration of alveolar lung clearance and non-neoplastic effects in the lung (bronchioalveolar hyperplasia and interstitial fibrosis) were also reported. No increased lung tumour rate was reported in mice. However, the high background tumour response in the control group might have limited the ability to detect any carcinogenic effects in this study.

Similar types of lung tumours were reported in rats intra-tracheally exposed to P25 (Pott and Roller 2005). Finally, two other intra-tracheal studies assessing the promotor potential of TiO_2-NP (rutile 20 nm or TiO_2-NP 80 nm) did not report any effect. However, the protocols used as not judged reliable and the studies have been disregarded (Xu et al. 2010, Yokohira et al. 2009).

An update of the literature search was performed until December 2017 focusing on studies carried out by the respiratory route (see annex 2). No reliable study was found in this update.

Conclusion on carcinogenicity of TiO_2-NP:

Based on the induction of lung tumours reported after inhalation and instillation exposures (Pott and Roller 2005, Heinrich et al. 1995), TiO_2-NP (P25 as material tested) is considered as a lung carcinogen in rats at a concentration resulting in pulmonary inflammation and altered clearance. This conclusion is in line with those of IARC (2006 & 2010), which classified TiO_2 (without more specifications) as possibly carcinogenic to humans (Group 2B), of NIOSH (2011), which considered ultrafine TiO_2 as potential occupational carcinogen and of RAC (ECHA, 2017), which concluded that TiO_2 (without further physico-chemical description) should be classified as Category 2 carcinogen (suspected human carcinogen) according to CLP Regulation. Especially, the RAC conclusion is based on a following weight-of-evidence approach:

- taking note that TiO_2 was not shown to be a multisite carcinogen,
- being aware that TiO_2 is a lung carcinogen especially in female rats,
- recognising that there are no robust carcinogenicity studies in species other than rats,
- recognising that the majority of rat lung tumours occurred late in life,
- recognising that rat lung tumours only developed under inhalation exposure conditions associated with marked particle loading of macrophages,
- presuming a practical threshold for lung tumour development (mutagenicity in lung cells is considered to depend on chronic inflammation and oxidative stress),
- taking note of experimental, mainly repeated dose toxicity data indicating a lower sensitivity of other small rodents, monkeys and humans compared to rats,
- being aware of TiO_2 epidemiology studies which do not consistently suggest an association between occupational exposure to TiO_2 and lung cancer mortality.

4.5 Toxicity to reproduction

The studies presented in the table below have been identified from the literature search performed up to 2017 focusing on studies carried out by the respiratory route (see annex 2).

No study performed with a standard protocol to assess fertility and development is available by respiratory route.

Table 9 : Developmental toxicity studies identified in the literature for TiO_2-NP

Method	Results	Remarks	Reference
INHALATION ROUTE			

Pregnant female C57BL/6BomTac mice (22-23/group) UV-Titan L181 (rutile 70,8% modified with Zr Si, NaO, Al, coating polyalcohol); 21 nm 40 mg/m^3 (measured: 42.4 ±2.9 mg/m^3; 1.70 ± 0.2 x10^6 part./cm^3; peak size: 97 nm) whole-body for 1h/d; GD8-18 Parameters: maternal lung inflammation, gestational and litter parameters; offspring neurofunction and fertility (exposure C57BL offspring cross-mated to naïve CBA/J mice)	<u>Time-mated adult female mice:</u> Lung contained 38 mg Ti/kg on day 5 and 33 mg Ti/kg on day 26-27 after exposure. Low or no Ti in liver. Decreased absolute and relative lung weight. No effect on gestational and litter parameters. Lung inflammation (BAL) 5 day and 26-27 days following exposure termination. <u>Gestationally exposed offspring:</u> Moderate neurobehavioral alteration (spent significantly less time in the central zone of the field and visited the central zone less frequently, startled less). <u>Fertility part of the study</u>: Low or no Ti in liver and milk. No significant effect on fertility.	Only one high concentration tested	Hougaard et al. (2010)
C57BL/6 mice UV-Titan L181 (rutile 70,8% modified with Zr Si, NaO, Al, coated with polyalcohol); 20.6 nm; BET = 107.7 m^2/g 42.4 mg/m^3 for one hour/day; GD8-18 by inhalation, whole body. Female offspring were mated with unexposed CBA males. F2 descendants collected on PND2-7 or PND80 and ESTR germline mutation rates estimates from full pedigrees of F1 female mice	No evidence for increased ESTR mutation rates in F1 and F2 offspring. No effect on viability, no effect on sex-ratio.	Only one high concentration tested	Boisen et al. (2012)
Pregnant female C57BL/6 mice (n=12-13) UV-Titan L181 (rutile 70,8% modified with Zr Si, NaO, Al, coated with polyalcohol); 20.6 nm Exposure to 42 mg/m^3 for 1h/d by inhalation whole body; GD8-18 F1 (C57BL/6J) offspring (n = 25) mated with unexposed CBA/J (cross-mating males/females) Assessment of male reproductive function in the two following generations (body and testicle weight, sperm content per g testicular parenchyma and daily sperm production (DSP))	Daily sperm production (DSP) not statistically affected in the F1 generation, although TiO_2 tended to reduce sperm counts (-12%). Time-to-first F2 litter increased with decreasing sperm production. Effect on sperm production in the F2 generation.	Only one high concentration chosen to correspond to half of the Danish OEL (8h). Need to optimize the method for measurement of DSP.	Kyjovska et al. (2013)

Time-mated C57BL/6Bom-Tac female mice (22-23/group) UV Titan L181 Rutile surface coated, 17 nm, surface area: 70 m^2/g Chemical composition: Na_2O (0.6%), SiO_2 (12.01%), Al_2O_3 (4.58%), ZrO_2 (1.17%), TiO_2 (70.81%). UV-Titan is coated with polyalcohol adding to the remaining wt %. Geometric mean size during inhalation exposure: 97 nm 42 mg/m^3 for 1h/day whole body during GD8-18. Parameters: Comet assay in BAL +/- liver; hepatic gene expression; lung inflammatory response	Persistent inflammation in mothers and affected gene expression in the liver of offspring, with increased response in female offspring. The observed changes in gene expression in the newborn offspring 2 days after birth suggest that anti-inflammatory processes were activated in the female offspring related to retinoic acid signalling. Negative *in vivo* comet assay (BAL and liver in the non-pregnant females and dams; liver in the newborn at PND 2 or weaned offspring at PND 22).	Only one high concentration.	Jackson et al. (2013)
Pregnant female Sprague-Dawley Aeroxide (anatase/rutile ; 21 nm) Whole body exposure to 11.3 ±0,039 mg/m^3 for 5h/d from GD10 for an average of 8.2±0.85 days Microvascular tissue isolation (GD20) and arteriolar reactivity studies of the uterine premyometrial and fetal tail arteries	Significant maternal and fetal microvascular dysfunction. Isolated maternal uterine arteriolar reactivity consistent with a metabolically impaired profile and hostile gestational environment that impacted fetal weight. Isolated fetal microvessels demonstrated significant impairments to signals of vasodilation specific to mechanistic signalling and shear stress.	Only one concentration. Even if not clearly stated in the publication, tested material corresponds to P25	Stapleton et al. (2013)
Pregnant female Sprague-Dawley (6/group) Aeroxide (anatase/rutile ; 21nm) Exposure to 10.6 ±0.3 mg/m^3 for 5h/d GD6-12 (average of 6.8±0.5 days) by inhalation, whole body. Maternal or litter characteristics (maternal weight, implantation site, pup/litter, progeny weight at w 8, 12, 16 and 20) Microvascular reactivity, mitochondrial respiration and hydrogen peroxide production of the coronary and uterine circulations of the female offspring evaluated between 11 and 16 weeks of age	No significant differences within the maternal or litter characteristics. No significant differences in spontaneous active diameter, passive diameter or vascular tone with respect to coronary arterioles. No oxidative stress (hydrogen peroxide production). Endothelium-dependent dilation and active mechanotransduction in both coronary and uterine arterioles abolished. Significant reduction in maximal mitochondrial respiration (state 3 – maximal mitochondrial state) in the left ventricle and uterus.	Only one concentration. May be attributed to altered NO signalling (decreased NO bioavailability associated with oxidative NO scavenging). Even if not clearly stated in the publication, tested material corresponds to P25	Stapleton, Nichols, et al. (2015)
Pregnant female Sprague-Dawley (4/group)	No effect on maternal weight, implantation site number or pup number per litter.	Few animals tested. Only one concentration.	Engler-Chiurazzi et al. (2016)

P25; count median aerodynamic diameter of 171 nm Exposure to 10.4 mg/m^3 for 5h/d; 4d/w; GD7-20 (for 7.8±0.5 days) by inhalation whole body Behaviour and cognitive functions of male pups at 5 months of age	No effect on locomotor, balance, affective, anxiety-like or depressive-like behaviour in the male pups. Reference memory learning, retention, and perseveration not markedly altered. Prenatal TiO_2-NP exposure induced significant working or short-term memory impairments and initial motivation: alteration in cognitive behaviour.		
Pregnant female Sprague-Dawley P25 (anatase/rutile ; 21nm) Whole body exposure to 10.35+/-0.13 mg/m^3; 5h/d treatments; from GD~6, with the last exposure given 24 h before birth, for a total of approximately 8 exposures (7.79+/-0.26 days) Physiological and bioenergetic effects on heart function and cardiomyocytes across three time points, fetal (GD20), neonatal (4–10 days), and young adult (6–12 wk).	Cardiac impairment of the progeny (systolic and diastolic abnormalities and cardiomyocyte contractile attenuation). Mitochondrial respiration dysfunction, with varying degrees of impairment across developmental stages.	Only one concentration.	Hathaway et al. (2017)
Pregnant female Sprague-Dawley P25 (anatase (80%) and rutile (20%)); primary particle size: 21 nm; specific surface area: 48.08 m^2/g; zeta potential: -56.6 mV Real-time TiO_2-NP mobility diameter: 129 nm, aerodynamic diameter: 143 nm Exposure by whole-body inhalation to 10 ± 0.5 mg/m^3, 4-6h/exposure, from GD5.78 ± 0.11 for 7-8 days (calculated, cumulative lung deposition = 217 ± 1 µg); isolation of 20 fetal hearts on GD20 Investigation of cardiovascular function.	No effect on progeny weight or total number of pups. Significant epigenetic and transcriptomic changes in the cardiac tissue (increased cardiac function); altered signalling liver and kidney pathways; increased inflammatory signalling and growth/survival	Only one concentration.	Stapleton et al. (2018)
INSTILLATION ROUTE			
C57BL/6 mice (5-6/group) TiO_2 anatase; about 6 nm (stable colloidal suspension of primary particles) – self-prepared in a Research laboratory	<u>Single dose</u>: inflammatory cell influx <u>3-doses</u>: increased inflammation and inhibition of lung development (increased mean linear intercept and decreased radial alveolar count) without effect on function	Only one concentration. Instillation route, worst case exposure (bolus administration)	Ambalavanan et al. (2013)

Treatment at 1 mg/kg on PND4 or PND4, 7 and 10 Measurement of lung function (compliance and resistance), development (morphology), inflammation (histology; multiplex analysis of BALF for cytokines) on PND14	Macrophages were noted to take up the TiO_2-NP, followed by polymorphonuclear infiltrate Inflammatory cytokines and matrix metalloproteinase-9 were increased in lung homogenates, and VEGF was reduced		
Pregnant female C57BL/6 mice TiO_2 anatase; 12.3 nm; BET: 96 m^2/g; zeta potential at pH7: 3.7 and hydrodynamic diameter: 1280 nm 3 instillations of a weekly dose of 100 µg on GD2.5, 9.5 and 16.5 Lung examination at GD17.5 (fetal stage); at PND 14.5 (pulmonary alveolarization) and at PND49.5 (lung maturity) Analysis of foetotoxicity on GD17.5 Measurement of cytokines on GD17.5 Chemical analysis of placenta and fetal lungs on GD17.5	Long-lasting impairment of lung development of the offspring. Increase of the alveolar space and a decrease of the number of alveoli on PND14.5 and 49.5. Decreased placental efficiency together with the presence of NPs in placenta, no increase of inflammatory mediators present in amniotic fluid, placenta or offspring lungs. Decreased pulmonary expression of vascular endothelial growth factor-a (VEGF-a) and matrix metalloproteinase 9 (MMP-9) at the fetal stage, and fibroblast growth factor-18 (FGF-18) at the alveolarization stage. No effect on uterine weight, fetal resorption rates and number of living fetuses. Decreased fetal weight. Increase of inflammatory cells in the lung of pregnant females.	Only one concentration. Instillation route, worst case exposure (bolus administration) Hypothesis: administration of NPs in pregnant mice is followed by an effect on the placenta with impact on the respiratory development of the offspring.	Paul et al. (2017)

Effects of in utero exposure of two forms of TiO_2-NP, UV-Titan L181 (Kyjovska et al. 2013, Jackson et al. 2013, Hougaard et al. 2010, Boisen et al. 2012) and P25 (Stapleton, Nichols, et al. 2015, Stapleton et al. 2013, Hathaway et al. 2017, Engler-Chiurazzi et al. 2016, Stapleton et al. 2018) were evaluated by inhalation by different groups .

Effects of surface-coated TiO_2-NP, UV-Titan L181 (rutile (70.8 wt%) modified with unspecific amounts of zirconium (0.86-1.17 wt%), silicon (12 wt%), aluminium (4.58 wt%) and sodium oxide (0.6 wt%), and coated with polyalcohols (crystallite primary particle size 20.6 nm; surface area 107 m^2/g; geometric mean diameter 97 nm)) was studied in C56BL/6 mice and their offspring by the same group (Kyjovska et al. 2013, Hougaard et al. 2010, Boisen et al. 2012). In these studies, pregnant females were whole-body exposed 1h/day to either filtered clean air, or a target concentration of 40 mg/m^3 of UV-Titan L181 (42.4 ± 2.9 mg/m3 measured over all the experiments) from gestational day 8 to 18.

548

549 Lung inflammation was noted in the BALF (with increased neutrophils) of the exposed dams, 5 and
550 26-27 days after exposure termination, relative to controls. In the offspring examined at age 11-15
551 weeks (males) or 12-16 weeks (females), cognitive functions were unaffected, while moderate
552 neurobehavioral changes were noted (in open field test). In contrast, no significant effect on
553 gestational and litter parameters or on fertility was reported (Hougaard et al. 2010).

554 No increased ESTR (expanded simple tandem repeat) mutations were measured in the F1 females
555 exposed in utero to UV-Titan 181 and in the F2 offspring of prenatally exposed female mice, as
556 compared to controls. There was also no effect on viability or sex ratio (Boisen et al. 2012).

557 Daily sperm production was not statistically significantly affected in F1 and F2 offspring. Only a trend
558 in reduced sperm counts was recorded in the F1 generation with an increase in time-to-first F2 litter
559 (Kyjovska et al. 2013).

560 No increase of DNA strand breaks was noted in BALF of time-mated mice or in the liver of both time-
561 mated mice and their offspring. In contrast, exposure to UV-Titan 181 induced a persistent
562 inflammation in mothers and affected gene expression in the liver of female offspring (Jackson et al.
563 2013).

564 It is worth to notice that the relevance of the results from the above mentioned studies is questionable
565 given the high concentration used (42 mg/m^3).

566

567 Aeroxide TiO_2 P25 (anatase 80%, rutile 20%; primary particle diameter 21 nm, average aerodynamic
568 diameter of agglomerates formed during aerolization: 149.1 ± 3.7 nm; surface area 48 m^2/g; zeta
569 potential -56.6 mV) was studied on female Sprague Dawley rats and their offspring in different
570 studies performed by the same group (Stapleton, Nichols, et al. 2015, Stapleton et al. 2013,
571 Hathaway et al. 2017, Engler-Chiurazzi et al. 2016, Stapleton et al. 2018). In these studies, pregnant
572 females were exposed whole-body 5h/day to either filtered clean air (control ; 0 mg/m^3) or a target
573 concentration of 10 mg/m^3 after implantation (from gestation day 6, 7 or 10), with an average duration
574 of about 7-8 days. The generated aerosols excluded agglomerates > 400 nm, and exposure started
575 once the steady state aerosol concentration was achieved. Concentrations were monitored in real
576 time, as well as the particle size distribution (using a scanning device).

577 Impact of the duration of exposure (≤ 7 days versus ≥ 7 days) was investigated in Stapleton et al.
578 (2013). The authors showed significant decreases in the average litter size and weight, and in pup
579 weight after 11 days of inhalation exposure, while an exposure of 7 days had no effects on maternal
580 weight, implantation sites, litter size, sex of pups or female progeny weight gain. No significant
581 differences within the maternal or litter characteristics (maternal weight, implantation site, number
582 and weight of pups) were noted in the consecutive studies (Stapleton et al. 2013, Engler-Chiurazzi
583 et al. 2016, Stapleton et al. 2018).

584 Microvascular characteristics were analysed by Stapleton, Nichols, et al. (2015), Stapleton et al.
585 (2013). The authors reported fetal microvascular dysfunction after in utero P25 exposure, with an

impaired ability of uterine arterioles to proper dilate and an impaired microvascular function. Endothelium-dependent dilation and active mechanotransduction in both coronary and uterine arterioles were significantly altered in the female progeny studied at 11-16 weeks of age. In addition, a significant reduction in maximal mitochondrial respiration in the left ventricle and uterus was noted. Hathaway et al. (2017) confirmed this decrease in basal and maximal respiration, and related this to the systolic and diastolic abnormalities and cardiomyocytes contractile attenuation observed in the progeny. Finally, Stapleton et al. (2018) reported a decreased cardiac dysfunction (characterized by epigenetic and transcriptomic changes) in foetuses while showing a propensity toward hepatic and renal disease and increased inflammatory signalling.

Regarding neurotoxicity, the behaviour and cognitive functions of pups were evaluated at 5 months of age by Engler-Chiurazzi et al. (2016). They showed significant working memory impairments, especially under maximal mnemonic challenge, and possible deficits in initial motivation in male F1 adults. According to the authors, these results indicate that maternal exposure during gestation produces psychological deficits that persist into adulthood in male rats.

Two additional studies carried out by the instillation route have been identified. Both focused on effects of TiO_2-NP (anatase) on the development of the lungs.

Intranasal instillation of TiO_2-NP (anatase, 6 nm) were used in newborn C57BL/6 mice to study effects on lung development (Ambalavanan et al. 2013). A dose equivalent to 1 mg/kg body weight (5 µl of NP suspension) was instilled either at post-natal day (PND) 4 (single-dose experiment) or at PND 4, 7, 10 (multiple-dose experiment) and compared to mice exposed to vehicle. Administration of anatase caused inflammatory cell infiltrates and inhibited lung development in both single- and multiple-dose experiments. Inflammatory cells consisted of macrophages containing accumulation of TiO_2-NP surrounded by other inflammatory cells (polymorphonuclear and some mononuclear cells). No alteration of lung function or pulmonary vascular modeling was recorded, but gene expression and protein amounts of specific cytokines were increased in lung homogenates, as well as MMP-9 (known to be involved in lung injury and inhibition of development). There was also an overexpression of proinflammatory cytokines such as IL-1β, known to impair lung alveolarization. VEGF (vascular endothelial growth factor), important for normal lung development, was decreased, this decrease may contribute to impairment of alveolarization. The authors concluded that these effects possibly impact the risk of respiratory disorders in later life.

TiO_2-NP (anatase, 12 nm) was also shown to impair lung development of the offspring of C57BL/6 female mice (Paul et al. 2017). The pregnant mice were anesthetized and treated with 10 µl of nanoparticle suspension (10 mg/ml) by non-surgical intratracheal instillation at gestational day (GD) 2.5, 9.5, 16.5, while vehicle alone was injected for the saline group (control). The fetal resorption rate and the number of fetus/litter were not affected, but the fetus weight was decreased at GD 17.5 as well as placental efficiency (fetal weight/placental weight). Lung morphometric measurement (at PND 14.5 and 49.5) indicated a decrease in lung alveolar surface in offspring after anatase exposure

during pregnancy. TiO_2-NP was significantly higher in the placenta of the treated group. Yet, no inflammatory response was detected in the amniotic fluid, placenta and lungs of fetuses from dams exposed to anatase. Therefore inflammation of dams' lungs did not appear to be the underlying mechanism contributing to lung impairment in the offspring. Decreased pulmonary expression of VEGF-α could be the mechanism leading to impairment of the lung. Other genes involved in lung development such as MMP-9 at the fetal stage and FGF-18 (fibroblast growth factor-18) at the alveolarization stage were shown to be downregulated. Contrary to Ambalavanan et al. (2013) who observed an increased MMP-9 expression in a context of a pro-inflammatory response, a decrease was recorded in the present study.

In all the studies described above, only a single concentration of TiO_2-NP was investigated and compared to the corresponding controls. Therefore, no dose-response relationship can be established. The results are useful to highlight mechanisms, but not to derive a 8h-OEL.

4.6 Sensitive population

Only few studies provide information on potential populations which may be particularly sensitive to TiO_2-NP.

Roulet et al. (2012) induced emphysema in rats by instillation of elastase. Seven days after elastase or saline instillation (control), rats underwent intratracheal instillation with TiO_2-NP (100 µg/rat) or bovine serum albumin (BSA) (0.5 mg/ml). The authors showed that TiO_2-NPs did not aggravate elastase-induced pulmonary inflammation and emphysema. This result suggests that people with lung pathology may not be particularly at risk in case of TiO_2-NP exposure, but this need to be confirmed by further studies.

Scuri et al. (2010), as stated in section 3.4.1.4, suggested a greatest sensitivity of young rats (newborn, 1-2 day old and weanling, 2 week old) compared with adults (12 week old), as evidenced by increased in lung neurotrophins, after 3-day inhalation to P25. In contrast, Gate et al. (2017) compared young adults (12–13-week old) and elderly rats (19-month old) in a biopersistence and translocation study (see 3.2) and showed that the amount of TiO_2 recovered in spleen and liver were higher in elderly rats. The study from Disdier et al. (2017) with rats of the same age, also underlines the age susceptibility for a potential neurotoxicity of inhaled TiO_2-NP, with older rat being more susceptible.

5 Construction of the OELs

For the time being, in most of available studies described above, concentrations of TiO_2-NP are expressed in mg/m^3. There are currently ongoing discussions on the choice of the relevant dose metrics to use for poorly soluble particle and specifically regarding nanometric forms. Other metrics are currently identified such as surface area, particle number, particle void volume... Indeed, several toxicology studies have suggested that the biological response following deposition of particles in the lung is dependent on particle area, rather than on mass concentration (Oberdorster (2002), NIOSH (2011)). Sager and Castranova (2009) and Noel et al. (2017) compared different exposure metrics after studying different size and agglomeration state (only in the study of Noel et al.) of TiO_2-NP. They confirmed that toxicity is related, at least in part, to surface area. However, regarding pulmonary effects, they also concluded that mass concentration, associated with agglomeration state could be appropriate, as it presents a good correlation with effects observed, and has the advantage of being commonly used and easier to determine. NIOSH (2011) reached similar conclusions.

Therefore, given the current lack of consensus on the dose metric to be used, the mass concentration (mg/m^3) is retained for the derivation of the 8h-OEL, as it is the most commonly used metric so far.

5.1 Construction of an 8 hour occupational exposure limit (8 hour-OEL)

5.1.1 Critical effect

From the available repeated-dose toxicity studies in animals, TiO_2-NP can induce adverse effects in: lung (both non-neoplastic and neoplastic lesions), cardiovascular system, brain, liver and kidney. Developmental toxicity (neurotoxicity, impaired microvascular function) is also reported when TiO_2-NP is administered during gestation.

Considering all the repeated dose toxicity studies performed by inhalation, the most sensitive effect seems to be lung inflammation, which is observed at concentrations from 2 mg/m^3 in rats. More severe pulmonary effects including lung tumorigenesis occurred at higher concentration (≥ 10 mg/m^3). Effects on other organs are also reported at concentrations higher than 2 mg/m^3. For example, effects on the cardiovascular system were noted at 6 mg/m^3 but lower concentrations were not tested. Similarly, neurotoxicity and developmental effects were observed at a single tested concentration of 10 mg/m^3. Regarding toxicity on the liver and the kidney, the studies identified were all performed by instillation and cannot be adequately compared to inhalation conditions.

Based on the available data, lung inflammation is identified as the critical effect after TiO_2-NP exposure. However, most studies only focused on pulmonary response and the few assessing other potential target organs only tested a single high concentration. In this context, it cannot be completely ruled out that other adverse effects can occur at non-inflammatory concentrations.

Interspecies differences from experimental studies

Bermudez et al. (2004) compared the sensitivity of three rodent species to the lung toxicity of P25. The experimental findings suggest that the rat is particularly sensitive to lung toxicity of TiO_2-NP compared to other rodents. Indeed, clear species differences in pulmonary clearance and lung lesions were observed after inhalation exposure to P25 for 13 weeks in rats, mice and hamsters (Bermudez et al. 2004). In particular, pulmonary lesions were more severe and occurred at a lower concentration in rats, which was the only species developing progressive fibro proliferative lesions and alveolar epithelial metaplasia. The differences may be explained, at least partially, by biological diversity of detoxification systems, such as anti-oxidant defences, as described below.

Despite a lung burden similar to rats, inflammatory response occurred in mice at higher concentration without developing metaplasia or fibrosis. This lower responsiveness could be explained by a lower sensitivity of this species to oxidative damage compared to rats. For instance, an increase of antioxidant (glutathione) levels in lung tissue was found during particle exposure in mice but not in rats (Oberdorster 1995).

In hamsters, the lack of lung adverse effect reported in this study can be related to a more efficient lung clearance system. Indeed, a markedly lower retention lung half-time was noted in hamsters compared to rats and mice. Furthermore, hamsters have antioxidant protection mechanisms different from rats and humans, suggesting that this species is not appropriate for testing particulate substances which may elicit inflammatory oxidative damage (ANSES 2016).

Extrapolation to humans

Regarding general particle mode of action, there are anatomical differences between the lungs of rodents and humans (e.g. lack of well-defined respiratory bronchioles in rats), resulting in different patterns of particle retention. In rats, the deposition of particles is rather uniform and principally observed in the alveolar lumen. In contrast, the deposition of particles in humans is more pronounced at bifurcations in the terminal airways (with observation of hot-spots) and in the interstitium. In addition, it has been shown that lung clearance of particles is slower in humans than in rats by approximately an order of magnitude, with about 60 days in rats (Brown, Wilson, and Grant 2005) and 400 days in humans (Kreyling WG and Scheuch G 2000).

Nevertheless, despite these differences, humans and rats display some consistency in response to dust exposure, including inflammatory reaction, lipoproteinosis, fibrosis and hyperplasia. These effects were not reported in mice and hamsters confirming that these species do not appear to be the most appropriate to predict the pulmonary toxicity of TiO_2-NP in humans (NIOSH, 2011).

In conclusion, considering:

- the lack of specific mechanistic data to adequately compare humans to rats and their sensitivity to TiO$_2$-NP exposure;
- a slower lung clearance of particles in humans compared to rats;
- similar qualitative lung response to dust between humans and rats.

The findings reported in rats with TiO$_2$-NP (P25) are considered relevant for humans.

5.1.2 Choice of the construction hypothesis

Carcinogenic chemicals have conventionally been divided into two categories according to the presumed mode of action: genotoxic or non-genotoxic.

As stated in the section above (3.3.2), based on the most recent studies, it can be concluded that TiO$_2$-NP is a weak genotoxic, with effects appearing only at high doses but showing a dose-response in numbers of positive studies. Carcinogenic effects, as evidenced by lung tumours, appear only at high concentrations, associated with altered clearance and inflammatory response (cf. 3.3.3).

Genotoxicity data on TiO$_2$-NP point to secondary genotoxicity as the main mechanism of action. Indeed, several publications suggest a correlation between inflammation and/or oxidative stress and genotoxicity. However, other mechanisms of action cannot be formally disregarded, but data currently available do not allow to demonstrate this. These conclusions are in line with those from other authorities or reviews (IARC, 2010; NIOSH, 2011; Anses 2016; Charles & Jomini et al., 2018; OECD 2018).

Weight of evidence is of importance in assessing genotoxicity of a chemical and choosing between the derivation of threshold or non-threshold toxicological reference value.

For TiO$_2$-NP, the majority of positive results are obtained from comet assays. First, many of the available comet assays are in vitro tests for which a harmonized protocol is not currently recommended as such by European legislation, in contrast to studies with validated OECD guidelines that are considered as standard protocols to assess mutagenicity of chemicals. In addition, one of the issues to be considered with comet assay is the biological relevance of results, since this assay measures early DNA lesion that may subsequently be repaired (Charles & Jomini et al., 2018). Applying a weight of evidence approach for genotoxicity, Brusick et al. (2016), reached a similar conclusion by allocating a low weight to “SSB/DSB (single strand break/double strand break) in vitro (including comet)” endpoint.

OECD stated that “*When evaluating the mutagenic potential of a test chemical, more weight should be given to the measurement of permanent DNA changes (i.e. mutations) than to DNA damage events that are reversible*” (OECD 2017). Therefore, positive responses in "indicator" tests (i.e. the measurement of DNA breaks, sister chromatid exchanges, etc.) are certainly associated with exposure but are to be considered insufficient to determine a mutagenic effect.

In summary, based on the available data, a threshold approach is considered to be the most relevant approach to derive the reference values.

5.1.3 Choice of the key study

Human studies available on TiO_2-NP, all considered as inadequate, do not allow the establishment of a 8h-OEL.

In animals, only few studies with repeated exposure are available by inhalation. Repeated-dose toxicity studies conducted by instillation were also found in the literature. As stated in OECD (2018), those studies cannot be used for risk assessment, mainly because this exposure bypassed the upper respiratory tract and is therefore not representative of inhalation exposure.

Therefore, among inhalation studies, the one of Bermudez et al. (2004) is selected as the key study. This is indeed the most robust study available by inhalation with the longest duration of exposure (13 weeks). The TiO_2-NP used (P25; 80% anatase/20% rutile; about 21 nm) is one of the OECD reference forms of TiO_2-NP and is fully characterized (OECD 2015). An adequate characterization of the tested material is a critical point considering the wide variety of forms of TiO_2-NP (different products varying in composition, coating, sizing etc.) available on the European market. Since intrinsic physicochemical properties of a nanomaterial, such as particle crystallinity, size, surface area and surface modification, are presumed to influence its reactivity and behaviour, it is essential to have information on these parameters for the tested substance. Moreover, compared to most other studies available, the concentrations used (0.5, 2 and 10 mg/m^3) are adequate to observe a dose-response relationship and identify a no-observed effect concentration. Finally, three rodent species were included (mice, rats and hamsters). This feature is very interesting as it allows an assessment of the sensitivity of different species to TiO_2-NP rigorously under the same protocol.

However, this study has also some drawbacks:

- only local/pulmonary toxicity was evaluated with limited details on the results of lung histopathology. This is a critical point in the assessment of TiO_2-NP, as the majority of the repeated-dose toxicity studies available only focused on lung response. No repeated dose toxicity study with a full investigation of various organs, according to OECD guidelines, is available. This limitation of the database is important to keep in mind for the establishment of the 8h-OEL and especially in the setting of the adjustment factors;
- only females were exposed. However, considering the rest of the database, it is not considered as a major deficiency since a significant variability in the inflammatory lung response between sexes is not expected after TiO_2-NP inhalation as this is a local effect;
- this study was performed by whole-body inhalation. Nose-only is however the preferred mode of exposure recommended in the Test Guidelines as this mode of exposure minimizes exposure or uptake by non-inhalation routes and thus allow evaluating the particle effect by inhalation only (OECD 2018b). However, in the case of TiO_2-NP, the critical effect identified

from the available studies is lung inflammation. Thus, a significant impact of the mode of administration between nose-only and whole-body is not expected. This is confirmed by Oyabu et al. (2016) showing that the difference on pulmonary effects after whole-body and nose-only inhalation of TiO_2-NP is minimal or even non-existent;

- P25 was not dispersed (by sonication for example) before exposure. In this context, it is considered that animals are rather exposed to large agglomerate particles instead of free and/or small aggregate particles. However, this protocol can be considered close to real exposure scenario compared to protocols with dispersion of particles.

Considering all these elements, the study of Bermudez et al. (2004) remains the most reliable study for the establishment of the TiO_2-NP 8h-OEL. It has to be noted that all other repeated-dose toxicity studies performed with several concentrations by inhalation, even if performed on other forms of TiO_2-NP (Oyabu et al. 2017, Ma-Hock et al. 2009, Landsiedel et al. 2014, Yu et al. 2015), support qualitatively and quantitatively the results obtained by Bermudez et al. (2004).

5.1.4 Choice of the critical dose

Histopathological observations in rats, identified as the most sensitive species, are used as a basis for the establishment of the point of departure. At the tested concentration of 0.5 mg/m^3, the only effects reported were a reversible decrease in body weight, the presence of particles within alveolar macrophages and very minimal changes in the patterns of alveolar macrophage accumulation in the lungs. Lesions at 2 mg/m^3 were minimal to mild in severity and consisted primarily of particle laden macrophage accumulation and aggregation in subpleural regions and in centriacinar zones, associated with minimal hypertrophy and hyperplasia of type II alveolar epithelial cells. A significant but reversible increase in terminal bronchiolar and alveolar cell replication was also found at this concentration. At 10 mg/m^3, there were more severe epithelial proliferative changes, including metaplastic changes in the centriacinar region (bronchiolization of alveolar epithelium) associated with particle-laden macrophage accumulation and increase of inflammation markers in the BALF. The histopathological findings were progressive with increase of concentration and time also after cessation of exposure and decrease in inflammatory response.

Based on the increased of cellular proliferation, a Benchmark Dose (BMD) modelisation was performed. Although a dose response was observed, the results of the BMD modelling cannot be accepted because of the low number of animals per dose (n=5) and the large inter-individual variability of the data set.

Indeed, several criteria of acceptance of the BMD are not fulfilled (US EPA 2012):

- the BMD/BMDL ratio is around 10 which means a too large uncertainty;
- the BMDL is 10 times lower than the minimum non-zero dose;
- the BMD stands between control and first dose.

In conclusion, a BMDL cannot be established based on these data.

Based on the abovementioned effects, the LOAEC is established at 2 mg/m^3 and the **NOAEC at 0.5 mg/m^3.**

5.1.5 Adjustments

To reduce the value of uncertainty on toxicokinetics inter-species variability, an allometric adjustment was performed. A Human Equivalent Concentration (HEC) was calculated.

The calculation of the HEC for TiO_2-NP, detailed below, has been mainly based on the methodology used by DFG for the derivation of the limit value for the respirable dust fraction of biopersistent granular dusts (MAK 2012).

This methodology is based on the assumption that the sensitivity to TiO_2-NP of rats and humans does not differ at the same dose per lung surface area.

The first step is the evaluation of the particle fraction deposited in the lung. Deposition fraction is the ratio of number of particles deposited on the lung to the number of particles entering respiratory tract. To estimate this fraction, the Multiple Path Particle Dosimetry (MPPD) model (v 3.04)[16] was used. This model was developed by the Chemical Industry Institute of Toxicology (CIIT), NC, USA, and the Dutch National Institute for Public Health and the Environment (RIVM). The MPPD model calculates the deposition and clearance of monodisperse and polydisperse aerosols in the respiratory tracts of laboratory animals and human adults and children (deposition only) for particles ranging in size from ultrafine (1 nm) to coarse (100 µm). Respiratory tract dosimetry models have been developed for several laboratory animal species including rat, mouse, rhesus monkey, pig and rabbit.

The second step is the calculation of the deposition rate, in m^3/day:

$$\textit{Deposition volume} = \textit{deposition fraction} \times \textit{tidal volume} \times \textit{respiratory rate} \times \textit{exposure time}$$

The elimination constant can be calculated, expressed in days:

$$\textit{Elimination constant} = -\ln(0.5)/\textit{elimination half-time}$$

The steady state lung load is calculated, in m^3:

[16] https://www.ara.com/products/multiple-path-particle-dosimetry-model-mppd-v-304

Steady state lung load = deposition volume/elimination constant

It has to be noted that the steady state load in mg per lung is obtained by multiplying this value by the dust concentration in mg/m^3, that is to say the NOAEC.

Finally, the lung load related to the lung surface area can be calculated for rats and humans and the ratio of these values is used for the calculation of the HEC by multiplying by the NOAEC:

$$HEC = NOAEC \times (steady\ state\ load/lung\ surface\ area)_{rat}/(steady\ state\ load/lung\ surface\ area)_{human}$$

The calculation of the HEC is presented below along with the graph modelling of the calculations. The details and references of the parameters and data used for calculation, such as options selected in MPPD program, are presented in annex 2.

Rat:

- Deposition $fraction_{rat}$: 0.056 (unitless)
- Deposition $rate_{rat}$ = 0.056 x (2.1/1000000) x 102 x 60 x 6 x 5/7 = 0.003084 m^3/day

  2.1 mL = tidal volume of the rat

  102/min = respiratory rate of the rat

  60 min x 6h x 5/7j = exposure time of the study, expressed in days
- Elimination $constant_{rat}$ = -(ln0.5)/60 = 0.0116/day

  60 days = elimination half-time of TiO_2-NP for the rat
- Steady state lung $load_{rat}$ = 0.003084/0.0116 = 0.2659 m^3

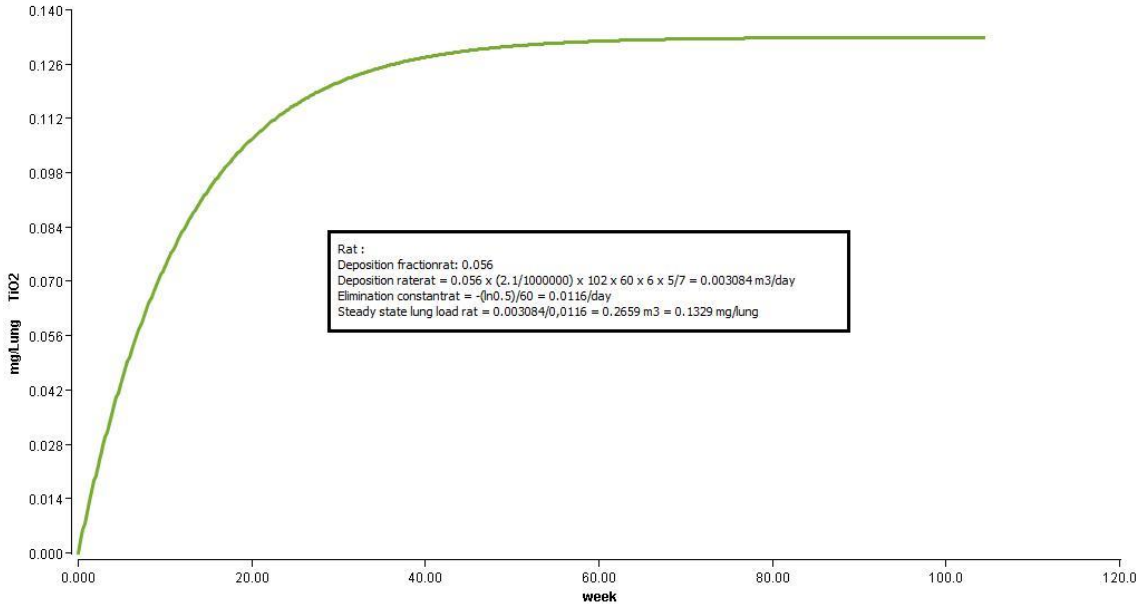


Figure 2: Modelling of steady state lung load in mg/lung in rats

Human:

- Deposition fraction$_{human}$: 0.1032 (unitless)
- Deposition rate$_{human}$ = 0.1032 x (1040/1000000) x 20 x 60 x 8 x 240/365 = 0.677 m^3/day

  1040 mL = tidal volume of human

  20/min = respiratory rate of human

  60 min x 8h x 240/365 = exposure time, expressed in days
- Elimination constant$_{human}$ = -(ln0.5)/400 = 0.00173/day

  400 days = elimination half-time of TiO_2-NP for human
- Steady state lung load$_{human}$ = 0.677/0.00173 = 391.61 m^3

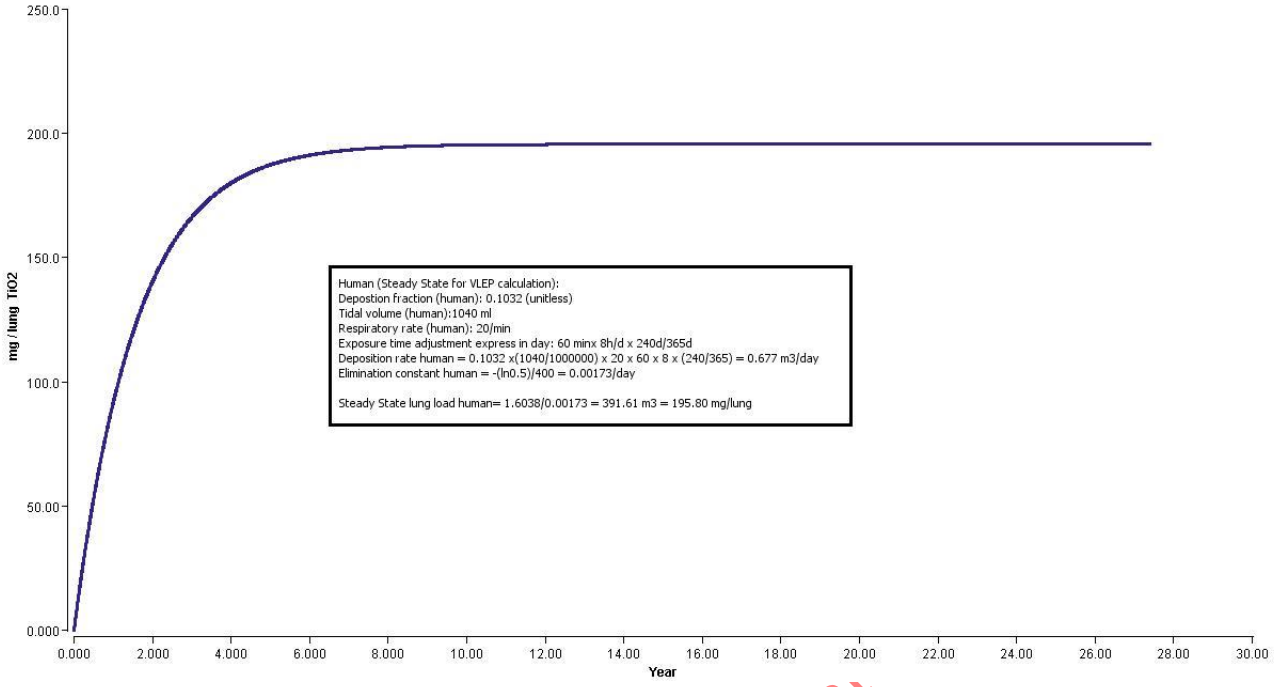


Figure 3: Modelling of steady state lung load in mg/lung in humans

Human Equivalent Concentration:

- **$NOAEC_{HEC}$** = NOAEC x (0.2659/0.297)/(391.61/57.22) = 0.5 x (0.8953/6.84) = 0.5 x 0.1309

  0.297 = lung surface area of rats, in m²

  57.22 = lung surface area of human, in m²

- **$NOAEC_{HEC}$** = 0.065 mg/m³

5.1.6 Adjustment factors

The chronic inhalation 8h-OEL was calculated from the $NOAEC_{HEC}$ using the following adjustment factors (AF) (ANSES, 2017):

- ***Inter-species variability (AF_A) = 3***

The allometric adjustment performed by modelling enabled a human equivalent concentration to be calculated. As provided for in the methodological document, to take toxicodynamic variability and residual uncertainties into account, an additional adjustment factor was set at 3.

- ***Inter-individual variability (AF_H) = 3***

In the absence of quantified data on inter-individual variability, this factor is assigned a default value of 3.

- ***Subchronic to chronic transposition (AF_S) = 3***

Because the key study was a subchronic study with animals exposed for 13 weeks and no data is available showing that with a longer exposure a lower concentration is not sufficient to induce effect, the value of 3 was used for exposure duration extrapolation.

- ***Use of a BMDL, LOAEC or NOAEC (AF_L) = 1***

Because establishment of the 8h-OEL is based on a NOAEC, this factor does not apply.

- ***Inadequacy of the database (AF_D) = 3***

Most of the studies performed on TiO$_2$-P25 are not judged fully reliable for chronic risk assessment (e.g. intratracheal administration, single high concentration tested, no chronic study). In addition, several repeated-dose toxicity studies have shown effect on other organs than lungs (cardiovascular system, liver, kidneys…). However, as the majority of repeated-dose toxicity studies by inhalation investigated only one endpoint at the time, it cannot be ruled out that other adverse effects could occur at sub-inflammatory concentrations. In this context, the value of 3 was selected.

A global adjustment factor of 81 is therefore used for the derivation of the OEL.

5.1.7 8h-OEL proposal

The 8h-OEL for TiO$_2$-P25 is calculated as it follows:

$$OEL = NOAEC_{HEC}/AF$$

$$OEL = 0.00085\ mg/m^3 = 0.80\ \mu g/m^3$$

This 8h-OEL is only applicable to TiO$_2$-NP as P25 (80% anatase/20% rutile; 21 nm) which is the substance tested in Bermudez et al. (2004) study.

In the present assessment, the relevance of this 8h-OEL to all forms of TiO$_2$-NP cannot be evaluated considering the presence of more than 350 different TiO$_2$ products on the European market (varying in composition, coating, size etc., - parameters which are presumed to influence the reactivity and behavior of TiO$_2$-NP). Thus, it cannot be verified whether P25 is the most potent form and to what extent the data provided are representative for all forms produced, processed and placed on the market.

It has to be highlighted that 4 adjustment factors are used for the derivation of the 8h-OEL. However, in the reference document for the derivation of OEL (Anses, 2017), it is indicated that "*If all the factors applied exceed 1000 or if in total more than 3 adjustment factors are applied, the key study is considered by the CES as inadequate for the construction of a VLEP*". Accordingly, the 8h-OEL should be disregarded. However, the CES considered that regarding the value of the global adjustment factor of 81, this recommendation of the guidance could reasonably be overlooked for this assessment.

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

Regarding the lack of relevant data on short term effect of TiO_2-NP for the construction of a STEL, and to limit the magnitude and the number of pic exposure, the CES recommends, according to its methodology (Anses, 2017), not to exceed over a period of 15 minutes 5 times the 8h-OEL, i.e. **4 µg.m^{-3}**.

5.3 « Skin » notation

Considering the lack of penetration of TiO_2-NP through skin, as concluded by the SCCS (SCCS, 2014), the “skin” notation is not recommended.

5.4 « Noise » notation

No available study suggests an ototoxic effect of TiO_2-NP. Consequently, the “noise” notation is not recommended.

6 Conclusions of the collective expert appraisal

8h-OEL: 0.80 µg/m^3

15min STEL: 4 µg.m^{-3}

« skin » notation: not recommended

« noise » notation: not recommended

7 References

Ambalavanan, N., A. Stanishevsky, A. Bulger, B. Halloran, C. Steele, Y. Vohra, and S. Matalon. 2013. "Titanium oxide nanoparticle instillation induces inflammation and inhibits lung development in mice." *Am J Physiol Lung Cell Mol Physiol* 304 (3):L152-61. doi: 10.1152/ajplung.00013.2012.

ANSES. 2016. CLH Report Proposal for Titanium Dioxide, Based on Regulation (EC) No 1272/2008 Annex VI, Part 2.

Anses. 2017. Document de référence pour l'élaboration de valeurs limites d'exposition à des agents chimiques en milieu professionnel. (Agence nationale de sécurité sanitaire pour l'alimentation, l'environnement et le travail, France). 142 p

Anses. 2019. AVIS de l'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail relatif à la proposition de VTR chronique par voie respiratoire pour le dioxyde de titane sous forme nanométrique (CAS n°13463-67-7).

Aragao-Santiago, L., H. Hillaireau, N. Grabowski, S. Mura, T. L. Nascimento, S. Dufort, J. L. Coll, N. Tsapis, and E. Fattal. 2016. "Compared in vivo toxicity in mice of lung delivered biodegradable and non-biodegradable nanoparticles." *Nanotoxicology* 10 (3):292-302. doi: 10.3109/17435390.2015.1054908.

Armand, L., A. Tarantini, D. Beal, M. Biola-Clier, L. Bobyk, S. Sorieul, K. Pernet-Gallay, C. Marie-Desvergne, I. Lynch, N. Herlin-Boime, and M. Carriere. 2016. "Long-term exposure of A549 cells to titanium dioxide nanoparticles induces DNA damage and sensitizes cells towards genotoxic agents." *Nanotoxicology* 10 (7):913-23. doi: 10.3109/17435390.2016.1141338.

Bermudez, E., J. B. Mangum, B. A. Wong, B. Asgharian, P. M. Hext, D. B. Warheit, and J. I. Everitt. 2004. "Pulmonary responses of mice, rats, and hamsters to subchronic inhalation of ultrafine titanium dioxide particles." *Toxicological Sciences* 77 (2):347-357. doi: 10.1093/toxsci/kfh019.

Boffetta, P., V. Gaborieau, L. Nadon, M. F. Parent, E. Weiderpass, and J. Siemiatycki. 2001. "Exposure to titanium dioxide and risk of lung cancer in a population-based study from Montreal." *Scand J Work Environ Health* 27 (4):227-32.

Boffetta, P., A. Soutar, J. W. Cherrie, F. Granath, A. Andersen, A. Anttila, M. Blettner, V. Gaborieau, S. J. Klug, S. Langard, D. Luce, F. Merletti, B. Miller, D. Mirabelli, E. Pukkala, H. O. Adami, and E. Weiderpass. 2004. "Mortality among workers employed in the titanium dioxide production industry in Europe." *Cancer Causes Control* 15 (7):697-706. doi: 10.1023/b:Caco.0000036188.23970.22.

Boisen, A. M., T. Shipley, P. Jackson, K. S. Hougaard, H. Wallin, C. L. Yauk, and U. Vogel. 2012. "NanoTiO(2) (UV-Titan) does not induce ESTR mutations in the germline of prenatally exposed female mice." *Part Fibre Toxicol* 9:19. doi: 10.1186/1743-8977-9-19.

Brown, J. S., W. E. Wilson, and L. D. Grant. 2005. "Dosimetric comparisons of particle deposition and retention in rats and humans." *Inhal Toxicol* 17 (7-8):355-85. doi: 10.1080/08958370590929475.

Brusick, David, Marilyn Aardema, Larry Kier, David Kirkland, and Gary Williams. 2016. "Genotoxicity Expert Panel review: weight of evidence evaluation of the genotoxicity of glyphosate, glyphosate-based formulations, and aminomethylphosphonic acid." *Critical Reviews in Toxicology* 46 (sup1):56-74. doi: 10.1080/10408444.2016.1214680.

Carp, O., C. L. Huisman, and A. Reller. 2004. "Photoinduced reactivity of titanium dioxide." *Progress in Solid State Chemistry* 32 (1):33-177. doi: https://doi.org/10.1016/j.progsolidstchem.2004.08.001.

1058 Chang, X., Y. Xie, J. Wu, M. Tang, and B. Wang. 2015. "Toxicological Characteristics of Titanium
1059 Dioxide Nanoparticle in Rats." *J Nanosci Nanotechnol* 15 (2):1135-42.

1060 Charles, S., S. Jomini, V. Fessard, E. Bigorgne-Vizade, C. Rousselle, and C. Michel. 2018.
1061 "Assessment of the in vitro genotoxicity of TiO2 nanoparticles in a regulatory context."
1062 *Nanotoxicology* 12 (4):357-374. doi: 10.1080/17435390.2018.1451567.

1063 Chen, H. W., S. F. Su, C. T. Chien, W. H. Lin, S. L. Yu, C. C. Chou, J. J. Chen, and P. C. Yang.
1064 2006. "Titanium dioxide nanoparticles induce emphysema-like lung injury in mice." *Faseb j*
1065 20 (13):2393-5. doi: 10.1096/fj.06-6485fje.

1066 Chen, J. L., and W. E. Fayerweather. 1988. "Epidemiologic study of workers exposed to titanium
1067 dioxide." *J Occup Med* 30 (12):937-42.

1068 Chen, T., J. Hu, C. Chen, J. Pu, X. Cui, and G. Jia. 2013. "Cardiovascular effects of pulmonary
1069 exposure to titanium dioxide nanoparticles in ApoE knockout mice." *J Nanosci Nanotechnol*
1070 13 (5):3214-22.

1071 Chen, T., J. Yan, and Y. Li. 2014. "Genotoxicity of titanium dioxide nanoparticles." *J Food Drug Anal*
1072 22 (1):95-104. doi: 10.1016/j.jfda.2014.01.008.

1073 Cho, W. S., R. Duffin, C. A. Poland, S. E. Howie, W. MacNee, M. Bradley, I. L. Megson, and K.
1074 Donaldson. 2010. "Metal oxide nanoparticles induce unique inflammatory footprints in the
1075 lung: important implications for nanoparticle testing." *Environ Health Perspect* 118
1076 (12):1699-706. doi: 10.1289/ehp.1002201.

1077 Choi, G. S., C. Oak, B. K. Chun, D. Wilson, T. W. Jang, H. K. Kim, M. Jung, E. Tutkun, and E. K.
1078 Park. 2014. "Titanium dioxide exposure induces acute eosinophilic lung inflammation in
1079 rabbits." *Ind Health* 52 (4):289-95.

1080 De Haar, C., I. Hassing, M. Bol, R. Bleumink, and R. Pieters. 2006. "Ultrafine but not fine particulate
1081 matter causes airway inflammation and allergic airway sensitization to co-administered
1082 antigen in mice." *Clinical and Experimental Allergy* 36 (11):1469-1479. doi: 10.1111/j.1365-
1083 2222.2006.02586.x.

1084 Disdier, C., M. Chalansonnet, F. Gagnaire, L. Gaté, F. Cosnier, J. Devoy, W. Saba, A. K. Lund, E.
1085 Brun, and A. Mabondzo. 2017. "Brain Inflammation, Blood Brain Barrier dysfunction and
1086 Neuronal Synaptophysin Decrease after Inhalation Exposure to Titanium Dioxide Nano-
1087 aerosol in Aging Rats." *Scientific Reports* 7 (1). doi: 10.1038/s41598-017-12404-5.

1088 Drew, Nathan M., Eileen D. Kuempel, Ying Pei, and Feng Yang. 2017. "A quantitative framework to
1089 group nanoscale and microscale particles by hazard potency to derive occupational exposure
1090 limits: Proof of concept evaluation." *Regulatory Toxicology and Pharmacology* 89:253-267.
1091 doi: https://doi.org/10.1016/j.yrtph.2017.08.003.

1092 ECHA. 2017. Committee for Risk Assessment (RAC). Opinion proposing harmonised classification
1093 and labelling

1094 at EU level of Titanium dioxide.

1095 Elder, Alison, Gunnar F. Nordberg, and Michael Kleinman. 2015. "Chapter 3 - Routes of Exposure,
1096 Dose, and Toxicokinetics of Metals∗∗This chapter is based on the chapter Routes of
1097 Exposure, Dose, and Metabolism of Metals by W.S. Beckett, G.F. Nordberg, and T.W.
1098 Clarkson in the third edition of this handbook." In *Handbook on the Toxicology of Metals*
1099 *(Fourth Edition)*, edited by Gunnar F. Nordberg, Bruce A. Fowler and Monica Nordberg, 45-
1100 74. San Diego: Academic Press.

1101 Ellis, E. D., J. P. Watkins, W. G. Tankersley, J. A. Phillips, and D. J. Girardi. 2013. "Occupational
1102 exposure and mortality among workers at three titanium dioxide plants." *Am J Ind Med* 56
1103 (3):282-91. doi: 10.1002/ajim.22137.

Ellis, E. D., J. Watkins, W. Tankersley, J. Phillips, and D. Girardi. 2010. "Mortality among titanium dioxide workers at three DuPont plants." *J Occup Environ Med* 52 (3):303-9. doi: 10.1097/JOM.0b013e3181d0bee2.

Engler-Chiurazzi, E. B., P. A. Stapleton, J. J. Stalnaker, X. Ren, H. Hu, T. R. Nurkiewicz, C. R. McBride, J. Yi, K. Engels, and J. W. Simpkins. 2016. "Impacts of prenatal nanomaterial exposure on male adult Sprague-Dawley rat behavior and cognition." *J Toxicol Environ Health A* 79 (11):447-52. doi: 10.1080/15287394.2016.1164101.

Eydner, M., D. Schaudien, O. Creutzenberg, H. Ernst, T. Hansen, W. Baumgartner, and S. Rittinghausen. 2012. "Impacts after inhalation of nano- and fine-sized titanium dioxide particles: morphological changes, translocation within the rat lung, and evaluation of particle deposition using the relative deposition index." *Inhal Toxicol* 24 (9):557-69. doi: 10.3109/08958378.2012.697494.

Fryzek, Jon, Bandana Chadda, Donald Marano, Kenneth White, Sarah Schweitzer, Joseph K McLaughlin, and William Blot. 2003. *A Cohort Mortality Study among Titanium Dioxide Manufacturing Workers in the United States*. Vol. 45.

Fu, Y., Y. Zhang, X. Chang, Y. Zhang, S. Ma, J. Sui, L. Yin, Y. Pu, and G. Liang. 2014. "Systemic immune effects of titanium dioxide nanoparticles after repeated intratracheal instillation in rat." *Int J Mol Sci* 15 (4):6961-73. doi: 10.3390/ijms15046961.

Gate, L., C. Disdier, F. Cosnier, F. Gagnaire, J. Devoy, W. Saba, E. Brun, M. Chalansonnet, and A. Mabondzo. 2017. "Biopersistence and translocation to extrapulmonary organs of titanium dioxide nanoparticles after subacute inhalation exposure to aerosol in adult and elderly rats." *Toxicol Lett* 265:61-69. doi: 10.1016/j.toxlet.2016.11.009.

Grassian, V. H., A. Adamcakova-Dodd, J. M. Pettibone, P. I. O'Shaughnessy, and P. S. Thorne. 2007. "Inflammatory response of mice to manufactured titanium dioxide nanoparticles: Comparison of size effects through different exposure routes." *Nanotoxicology* 1 (3):211-226. doi: 10.1080/17435390701694295.

Grassian, V. H., T. O'Shaughnessy P, A. Adamcakova-Dodd, J. M. Pettibone, and P. S. Thorne. 2007. "Inhalation exposure study of titanium dioxide nanoparticles with a primary particle size of 2 to 5 nm." *Environ Health Perspect* 115 (3):397-402. doi: 10.1289/ehp.9469.

Gustafsson, A., E. Lindstedt, L. S. Elfsmark, and A. Bucht. 2011. "Lung exposure of titanium dioxide nanoparticles induces innate immune activation and long-lasting lymphocyte response in the Dark Agouti rat." *J Immunotoxicol* 8 (2):111-21. doi: 10.3109/1547691x.2010.546382.

Halappanavar, S., P. Jackson, A. Williams, K. A. Jensen, K. S. Hougaard, U. Vogel, C. L. Yauk, and H. Wallin. 2011. "Pulmonary response to surface-coated nanotitanium dioxide particles includes induction of acute phase response genes, inflammatory cascades, and changes in microRNAs: a toxicogenomic study." *Environ Mol Mutagen* 52 (6):425-39. doi: 10.1002/em.20639.

Halappanavar, S., A. T. Saber, N. Decan, K. A. Jensen, D. Wu, N. R. Jacobsen, C. Guo, J. Rogowski, I. K. Koponen, M. Levin, A. M. Madsen, R. Atluri, V. Snitka, R. K. Birkedal, D. Rickerby, A. Williams, H. Wallin, C. L. Yauk, and U. Vogel. 2015. "Transcriptional profiling identifies physicochemical properties of nanomaterials that are determinants of the in vivo pulmonary response." *Environ Mol Mutagen* 56 (2):245-64. doi: 10.1002/em.21936.

Hamilton, R. F., N. Wu, D. Porter, M. Buford, M. Wolfarth, and A. Holian. 2009. "Particle length-dependent titanium dioxide nanomaterials toxicity and bioactivity." *Part Fibre Toxicol* 6:35. doi: 10.1186/1743-8977-6-35.

Hashizume, N., Y. Oshima, M. Nakai, T. Kobayashi, T. Sasaki, K. Kawaguchi, K. Honda, M. Gamo, K. Yamamoto, Y. Tsubokura, S. Ajimi, Y. Inoue, and N. Imatanaka. 2016. "Categorization of nano-structured titanium dioxide according to physicochemical characteristics and pulmonary toxicity." *Toxicol Rep* 3:490-500. doi: 10.1016/j.toxrep.2016.05.005.

Hathaway, Q. A., C. E. Nichols, D. L. Shepherd, P. A. Stapleton, S. L. McLaughlin, J. C. Stricker, S. L. Rellick, M. V. Pinti, A. B. Abukabda, C. R. McBride, J. Yi, S. M. Stine, T. R. Nurkiewicz, and J. M. Hollander. 2017. "Maternal-engineered nanomaterial exposure disrupts progeny cardiac function and bioenergetics." *Am J Physiol Heart Circ Physiol* 312 (3):H446-h458. doi: 10.1152/ajpheart.00634.2016.

Heinrich, U., R. Fuhst, S. Rittinghausen, O. Creutzenberg, B. Bellmann, W. Koch, and K. Levsen. 1995. "Chronic Inhalation Exposure of Wistar Rats and two Different Strains of Mice to Diesel Engine Exhaust, Carbon Black, and Titanium Dioxide." *Inhalation Toxicology* 7 (4):533-556. doi: 10.3109/08958379509015211.

Hong, F., L. Ji, Y. Zhou, and L. Wang. 2017. "Chronic nasal exposure to nanoparticulate TiO2 causes pulmonary tumorigenesis in male mice." *Environ Toxicol* 32 (5):1651-1657. doi: 10.1002/tox.22393.

Horie, M., H. Fukui, S. Endoh, J. Maru, A. Miyauchi, M. Shichiri, K. Fujita, E. Niki, Y. Hagihara, Y. Yoshida, Y. Morimoto, and H. Iwahashi. 2012. "Comparison of acute oxidative stress on rat lung induced by nano and fine-scale, soluble and insoluble metal oxide particles: NiO and TiO2." *Inhal Toxicol* 24 (7):391-400. doi: 10.3109/08958378.2012.682321.

Horvath, T., A. Papp, D. Kovacs, L. Kalomista, G. Kozma, and T. Vezer. 2017. "Electrophysiological alterations and general toxic signs obtained by subacute administration of titanium dioxide nanoparticles to the airways of rats." *Ideggyogyaszati Szemle* 70 (3-4):127-135. doi: 10.18071/isz.70.0127.

Hougaard, K. S., P. Jackson, K. A. Jensen, J. J. Sloth, K. Loschner, E. H. Larsen, R. K. Birkedal, A. Vibenholt, A. M. Boisen, H. Wallin, and U. Vogel. 2010. "Effects of prenatal exposure to surface-coated nanosized titanium dioxide (UV-Titan). A study in mice." *Part Fibre Toxicol* 7:16. doi: 10.1186/1743-8977-7-16.

Huang, K. T., C. T. Wu, K. H. Huang, W. C. Lin, C. M. Chen, S. S. Guan, C. K. Chiang, and S. H. Liu. 2015. "Titanium nanoparticle inhalation induces renal fibrosis in mice via an oxidative stress upregulated transforming growth factor-beta pathway." *Chem Res Toxicol* 28 (3):354-64. doi: 10.1021/tx500287f.

Hurbankova, M., S. Cerna, Z. Kovacikova, S. Wimmerova, D. Hraskova, J. Marcisiakova, and S. Moricova. 2013. "Effect of TiO2 nanofibres on selected bronchoalveolar parameters in acute and subacute phase--experimental study." *Cent Eur J Public Health* 21 (3):165-70.

Husain, M., A. T. Saber, C. Guo, N. R. Jacobsen, K. A. Jensen, C. L. Yauk, A. Williams, U. Vogel, H. Wallin, and S. Halappanavar. 2013. "Pulmonary instillation of low doses of titanium dioxide nanoparticles in mice leads to particle retention and gene expression changes in the absence of inflammation." *Toxicol Appl Pharmacol* 269 (3):250-62. doi: 10.1016/j.taap.2013.03.018.

Husain, M., D. Wu, A. T. Saber, N. Decan, N. R. Jacobsen, A. Williams, C. L. Yauk, H. Wallin, U. Vogel, and S. Halappanavar. 2015. "Intratracheally instilled titanium dioxide nanoparticles translocate to heart and liver and activate complement cascade in the heart of C57BL/6 mice." *Nanotoxicology* 9 (8):1013-22. doi: 10.3109/17435390.2014.996192.

Hussain, S., J. A. Vanoirbeek, K. Luyts, V. De Vooght, E. Verbeken, L. C. Thomassen, J. A. Martens, D. Dinsdale, S. Boland, F. Marano, B. Nemery, and P. H. Hoet. 2011. "Lung exposure to nanoparticles modulates an asthmatic response in a mouse model." *Eur Respir J* 37 (2):299-309. doi: 10.1183/09031936.00168509.

IARC. 2010. Monographs on the Evaluation of Carcinogenic Risks to Humans.

Ichihara, S., W. Li, S. Omura, Y. Fujitani, Y. Liu, Q. Wang, Y. Hiraku, N. Hisanaga, K. Wakai, X. Ding, T. Kobayashi, and G. Ichihara. 2016. "Exposure assessment and heart rate variability monitoring in workers handling titanium dioxide particles: a pilot study." *Journal of Nanoparticle Research* 18 (3):1-14. doi: 10.1007/s11051-016-3340-2.

1201 INERIS. 2016. Rapport d'étude: proposition d'un repère toxicologique pour l'oxyde de titane
1202 nanométrique pour des expositions environnementales par voie respiratoire ou orale.

1203 Jackson, P., S. Halappanavar, K. S. Hougaard, A. Williams, A. M. Madsen, J. S. Lamson, O.
1204 Andersen, C. Yauk, H. Wallin, and U. Vogel. 2013. "Maternal inhalation of surface-coated
1205 nanosized titanium dioxide (UV-Titan) in C57BL/6 mice: effects in prenatally exposed
1206 offspring on hepatic DNA damage and gene expression." *Nanotoxicology* 7 (1):85-96. doi:
1207 10.3109/17435390.2011.633715.

1208 Jugan, M. L., S. Barillet, A. Simon-Deckers, N. Herlin-Boime, S. Sauvaigo, T. Douki, and M. Carriere.
1209 2012. "Titanium dioxide nanoparticles exhibit genotoxicity and impair DNA repair activity in
1210 A549 cells." *Nanotoxicology* 6 (5):501-13. doi: 10.3109/17435390.2011.587903.

1211 Kim, B. G., P. H. Lee, S. H. Lee, M. K. Park, and A. S. Jang. 2017. "Effect of TiO(2) Nanoparticles
1212 on Inflammasome-Mediated Airway Inflammation and Responsiveness." *Allergy Asthma*
1213 *Immunol Res* 9 (3):257-264. doi: 10.4168/aair.2017.9.3.257.

1214 Knuckles, T. L., J. Yi, D. G. Frazer, H. D. Leonard, B. T. Chen, V. Castranova, and T. R. Nurkiewicz.
1215 2012. "Nanoparticle inhalation alters systemic arteriolar vasoreactivity through sympathetic
1216 and cyclooxygenase-mediated pathways." *Nanotoxicology* 6 (7):724-35. doi:
1217 10.3109/17435390.2011.606926.

1218 Kobayashi, N., M. Naya, S. Endoh, J. Maru, K. Yamamoto, and J. Nakanishi. 2009. "Comparative
1219 pulmonary toxicity study of nano-TiO(2) particles of different sizes and agglomerations in
1220 rats: different short- and long-term post-instillation results." *Toxicology* 264 (1-2):110-8. doi:
1221 10.1016/j.tox.2009.08.002.

1222 Kobayashi, T., Y. Oshima, Y. Tsubokura, N. Hashizume, S. Ajimi, T. Kayashima, M. Nakai, T. Sasaki,
1223 K. Kawaguchi, and N. Imatanaka. 2016. "Effects of dose volume and delivery device on
1224 bronchoalveolar lavage parameters of intratracheally administered nano-sized TiO2 in rats."
1225 *Regul Toxicol Pharmacol* 81:233-241. doi: 10.1016/j.yrtph.2016.08.018.

1226 Kreyling, W. G., U. Holzwarth, N. Haberl, J. Kozempel, S. Hirn, A. Wenk, C. Schleh, M. Schaffler, J.
1227 Lipka, M. Semmler-Behnke, and N. Gibson. 2017. "Quantitative biokinetics of titanium
1228 dioxide nanoparticles after intravenous injection in rats: Part 1." *Nanotoxicology* 11 (4):434-
1229 442. doi: 10.1080/17435390.2017.1306892.

1230 Kreyling, W. G., U. Holzwarth, N. Haberl, J. Kozempel, A. Wenk, S. Hirn, C. Schleh, M. Schaffler, J.
1231 Lipka, M. Semmler-Behnke, and N. Gibson. 2017. "Quantitative biokinetics of titanium
1232 dioxide nanoparticles after intratracheal instillation in rats: Part 3." *Nanotoxicology* 11
1233 (4):454-464. doi: 10.1080/17435390.2017.1306894.

1234 Kreyling, W. G., U. Holzwarth, C. Schleh, J. Kozempel, A. Wenk, N. Haberl, S. Hirn, M. Schaffler, J.
1235 Lipka, M. Semmler-Behnke, and N. Gibson. 2017. "Quantitative biokinetics of titanium
1236 dioxide nanoparticles after oral application in rats: Part 2." *Nanotoxicology* 11 (4):443-453.
1237 doi: 10.1080/17435390.2017.1306893.

1238 Kreyling WG, and Scheuch G. 2000. "Clearance of particles deposited in the lungs. In: Gehr P,
1239 Heyder J (Eds) Particle-Lung Interactions
1240 " *Marcel Dekker, New York, Basel, 232–376*.

1241 Kwon, S., Y. S. Yang, H. S. Yang, J. Lee, M. S. Kang, B. S. Lee, K. Lee, and C. W. Song. 2012.
1242 "Nasal and pulmonary toxicity of titanium dioxide nanoparticles in rats." *Toxicol Res* 28
1243 (4):217-24. doi: 10.5487/tr.2012.28.4.217.

1244 Kyjovska, Z. O., A. M. Boisen, P. Jackson, H. Wallin, U. Vogel, and K. S. Hougaard. 2013. "Daily
1245 sperm production: application in studies of prenatal exposure to nanoparticles in mice."
1246 *Reprod Toxicol* 36:88-97. doi: 10.1016/j.reprotox.2012.12.005.

1247 Landsiedel, R., L. Ma-Hock, T. Hofmann, M. Wiemann, V. Strauss, S. Treumann, W. Wohlleben, S.
1248 Groters, K. Wiench, and B. van Ravenzwaay. 2014. "Application of short-term inhalation

studies to assess the inhalation toxicity of nanomaterials." *Part Fibre Toxicol* 11:16. doi: 10.1186/1743-8977-11-16.

Larsen, S. T., P. Jackson, S. S. Poulsen, M. Levin, K. A. Jensen, H. Wallin, G. D. Nielsen, and I. K. Koponen. 2016. "Airway irritation, inflammation, and toxicity in mice following inhalation of metal oxide nanoparticles." *Nanotoxicology* 10 (9):1254-1262. doi: 10.1080/17435390.2016.1202350.

LeBlanc, A. J., J. L. Cumpston, B. T. Chen, D. Frazer, V. Castranova, and T. R. Nurkiewicz. 2009. "Nanoparticle inhalation impairs endothelium-dependent vasodilation in subepicardial arterioles." *J Toxicol Environ Health A* 72 (24):1576-84. doi: 10.1080/15287390903232467.

LeBlanc, A. J., A. M. Moseley, B. T. Chen, D. Frazer, V. Castranova, and T. R. Nurkiewicz. 2010. "Nanoparticle inhalation impairs coronary microvascular reactivity via a local reactive oxygen species-dependent mechanism." *Cardiovasc Toxicol* 10 (1):27-36. doi: 10.1007/s12012-009-9060-4.

Lee, J. F., S. P. Tung, D. Wang, D. Y. Yeh, Y. Fong, Y. C. Young, and F. J. Leu. 2014. "Lipoxygenase pathway mediates increases of airway resistance and lung inflation induced by exposure to nanotitanium dioxide in rats." *Oxid Med Cell Longev* 2014:485604. doi: 10.1155/2014/485604.

Leppänen, M., A. Korpi, M. Miettinen, J. Leskinen, T. Torvela, E. M. Rossi, E. Vanhala, H. Wolff, H. Alenius, V. M. Kosma, J. Joutsensaari, J. Jokiniemi, and P. Pasanen. 2011. "Nanosized TiO2 caused minor airflow limitation in the murine airways." *Archives of Toxicology* 85 (7):827-839. doi: 10.1007/s00204-011-0644-y.

Leppanen, M., A. Korpi, S. Mikkonen, P. Yli-Pirila, M. Lehto, L. Pylkkanen, H. Wolff, V. M. Kosma, H. Alenius, J. Joutsensaari, and P. Pasanen. 2015. "Inhaled silica-coated TiO2 nanoparticles induced airway irritation, airflow limitation and inflammation in mice." *Nanotoxicology* 9 (2):210-8. doi: 10.3109/17435390.2014.914260.

Li, B., Y. Ze, Q. Sun, T. Zhang, X. Sang, Y. Cui, X. Wang, S. Gui, D. Tan, M. Zhu, X. Zhao, L. Sheng, L. Wang, F. Hong, and M. Tang. 2013. "Molecular mechanisms of nanosized titanium dioxide-induced pulmonary injury in mice." *PLoS One* 8 (2):e55563. doi: 10.1371/journal.pone.0055563.

Li, J., Q. Li, J. Xu, J. Li, X. Cai, R. Liu, Y. Li, J. Ma, and W. Li. 2007. "Comparative study on the acute pulmonary toxicity induced by 3 and 20 nm TiO2 primary particles in mice." *Environmental Toxicology and Pharmacology* 24 (3):239-244. doi: 10.1016/j.etap.2007.06.004.

Liang, G., Y. Pu, L. Yin, R. Liu, B. Ye, Y. Su, and Y. Li. 2009. "Influence of different sizes of titanium dioxide nanoparticles on hepatic and renal functions in rats with correlation to oxidative stress." *J Toxicol Environ Health A* 72 (11-12):740-5. doi: 10.1080/15287390902841516.

Lide, DR (ed.). 2000. *CRC Handbook of Chemistry and Physics*. 81st Edition ed: CRC Press: Boca Raton.

Liu, R., X. Zhang, Y. Pu, L. Yin, Y. Li, X. Zhang, G. Liang, X. Li, and J. Zhang. 2010. "Small-sized titanium dioxide nanoparticles mediate immune toxicity in rat pulmonary alveolar macrophages in vivo." *J Nanosci Nanotechnol* 10 (8):5161-9.

Ma-Hock, L., S. Burkhardt, V. Strauss, A. O. Gamer, K. Wiench, B. van Ravenzwaay, and R. Landsiedel. 2009. "Development of a short-term inhalation test in the rat using nano-titanium dioxide as a model substance." *Inhal Toxicol* 21 (2):102-18. doi: 10.1080/08958370802361057.

Magdolenova, Z., D. Bilanicova, G. Pojana, L. M. Fjellsbo, A. Hudecova, K. Hasplova, A. Marcomini, and M. Dusinska. 2012. "Impact of agglomeration and different dispersions of titanium dioxide nanoparticles on the human related in vitro cytotoxicity and genotoxicity." *J Environ Monit* 14 (2):455-64. doi: 10.1039/c2em10746e.

1297 MAK. 2012. "General threshold limit value for dust (R fraction) (Biopersistent granular dusts)[MAK
1298 Value Documentation, 2012] " In *The MAK-Collection for Occupational Health and Safety*
1299 *(eds)*.

1300 Mikkelsen, L., M. Sheykhzade, K. A. Jensen, A. T. Saber, N. R. Jacobsen, U. Vogel, H. Wallin, S.
1301 Loft, and P. Moller. 2011. "Modest effect on plaque progression and vasodilatory function in
1302 atherosclerosis-prone mice exposed to nanosized TiO(2)." *Part Fibre Toxicol* 8:32. doi:
1303 10.1186/1743-8977-8-32.

1304 Nemmar, A., K. Melghit, S. Al-Salam, S. Zia, S. Dhanasekaran, S. Attoub, I. Al-Amri, and B. H. Ali.
1305 2011. "Acute respiratory and systemic toxicity of pulmonary exposure to rutile Fe-doped
1306 TiO(2) nanorods." *Toxicology* 279 (1-3):167-75. doi: 10.1016/j.tox.2010.10.007.

1307 Nemmar, A., K. Melghit, and B. H. Ali. 2008. "The acute proinflammatory and prothrombotic effects
1308 of pulmonary exposure to rutile TiO2 nanorods in rats." *Exp Biol Med (Maywood)* 233
1309 (5):610-9. doi: 10.3181/0706-rm-165.

1310 NIOSH. 2011. CURRENT INTELLIGENCE BULLETIN 63: Occupational Exposure to Titanium
1311 Dioxide.

1312 Noel, A., M. Charbonneau, Y. Cloutier, R. Tardif, and G. Truchon. 2013. "Rat pulmonary responses
1313 to inhaled nano-TiO(2): effect of primary particle size and agglomeration state." *Part Fibre*
1314 *Toxicol* 10:48. doi: 10.1186/1743-8977-10-48.

1315 Noel, A., K. Maghni, Y. Cloutier, C. Dion, K. J. Wilkinson, S. Halle, R. Tardif, and G. Truchon. 2012.
1316 "Effects of inhaled nano-TiO2 aerosols showing two distinct agglomeration states on rat
1317 lungs." *Toxicol Lett* 214 (2):109-19. doi: 10.1016/j.toxlet.2012.08.019.

1318 Noel, A., G. Truchon, Y. Cloutier, M. Charbonneau, K. Maghni, and R. Tardif. 2017. "Mass or total
1319 surface area with aerosol size distribution as exposure metrics for inflammatory, cytotoxic
1320 and oxidative lung responses in rats exposed to titanium dioxide nanoparticles." *Toxicol Ind*
1321 *Health* 33 (4):351-364. doi: 10.1177/0748233716651560.

1322 Numano, T., J. Xu, M. Futakuchi, K. Fukamachi, D. B. Alexander, F. Furukawa, J. Kanno, A. Hirose,
1323 H. Tsuda, and M. Suzui. 2014. "Comparative study of toxic effects of anatase and rutile type
1324 nanosized titanium dioxide particles in vivo and in vitro." *Asian Pac J Cancer Prev* 15 (2):929-
1325 35.

1326 Nurkiewicz, T. R., D. W. Porter, A. F. Hubbs, J. L. Cumpston, B. T. Chen, D. G. Frazer, and V.
1327 Castranova. 2008. "Nanoparticle inhalation augments particle-dependent systemic
1328 microvascular dysfunction." *Particle and Fibre Toxicology* 5. doi: 10.1186/1743-8977-5-1.

1329 Nurkiewicz, T. R., D. W. Porter, A. F. Hubbs, S. Stone, B. T. Chen, D. G. Frazer, M. A. Boegehold,
1330 and V. Castranova. 2009. "Pulmonary nanoparticle exposure disrupts systemic
1331 microvascular nitric oxide signaling." *Toxicol Sci* 110 (1):191-203. doi:
1332 10.1093/toxsci/kfp051.

1333 Oberdorster, G. 1995. "Lung particle overload: implications for occupational exposures to particles."
1334 *Regul Toxicol Pharmacol* 21 (1):123-35. doi: 10.1006/rtph.1995.1017.

1335 Oberdörster, G., J. N. Finkelstein, C. Johnston, R. Gelein, C. Cox, R. Baggs, and A. C. Elder. 2000.
1336 "Acute pulmonary effects of ultrafine particles in rats and mice." *Research report (Health*
1337 *Effects Institute)* (96):5-74; disc. 75-7486.

1338 OECD. 2015. Testing Programme of Manufactured Nanomaterials - Titanium dioxide In *Series on*
1339 *the Safety of Manufactured Nanomaterials No. 73*.

1340 OECD. 2017. Overview of the set of OECD Genetic Toxicology Test Guidelines and updates
1341 performed in 2014-2015. In *Series on Testing & Assessment No. 238*

1342 OECD. 2018a. CASE STUDY ON GROUPING AND READ-ACROSS FOR NANOMATERIALS
1343 GENOTOXICITY OF NANO- TiO2.

OECD. 2018b. Guidance document on inhalation toxicity studies. In *Series on Testing and Assessment*

Okada, T., A. Ogami, B. W. Lee, C. Kadoya, T. Oyabu, and T. Myojo. 2016. "Pulmonary responses in rat lungs after intratracheal instillation of 4 crystal forms of titanium dioxide nanoparticles." *J Occup Health* 58 (6):602-611. doi: 10.1539/joh.16-0094-OA.

Oyabu, T., Y. Morimoto, M. Hirohashi, M. Horie, T. Kambara, B. W. Lee, M. Hashiba, Y. Mizuguchi, T. Myojo, and E. Kuroda. 2013. "Dose-dependent pulmonary response of well-dispersed titanium dioxide nanoparticles following intratracheal instillation." *Journal of Nanoparticle Research* 15 (4). doi: 10.1007/s11051-013-1600-y.

Oyabu, T., Y. Morimoto, H. Izumi, Y. Yoshiura, T. Tomonaga, B. W. Lee, T. Okada, T. Myojo, M. Shimada, M. Kubo, K. Yamamoto, K. Kawaguchi, and T. Sasaki. 2016. "Comparison between whole-body inhalation and nose-only inhalation on the deposition and health effects of nanoparticles." *Environ Health Prev Med* 21 (1):42-8. doi: 10.1007/s12199-015-0493-z.

Oyabu, T., T. Myojo, B. W. Lee, T. Okada, H. Izumi, Y. Yoshiura, T. Tomonaga, Y. S. Li, K. Kawai, M. Shimada, M. Kubo, K. Yamamoto, K. Kawaguchi, T. Sasaki, and Y. Morimoto. 2017. "Biopersistence of NiO and TiO2 nanoparticles following intratracheal instillation and inhalation." *International Journal of Molecular Sciences* 18 (12). doi: 10.3390/ijms18122757.

Park, E. J., G. H. Lee, H. W. Shim, J. H. Kim, M. H. Cho, and D. W. Kim. 2014. "Comparison of toxicity of different nanorod-type TiO2 polymorphs in vivo and in vitro." *J Appl Toxicol* 34 (4):357-66. doi: 10.1002/jat.2932.

Paul, E., M. L. Franco-Montoya, E. Paineau, B. Angeletti, S. Vibhushan, A. Ridoux, A. Tiendrebeogo, M. Salome, B. Hesse, D. Vantelon, J. Rose, F. Canoui-Poitrine, J. Boczkowski, S. Lanone, C. Delacourt, and J. C. Pairon. 2017. "Pulmonary exposure to metallic nanomaterials during pregnancy irreversibly impairs lung development of the offspring." *Nanotoxicology* 11 (4):484-495. doi: 10.1080/17435390.2017.1311381.

Pelclova, D., H. Barosova, J. Kukutschova, V. Zdimal, T. Navratil, Z. Fenclova, S. Vlckova, J. Schwarz, N. Zikova, P. Kacer, M. Komarc, J. Belacek, and S. Zakharov. 2015. "Raman microspectroscopy of exhaled breath condensate and urine in workers exposed to fine and nano TiO2 particles: A cross-sectional study." *Journal of Breath Research* 9 (3). doi: 10.1088/1752-7155/9/3/036008.

Pelclova, D., V. Zdimal, Z. Fenclova, S. Vlckova, F. Turci, I. Corazzari, P. Kacer, J. Schwarz, N. Zikova, O. Makes, K. Syslova, M. Komarc, J. Belacek, T. Navratil, M. Machajova, and S. Zakharov. 2016. "Markers of oxidative damage of nucleic acids and proteins among workers exposed to TiO2 (nano) particles." *Occup Environ Med* 73 (2):110-8. doi: 10.1136/oemed-2015-103161.

Pelclova, D., V. Zdimal, P. Kacer, Z. Fenclova, S. Vlckova, M. Komarc, T. Navratil, J. Schwarz, N. Zikova, O. Makes, K. Syslova, J. Belacek, and S. Zakharov. 2016. "Leukotrienes in exhaled breath condensate and fractional exhaled nitric oxide in workers exposed to TiO2 nanoparticles." *J Breath Res* 10 (3):036004. doi: 10.1088/1752-7155/10/3/036004.

Pelclova, D., V. Zdimal, P. Kacer, S. Vlckova, Z. Fenclova, T. Navratil, M. Komarc, J. Schwarz, N. Zikova, O. Makes, and S. Zakharov. 2016. "Markers of nucleic acids and proteins oxidation among office workers exposed to air pollutants including (nano)TiO2 particles." *Neuro Endocrinol Lett* 37 (Suppl1):13-16.

Pelclova, D., V. Zdimal, P. Kacer, N. Zikova, M. Komarc, Z. Fenclova, S. Vlckova, J. Schwarz, O. Makes, K. Syslova, T. Navratil, F. Turci, I. Corazzari, S. Zakharov, and D. Bello. 2017. "Markers of lipid oxidative damage in the exhaled breath condensate of nano TiO2 production workers." *Nanotoxicology* 11 (1):52-63. doi: 10.1080/17435390.2016.1262921.

Porter, D. W., N. Wu, A. F. Hubbs, R. R. Mercer, K. Funk, F. Meng, J. Li, M. G. Wolfarth, L. Battelli, S. Friend, M. Andrew, R. Hamilton, Jr., K. Sriram, F. Yang, V. Castranova, and A. Holian.

2013. "Differential mouse pulmonary dose and time course responses to titanium dioxide nanospheres and nanobelts." *Toxicol Sci* 131 (1):179-93. doi: 10.1093/toxsci/kfs261.

Pott, F., and M. Roller. 2005. "Carcinogenicity study with nineteen granular dusts in rats." *European Journal of Oncology* 10 (4):249-281.

Pujalte, I., D. Dieme, S. Haddad, A. M. Serventi, and M. Bouchard. 2017. "Toxicokinetics of titanium dioxide (TiO2) nanoparticles after inhalation in rats." *Toxicol Lett* 265:77-85. doi: 10.1016/j.toxlet.2016.11.014.

Pujalte, I., A. Serventi, A. Noel, D. Dieme, S. Haddad, and M. Bouchard. 2017. "Characterization of Aerosols of Titanium Dioxide Nanoparticles Following Three Generation Methods Using an Optimized Aerosolization System Designed for Experimental Inhalation Studies." *Toxics* 5 (3). doi: 10.3390/toxics5030014.

Rahman, L., D. Wu, M. Johnston, A. William, and S. Halappanavar. 2017. "Toxicogenomics analysis of mouse lung responses following exposure to titanium dioxide nanomaterials reveal their disease potential at high doses." *Mutagenesis* 32 (1):59-76. doi: 10.1093/mutage/gew048.

Ramanakumar, A. V., M. E. Parent, B. Latreille, and J. Siemiatycki. 2008. "Risk of lung cancer following exposure to carbon black, titanium dioxide and talc: results from two case-control studies in Montreal." *Int J Cancer* 122 (1):183-9. doi: 10.1002/ijc.23021.

Relier, C., M. Dubreuil, O. Lozano Garcia, E. Cordelli, J. Mejia, P. Eleuteri, F. Robidel, T. Loret, F. Pacchierotti, S. Lucas, G. Lacroix, and B. Trouiller. 2017. "Study of TiO2 P25 Nanoparticles Genotoxicity on Lung, Blood, and Liver Cells in Lung Overload and Non-Overload Conditions After Repeated Respiratory Exposure in Rats." *Toxicol Sci* 156 (2):527-537. doi: 10.1093/toxsci/kfx006.

Renwick, L. C., D. Brown, A. Clouter, and K. Donaldson. 2004. "Increased inflammation and altered macrophage chemotactic responses caused by two ultrafine particle types." *Occup Environ Med* 61 (5):442-7.

Roberts, J. R., R. S. Chapman, V. R. Tirumala, A. Karim, B. T. Chen, D. Schwegler-Berry, A. B. Stefaniak, S. S. Leonard, and J. M. Antonini. 2011. "Toxicological evaluation of lung responses after intratracheal exposure to non-dispersed titanium dioxide nanorods." *J Toxicol Environ Health A* 74 (12):790-810. doi: 10.1080/15287394.2011.567954.

Rossi, E. M., L. Pylkkanen, A. J. Koivisto, H. Nykasenoja, H. Wolff, K. Savolainen, and H. Alenius. 2010. "Inhalation exposure to nanosized and fine TiO2 particles inhibits features of allergic asthma in a murine model." *Part Fibre Toxicol* 7:35. doi: 10.1186/1743-8977-7-35.

Rossi, E. M., L. Pylkkänen, A. J. Koivisto, M. Vippola, K. A. Jensen, M. Miettinen, K. Sirola, H. Nykäsenoja, P. Karisola, T. Stjernvall, E. Vanhala, M. Kiilunen, P. Pasanen, M. Mäkinen, K. Hämeri, J. Joutsensaari, T. Tuomi, J. Jokiniemi, H. Wolff, K. Savolainen, S. Matikainen, and H. Alenius. 2009. "Airway exposure to silica-coated TiO2 nanoparticles induces pulmonary neutrophilia in mice." *Toxicological Sciences* 113 (2):422-433. doi: 10.1093/toxsci/kfp254.

Roulet, A., L. Armand, M. Dagouassat, F. Rogerieux, A. Simon-Deckers, E. Belade, J. T. Van Nhieu, S. Lanone, J. C. Pairon, G. Lacroix, and J. Boczkowski. 2012. "Intratracheally administered titanium dioxide or carbon black nanoparticles do not aggravate elastase-induced pulmonary emphysema in rats." *BMC Pulm Med* 12:38. doi: 10.1186/1471-2466-12-38.

Roursgaard, M., K. A. Jensen, S. S. Poulsen, N. E. Jensen, L. K. Poulsen, M. Hammer, G. D. Nielsen, and S. T. Larsen. 2011. "Acute and subchronic airway inflammation after intratracheal instillation of quartz and titanium dioxide agglomerates in mice." *ScientificWorldJournal* 11:801-25. doi: 10.1100/tsw.2011.67.

Rushton, E. K., J. Jiang, S. S. Leonard, S. Eberly, V. Castranova, P. Biswas, A. Elder, X. Han, R. Gelein, J. Finkelstein, and G. Oberdorster. 2010. "Concept of assessing nanoparticle hazards considering nanoparticle dosemetric and chemical/biological response metrics." *J Toxicol Environ Health A* 73 (5):445-61. doi: 10.1080/15287390903489422.

1442 Saber, Anne Thoustrup, Jacob Stuart Lamson, Nicklas Raun Jacobsen, Gitte Ravn-Haren, Karin
1443 Sørig Hougaard, Allen Njimeri Nyendi, Pia Wahlberg, Anne Mette Madsen, Petra Jackson,
1444 Håkan Wallin, and Ulla Vogel. 2013. "Particle-Induced Pulmonary Acute Phase Response
1445 Correlates with Neutrophil Influx Linking Inhaled Particles and Cardiovascular Risk." *PLOS*
1446 *ONE* 8 (7):e69020. doi: 10.1371/journal.pone.0069020.

1447 Sager, T. M., and V. Castranova. 2009. "Surface area of particle administered versus mass in
1448 determining the pulmonary toxicity of ultrafine and fine carbon black: comparison to ultrafine
1449 titanium dioxide." *Part Fibre Toxicol* 6:15. doi: 10.1186/1743-8977-6-15.

1450 Savi, M., S. Rossi, L. Bocchi, L. Gennaccaro, F. Cacciani, A. Perotti, D. Amidani, R. Alinovi, M.
1451 Goldoni, I. Aliatis, P. P. Lottici, D. Bersani, M. Campanini, S. Pinelli, M. Petyx, C. Frati, A.
1452 Gervasi, K. Urbanek, F. Quaini, A. Buschini, D. Stilli, C. Rivetti, E. Macchi, A. Mutti, M.
1453 Miragoli, and M. Zaniboni. 2014. "Titanium dioxide nanoparticles promote arrhythmias via a
1454 direct interaction with rat cardiac tissue." *Part Fibre Toxicol* 11:63. doi: 10.1186/s12989-014-
1455 0063-3.

1456 Scaffold. Stockmann-Juvala, H., P. Taxell, et T. Santonen. 2014. "Formulating occupational
1457 exposure limits values (OELs)(Inhalation & Dermal)." : Finnish Institute of Occupational
1458 Health, Helsinki Scaffold Public Documents-SPD7. http://scaffold.eu-
1459 vri.eu/filehandler.ashx?file=13717

1460 Scarino, A., A. Noel, P. M. Renzi, Y. Cloutier, R. Vincent, G. Truchon, R. Tardif, and M.
1461 Charbonneau. 2012. "Impact of emerging pollutants on pulmonary inflammation in asthmatic
1462 rats: ethanol vapors and agglomerated TiO2 nanoparticles." *Inhal Toxicol* 24 (8):528-38. doi:
1463 10.3109/08958378.2012.696741.

1464 SCCS. 2014. OPINION ON Titanium Dioxide (nano form) COLIPA n° S75. SCCS/1516/13 Revision
1465 of 22 April 2014

1466 Scuri, M., B. T. Chen, V. Castranova, J. S. Reynolds, V. J. Johnson, L. Samsell, C. Walton, and G.
1467 Piedimonte. 2010. "Effects of titanium dioxide nanoparticle exposure on neuroimmune
1468 responses in rat airways." *J Toxicol Environ Health A* 73 (20):1353-69. doi:
1469 10.1080/15287394.2010.497436.

1470 Shinohara, N., Y. Oshima, T. Kobayashi, N. Imatanaka, M. Nakai, T. Ichinose, T. Sasaki, G. Zhang,
1471 H. Fukui, and M. Gamo. 2014. "Dose-dependent clearance kinetics of intratracheally
1472 administered titanium dioxide nanoparticles in rat lung." *Toxicology* 325:1-11. doi:
1473 10.1016/j.tox.2014.08.003.

1474 Silva, R. M., C. Teesy, L. Franzi, A. Weir, P. Westerhoff, J. E. Evans, and K. E. Pinkerton. 2013.
1475 "Biological response to nano-scale titanium dioxide (TiO2): role of particle dose, shape, and
1476 retention." *J Toxicol Environ Health A* 76 (16):953-72. doi: 10.1080/15287394.2013.826567.

1477 Stapleton, P. A., Q. A. Hathaway, C. E. Nichols, A. B. Abukabda, M. V. Pinti, D. L. Shepherd, C. R.
1478 McBride, J. Yi, V. C. Castranova, J. M. Hollander, and T. R. Nurkiewicz. 2018. "Maternal
1479 engineered nanomaterial inhalation during gestation alters the fetal transcriptome." *Particle*
1480 *and Fibre Toxicology* 15 (1):3. doi: 10.1186/s12989-017-0239-8.

1481 Stapleton, P. A., C. R. McBride, J. Yi, and T. R. Nurkiewicz. 2015. "Uterine microvascular sensitivity
1482 to nanomaterial inhalation: An in vivo assessment." *Toxicol Appl Pharmacol* 288 (3):420-8.
1483 doi: 10.1016/j.taap.2015.08.013.

1484 Stapleton, P. A., V. C. Minarchick, J. Yi, K. Engels, C. R. McBride, and T. R. Nurkiewicz. 2013.
1485 "Maternal engineered nanomaterial exposure and fetal microvascular function: does the
1486 Barker hypothesis apply?" *Am J Obstet Gynecol* 209 (3):227.e1-11. doi:
1487 10.1016/j.ajog.2013.04.036.

1488 Stapleton, P. A., C. E. Nichols, J. Yi, C. R. McBride, V. C. Minarchick, D. L. Shepherd, J. M.
1489 Hollander, and T. R. Nurkiewicz. 2015. "Microvascular and mitochondrial dysfunction in the

female F1 generation after gestational TiO2 nanoparticle exposure." *Nanotoxicology* 9 (8):941-51. doi: 10.3109/17435390.2014.984251.

Sun, Q., D. Tan, Y. Ze, X. Sang, X. Liu, S. Gui, Z. Cheng, J. Cheng, R. Hu, G. Gao, G. Liu, M. Zhu, X. Zhao, L. Sheng, L. Wang, M. Tang, and F. Hong. 2012. "Pulmotoxicological effects caused by long-term titanium dioxide nanoparticles exposure in mice." *J Hazard Mater* 235-236:47-53. doi: 10.1016/j.jhazmat.2012.05.072.

Sun, Q., D. Tan, Q. Zhou, X. Liu, Z. Cheng, G. Liu, M. Zhu, X. Sang, S. Gui, J. Cheng, R. Hu, M. Tang, and F. Hong. 2012. "Oxidative damage of lung and its protective mechanism in mice caused by long-term exposure to titanium dioxide nanoparticles." *J Biomed Mater Res A* 100 (10):2554-62. doi: 10.1002/jbm.a.34190.

Tang, M., T. Zhang, Y. Xue, S. Wang, M. Huang, Y. Yang, M. Lu, H. Lei, L. Kong, Y. Wang, and Y. Pu. 2011. "Metabonomic studies of biochemical changes in the serum of rats by intratracheally instilled TiO2 nanoparticles." *J Nanosci Nanotechnol* 11 (4):3065-74.

US EPA. June 2012. Benchmark Dose Technical Guidance. EPA/100/R-12/001. Risk Assessment Forum. U.S. Environmental Protection Agency Washington, DC 20460

van Ravenzwaay, B., R. Landsiedel, E. Fabian, S. Burkhardt, V. Strauss, and L. Ma-Hock. 2009. "Comparing fate and effects of three particles of different surface properties: nano-TiO(2), pigmentary TiO(2) and quartz." *Toxicol Lett* 186 (3):152-9. doi: 10.1016/j.toxlet.2008.11.020.

Wallin, H., Z. O. Kyjovska, S. S. Poulsen, N. R. Jacobsen, A. T. Saber, S. Bengtson, P. Jackson, and U. Vogel. 2017. "Surface modification does not influence the genotoxic and inflammatory effects of TiO2 nanoparticles after pulmonary exposure by instillation in mice." *Mutagenesis* 32 (1):47-57. doi: 10.1093/mutage/gew046.

Wan, R., Y. Mo, Z. Zhang, M. Jiang, S. Tang, and Q. Zhang. 2017. "Cobalt nanoparticles induce lung injury, DNA damage and mutations in mice." *Part Fibre Toxicol* 14 (1):38. doi: 10.1186/s12989-017-0219-z.

Wang, J., C. Chen, Y. Liu, F. Jiao, W. Li, F. Lao, Y. Li, B. Li, C. Ge, G. Zhou, Y. Gao, Y. Zhao, and Z. Chai. 2008. "Potential neurological lesion after nasal instillation of TiO(2) nanoparticles in the anatase and rutile crystal phases." *Toxicol Lett* 183 (1-3):72-80. doi: 10.1016/j.toxlet.2008.10.001.

Wang, J., Y. Liu, F. Jiao, F. Lao, W. Li, Y. Gu, Y. Li, C. Ge, G. Zhou, B. Li, Y. Zhao, Z. Chai, and C. Chen. 2008. "Time-dependent translocation and potential impairment on central nervous system by intranasally instilled TiO(2) nanoparticles." *Toxicology* 254 (1-2):82-90. doi: 10.1016/j.tox.2008.09.014.

Warheit, D. B., T. R. Webb, K. L. Reed, S. Frerichs, and C. M. Sayes. 2007. "Pulmonary toxicity study in rats with three forms of ultrafine-TiO2 particles: differential responses related to surface properties." *Toxicology* 230 (1):90-104. doi: 10.1016/j.tox.2006.11.002.

Warheit, D. B., T. R. Webb, C. M. Sayes, V. L. Colvin, and K. L. Reed. 2006. "Pulmonary instillation studies with nanoscale TiO2 rods and dots in rats: toxicity is not dependent upon particle size and surface area." *Toxicol Sci* 91 (1):227-36. doi: 10.1093/toxsci/kfj140.

Weast, R.C. (ed.). 1991. *CRC Handbook of Chemistry and Physics* 69th Edition ed: CRC Press Inc., Boca Raton, Florida.

Xu, J., M. Futakuchi, M. Iigo, K. Fukamachi, D. B. Alexander, H. Shimizu, Y. Sakai, S. Tamano, F. Furukawa, T. Uchino, H. Tokunaga, T. Nishimura, A. Hirose, J. Kanno, and H. Tsuda. 2010. "Involvement of macrophage inflammatory protein 1alpha (MIP1alpha) in promotion of rat lung and mammary carcinogenic activity of nanoscale titanium dioxide particles administered by intra-pulmonary spraying." *Carcinogenesis* 31 (5):927-35. doi: 10.1093/carcin/bgq029.

Yin, J., C. Kang, Y. Li, Q. Li, X. Zhang, and W. Li. 2014. "Aerosol inhalation exposure study of respiratory toxicity induced by 20 nm anatase titanium dioxide nanoparticles." *Toxicology Research* 3 (5):367-374. doi: 10.1039/c4tx00040d.

Yokohira, M., N. Hashimoto, K. Yamakawa, S. Suzuki, K. Saoo, T. Kuno, and K. Imaida. 2009. "Lung Carcinogenic Bioassay of CuO and TiO(2) Nanoparticles with Intratracheal Instillation Using F344 Male Rats." *J Toxicol Pathol* 22 (1):71-8. doi: 10.1293/tox.22.71.

Yoshiura, Y., H. Izumi, T. Oyabu, M. Hashiba, T. Kambara, Y. Mizuguchi, B. W. Lee, T. Okada, T. Tomonaga, T. Myojo, K. Yamamoto, S. Kitajima, M. Horie, E. Kuroda, and Y. Morimoto. 2015. "Pulmonary toxicity of well-dispersed titanium dioxide nanoparticles following intratracheal instillation." *J Nanopart Res* 17 (6):241. doi: 10.1007/s11051-015-3054-x.

Yu, K. N., J. H. Sung, S. Lee, J. E. Kim, S. Kim, W. Y. Cho, A. Y. Lee, S. J. Park, J. Lim, C. Park, C. Chae, J. K. Lee, J. Lee, J. S. Kim, and M. H. Cho. 2015. "Inhalation of titanium dioxide induces endoplasmic reticulum stress-mediated autophagy and inflammation in mice." *Food Chem Toxicol* 85:106-13. doi: 10.1016/j.fct.2015.08.001.

Yu, X., X. Zhao, Y. Ze, L. Wang, D. Liu, J. Hong, B. Xu, A. Lin, C. Zhang, Y. Zhao, B. Li, and F. Hong. 2014. "Changes of serum parameters of TiO(2) nanoparticle-induced atherosclerosis in mice." *J Hazard Mater* 280:364-71. doi: 10.1016/j.jhazmat.2014.08.015.

Ze, X., M. Su, X. Zhao, H. Jiang, J. Hong, X. Yu, D. Liu, B. Xu, L. Sheng, Q. Zhou, J. Zhou, J. Cui, K. Li, L. Wang, Y. Ze, and F. Hong. 2016. "TiO2 nanoparticle-induced neurotoxicity may be involved in dysfunction of glutamate metabolism and its receptor expression in mice." *Environ Toxicol* 31 (6):655-62. doi: 10.1002/tox.22077.

Ze, Y., R. Hu, X. Wang, X. Sang, X. Ze, B. Li, J. Su, Y. Wang, N. Guan, X. Zhao, S. Gui, L. Zhu, Z. Cheng, J. Cheng, L. Sheng, Q. Sun, L. Wang, and F. Hong. 2014. "Neurotoxicity and gene-expressed profile in brain-injured mice caused by exposure to titanium dioxide nanoparticles." *J Biomed Mater Res A* 102 (2):470-8. doi: 10.1002/jbm.a.34705.

Ze, Y., L. Sheng, X. Zhao, J. Hong, X. Ze, X. Yu, X. Pan, A. Lin, Y. Zhao, C. Zhang, Q. Zhou, L. Wang, and F. Hong. 2014. "TiO2 nanoparticles induced hippocampal neuroinflammation in mice." *PLoS One* 9 (3):e92230. doi: 10.1371/journal.pone.0092230.

Ze, Y., L. Sheng, X. Zhao, X. Ze, X. Wang, Q. Zhou, J. Liu, Y. Yuan, S. Gui, X. Sang, Q. Sun, J. Hong, X. Yu, L. Wang, B. Li, and F. Hong. 2014. "Neurotoxic characteristics of spatial recognition damage of the hippocampus in mice following subchronic peroral exposure to TiO2 nanoparticles." *J Hazard Mater* 264:219-29. doi: 10.1016/j.jhazmat.2013.10.072.

Ze, Y., L. Zheng, X. Zhao, S. Gui, X. Sang, J. Su, N. Guan, L. Zhu, L. Sheng, R. Hu, J. Cheng, Z. Cheng, Q. Sun, L. Wang, and F. Hong. 2013. "Molecular mechanism of titanium dioxide nanoparticles-induced oxidative injury in the brain of mice." *Chemosphere* 92 (9):1183-9. doi: 10.1016/j.chemosphere.2013.01.094.

Zhang, G., N. Shinohara, H. Kano, H. Senoh, M. Suzuki, T. Sasaki, S. Fukushima, and M. Gamo. 2015. "Quantitative evaluation of the pulmonary microdistribution of TiO2 nanoparticles using X-ray fluorescence microscopy after intratracheal administration with a microsprayer in rats." *J Appl Toxicol* 35 (6):623-30. doi: 10.1002/jat.3109.

Zhang, G., N. Shinohara, H. Kano, H. Senoh, M. Suzuki, T. Sasaki, S. Fukushima, and M. Gamo. 2016. "Quantitative evaluation of local pulmonary distribution of TiO2 in rats following single or multiple intratracheal administrations of TiO2 nanoparticles using X-ray fluorescence microscopy." *J Appl Toxicol* 36 (10):1268-75. doi: 10.1002/jat.3287.

Zhang, L., R. Bai, B. Li, C. Ge, J. Du, Y. Liu, L. Le Guyader, Y. Zhao, Y. Wu, S. He, Y. Ma, and C. Chen. 2011. "Rutile TiO(2) particles exert size and surface coating dependent retention and lesions on the murine brain." *Toxicol Lett* 207 (1):73-81. doi: 10.1016/j.toxlet.2011.08.001.

Zhao, Lin, Yifang Zhu, Zhangjian Chen, Huadong Xu, Jingwen Zhou, Shichuan Tang, Zhizhen Xu, Fanling Kong, Xinwei Li, Yifei Zhang, Xianzuo Li, Ji Zhang, and Guang Jia. 2018.

"Cardiopulmonary effects induced by occupational exposure to titanium dioxide nanoparticles." *Nanotoxicology* 12 (2):169-184. doi: 10.1080/17435390.2018.1425502.

Zhen, S., Q. Qian, G. Jia, J. Zhang, C. Chen, and Y. Wei. 2012. "A panel study for cardiopulmonary effects produced by occupational exposure to inhalable titanium dioxide." *J Occup Environ Med* 54 (11):1389-94. doi: 10.1097/JOM.0b013e3182611a49.

ANNEXES

Annex 1: Bibliographic Search

Study question: Identification of toxicological studies performed with TiO_2-NP.

The ultimate goals of this systematic review are 1) the derivation of a chronic toxicological reference value by inhalation with TiO_2-NP; 2) Identification of toxicological concerns which needs to be clarified (by requesting new studies) during Substance Evaluation in the framework of Reach Regulation.

Description of the review method:

Publications were identified through two databases: PubMed and Scopus®. Secondary literature from IARC, OECD, NIOSH, ECHA, EFSA and SCCS was also taken into account.

The methodology of the review (eligibility criteria and key words) was defined between October and December 2017. The literature search was performed in January 2018. An update of the systematic review was performed in July 2018.

Key words and eligibility criteria for inclusion or exclusion:

The following key words were used for the records identification in the selected database (PubMed and Scopus®):

Identity:

"Titanium dioxide" OR Titania OR "TiO2" OR "TiO(2)" OR Rutile OR Anatase OR Brookite OR P25 OR "T-lite" OR "Titanium oxide*" OR "TiO2-NPs" OR Nanotitania OR Nanotitanium OR E171 OR NM101 OR NM102 OR NM103 OR NM104 OR NM105

"Titanium dioxide" OR titania OR "TiO2" OR "TiO(2)" OR rutile OR anatase OR brookite OR p25 OR "T-lite" OR "Titanium oxide*" OR "TiO2-NPs" OR nanotitania OR nanotitanium OR e171 OR nm101 OR nm102 OR nm103 OR nm104 OR nm105)) AND (ALL ("Ultra Fine" OR nanoscale OR nanomaterial OR "nanoparti*" OR nano OR nanocrystal OR nanosized OR "nanostructure*" OR synthetic OR nanobelt OR nanotube OR "nanofib*" OR "nanolayer*" OR modified OR coated OR "nanocomposite*" OR "functionali*" OR "nanopowder*" OR nanoamor OR nanotechnology OR "nanoadditive*" OR uncoated OR aggregate OR substituted OR agglomerate OR nm100 OR "Food-grade"

Exposure:

"Inhalat*" OR "respira*" OR airway OR nasal OR intranasal OR "intra tracheal" OR instillation OR lung OR chronic OR "pre natal" OR "post natal" OR subchronic OR "repeat*" OR "day*" OR "week*" OR olfactive OR "month*" OR "year*" OR "long term" OR subacute OR "short term" OR "nose only" OR acute OR oral OR gavage OR "drinking water" OR feed OR food OR diet OR "per oral"

"in vivo" OR animal OR "cohort*" OR "case control" OR epidemiology OR "review*" OR "chapter*" OR "poster*" OR "experiment*" OR occupational OR longitudinal OR "in vitro" OR cell OR "in silico" OR safety OR evaluation OR corona OR biokinetic OR "ex vivo"

Population:

Child*" OR "worker*" OR "adult*" OR occupational OR "rat*" OR mouse OR "rabbit*" OR "human*" OR "monkey*" OR "dog*" OR hen OR "guinea pig*" OR "animal*" OR "sensitive population" OR painter OR man OR woman OR men OR women OR "manufacturer*" OR asthmatic OR pregnant OR infant OR toddler OR male OR female OR mammalian OR mice OR elderly OR aging OR gestation

Outcome:

Toxicity OR toxicology OR "inflammat*" OR "neurotox*" OR "tumor*" OR neoplastic OR promotion OR cancer OR "oxidative stress" OR "reactive oxygen species" OR "reactive nitrogen species" OR ros OR rns OR fertility OR developmental OR effect OR "genotox*" OR "mutagen*" OR genetic OR aberration OR mutation OR "DNA damage" OR overload OR transformation OR diffusion OR translocation OR clastogenicity OR "micronucle*" OR comet OR "carcinogen*" OR hormone OR thyroid OR reproduction OR tumour OR non-neoplastic OR immunity OR metabolism OR heart OR brain OR lung OR kidney OR "blood barrier" OR "Blood-Brain-Barrier" OR "placental barrier" OR retention OR disease OR "adverse effect*" OR concern OR elimination OR kinetics OR absorption OR "reprotox*" OR safety OR noael OR loael OR loel OR noel OR mitochondria OR nucleus OR threshold OR bmd OR behavior OR reactivity OR benchmark OR hazard OR risk OR spleen OR irritation OR hematoencephalic OR assessment OR placenta OR development OR immune OR epigenetic OR promoter OR chromosome AND stability OR alveolar AND barrier OR injury OR lipid OR crossing OR excretion OR body AND burden OR distribution OR macrophage OR epigenome OR intestinal OR gut OR permeability OR renal

Only records published from 2000 were considered because it is generally considered that before this date, the tests often had missing information on the physicochemical characteristics of the testing nanomaterial and/or did not take into account nano-specificity. In addition, publications not written in English or French were excluded. A total of **1888** records were thus identified.

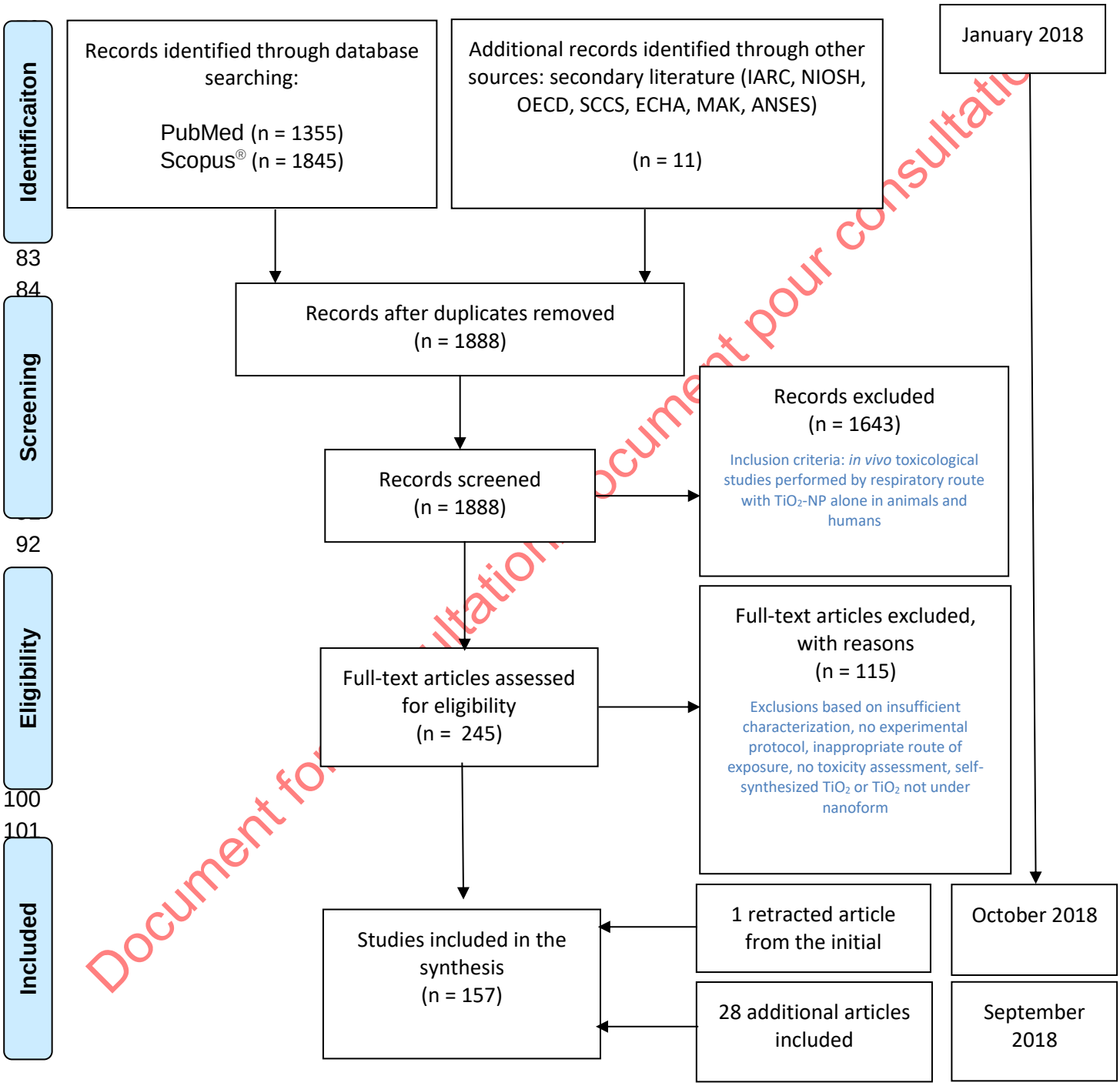
The following exclusion criteria were then applied leading to the exclusion of **1643** records based on title and abstract:

- Studies not performed with TiO$_2$-NP alone;
- Not toxicological studies (ex. ecotoxicological studies, studies on implants, water disinfection, biotechnology, nanomedicine, analytical method, etc.).

In addition, it was decided to only focus the assessment on *in vivo* toxicity of TiO$_2$-NP by respiratory route. Therefore, only full-text articles complying with this criterion were selected, leading to a total of **245** articles. In addition, in September 2018, **28** further full-text articles were included. Finally, **one** article sorted from the initial bibliographic research was retracted in October 2018.

Methodology quality assessment of included studies:

Among these 266 articles, 157 articles were judged relevant and included in the synthesis.



Annex 2: Details of parameters used for calculation of OEL

Information on the use of the MPPD model version 3.04

Parameter	Rat	Human	Reference
Airway Morphometry			
Model	Asymm. Multiple-Path long Evans	Yeh/Schum 5-lobe	/
FRC (ml)	4.0	3300	MPPD default value
URT Volume (ml)	0.42	50	MPPD default value
Inhalant properties – Aerosol			
Density (g/cm3)	4.26	4.26	P25 data, Sigma-Aldrich
Aspect ratio	1	1	/
Diameter (µm)	1.44 (MMAD)	1.44 (MMAD)	Bermudez et al. (2004)
GSD	2.6	2.6	
Inhability adjustment uncheck			Size < 3 µm
Equiv. Diam. Model uncheck			
Exposure condition – Constant exposure			
Aerosol concentration (mg/m3)	0.5	0.5	NOAEC Bermudez et al. 2004
Breathing Frequency (/min)	102	20	MPPD default value
Tidal Volume (ml)	2.1	1040	MPPD default value
Inspiration Fraction	0.5	0.5	Default value
Pause Fraction	0	0	Default value
Breeathing Scenario	Whole-Body inhalation	Oronasal – normal augmenter	
Deposition/Clearance - Deposition only			

Details and justification of the parameters and data used for calculation of the OEL

- Elimination half-life:
  - Rat: 60 days (Brown et al. 2005), confirmed by the results of the Bermudez et al., (2004) study where an elimination half-life of 63 days was calculated for the concentration of 0.5 mg/m^3 for rats.
  - Human: 400 days (Kreyling and Scheuch 2000)
- Lung surface area
  - Rat: 57.22 m² (U.S. EPA, 2009)
  - Human: 0.297 m² (U.S. EPA, 2009)

Annex 3: Minority opinions

Three experts from the HRV Committee expressed a minority opinion and one expert from the HRV Committee abstained on the collective expert appraisal report during the validation of the opinion.

Their position is laid out below.

"The calculation of the $NOAEC_{HEC}$ of 65 µg.m^{-3} based on the rat NOAEC (500 µg.m^{-3}), according to MAK methodology described in the report doesn't seem questionable to us; however, the choice or the justifications used for some adjustment factors are.

In particular:

- an AF_S of 3 was used whereas the $NOAEC_{HEC}$ takes into account:
    1. the calculation of the lung deposition fraction in human for a 8h/d, 5d/w, 240d/y, life-long exposure;
    2. the difference in the elimination half-time between human (400 days) and rats (60 days);
    3. the fact that it is based on a threshold effect in the most sensitive species. Indeed, experimental data suggest that the rat is particularly sensitive to pulmonary toxicity of TiO_2-NP compared to other rodent species (mice and hamster), but also to monkey and human (cf § 4.4 of collective expert appraisal)
- an AF_A of 3 was used while at the steady state of lung load, the sensitivity of the rat, considered as the most sensitive species, and of human is the same for a given dose of TiO_2-NP expressed per m² of pulmonary surface. Moreover, the toxicodynamic variability should be limited compared to soluble compounds or vapours as TiO_2 is almost insoluble.
- finally, an AF_D of 3 was also used based on the following justification: "it cannot be ruled out that other adverse effects could occur at sub-inflammatory concentrations". While it is always appropriate to question the overall quality of the database, the current scientific data do not suggest that some effect could occur at exposure concentrations lower than those without observed effects in the lungs. None of the OEL report dealt with so far provided fully exhaustive data on all organs and all biological functions. Therefore, it seems to us that the use of an AF_D of 3 in the present report on the sole basis "it cannot be ruled out" should involve applying the same factor in every OEL and TRV dossier systematically. From our point of view, this appears inappropriate."

153 # Annex 4: Follow-up of updates of the report

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Date	Version	Description of the changes
November 2019	01	version for public consultation

155