Scientific Committee on Health, Environmental and Emerging Risks

SCHEER

Opinion on the safety of titanium dioxide in toys

Scientific Committees

on Consumer Safety
on Health, Environmental and Emerging Risks

The SCHEER adopted this document at its plenary meeting on 9 June 2023

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ABSTRACT

Following the mandate from the European Commission, this scientific Opinion evaluates whether the uses of titanium dioxide in toys and toy materials can be considered to be safe in light of the exposure identified, and in light of the classification of titanium dioxide as carcinogenic category 2 after inhalation. Safe toys and toy materials, for which derogation is possible, should be indicated. Scientific data on the toxicity of TiO_2 from primary and secondary sources and additional information on the use of TiO_2 in toys, provided by the Toys Industries of Europe (TIE), was evaluated and included in the Opinion where appropriate. Several uses of TiO_2 in toys with the highest possible exposures were evaluated. In its scientific work, the SCHEER relies on its Memorandum on Weight of Evidence (WoE).

Inhalation exposure

When it can be demonstrated with high certainty that no ultrafine fraction is present in pigmentary TiO_2 preparations used in toys and toy materials, safe use with no or negligible risk for all products with a TiO_2 content above 1% is indicated based on the exposure estimations of this Opinion. However, if an ultrafine fraction is assumed to be present, safe use is not indicated for the use of casting kits (exposure scenario 1, realistic high and upper bound estimate), chalk (exposure scenario 2, upper bound estimate) and powder paints (exposure scenario 4, upper bound estimate). White colour pencils can be used with no or negligible risk (safe) by children of different age groups independent whether an ultrafine fraction is present in the TiO_2 preparation. The WoE for the inhalation risk characterisation is strong and for the exposure assessment weak to moderate.

Oral exposure

Based on the Margin-of-Safety values only, it can be concluded that toys containing pigmentary TiO_2 can be used with no or negligible risk in the worst-case exposure scenarios considered. However, the WoE for the oral risk characterisation is weak for the hazard characterisation and weak to moderate for the exposure assessment. Safe use is only indicated when the absence of an ultrafine fraction in the TiO_2 pigments can be demonstrated by an appropriate methodology.

ACKNOWLEDGMENTS

Members of the Working Group are acknowledged for their valuable contribution to this opinion. The members of the Working Group are:

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1. SUMMARY

Background

Following the mandate from the European Commission, this scientific Opinion evaluates whether the uses of pigmentary titanium dioxide in toys and toy materials can be considered to be safe in light of the exposure identified, and in light of the classification of titanium dioxide as carcinogenic category 2 after inhalation. Safe toys and toy materials, for which derogation is possible, should be indicated.

The Toy Safety Directive 2009/48/EC prohibits the use of substances in toys if those substances are classified as carcinogenic, mutagenic or toxic for reproduction (CMR). Under certain conditions, however, the use of such substances may be permitted. To permit the use of a CMR substance of category 2, the substance has to be evaluated by the relevant Scientific Committee and found to be safe, in particular in view of exposure. This Opinion describes the risk assessment for the use of pigmentary TiO_2 as colouring agent in toys and/or toy materials used for the production of toys.

To address the terms of reference of this Opinion, scientific data on the toxicity of TiO_2 and information regarding approaches to derive NOAEL values or other toxicological points of departure were collected from available open literature, websites and from documents of other Scientific Committees and International Organisations (*e.g.*, IARC (WHO), EPA (US), EFSA, SCCS, Health Canada). In addition, information on the use of TiO_2 in toys, provided by the Toys Industries of Europe (TIE), was evaluated and included in the Opinion where appropriate. In its scientific work, the SCHEER relies on its Memorandum on Weight of Evidence (WoE).

Application of TiO_2 in toys

Based on the information on different white pigments containing TiO_2, which was provided by the toy industry, inhalable particles below a size of 10 µm (which is the size indicated in EU 2020/217 for a carcinogenic hazard) are present in the white pigments used in the toy production. A number of products contain TiO_2 pigments with a particle size <10 µm at a percentage above 1%.

Particle size

Throughout this Opinion, nanoscale/nanosized particles (1-100 nm) will be indicated as ultrafine particles in line with conventions in inhalation toxicology. Microscale particles with an aerodynamic diameter above 0.1 µm will be indicated as fine particles. However, when referring to studies performed with TiO_2 as nanomaterials, the opinion retains the original wording of nanoparticle/nanomaterial/nanofraction.

Pigmentary fine TiO_2 should not contain a nanofraction. Limited industry data provided show an overall size distribution of pigmentary TiO_2 with a range of 141nm – 39811 nm Although it is not demonstrated that an ultrafine fraction would be present to a significant degree for the pigmentary TiO_2 as used in toys and toy materials, the presence of an ultrafine fraction in the pigments cannot be excluded because measurement methods may not have evaluated constituent particles and agglomerates. The weight of evidence is weak for the conclusion that there are no ultrafine particles present, based on limited data with medium consistency and medium quality. The data are provided by the toy industry while robust study reports on the measurement methods of the particle size distribution of TiO_2 pigment used in toys are not available.

Inhalation and ingestion exposure potential

Inhalation exposure to TiO_2 may potentially occur with the use of products that can lead to the release of TiO_2 particles, such as powder products and spray products, and products for which wear and tear occurs. Therefore, when considering exposure to TiO_2, particular attention needs to be given to inhalation exposures arising from use of such products. On

this basis, four exposure scenarios were selected that are intended to encompass the use of toys with highest potential for inhalation exposure: 1. casting kits, 2. chalk, 3. white colour pencils and 4. powder paint.

Regarding risks arising from oral exposure, recently EFSA expressed a concern for TiO$_2$ as food additive E171 after oral exposure in view of uncertainties regarding possible genotoxic effects. Based on this EFSA Opinion, the SCHEER concluded that there was a need to also assess the oral exposure to TiO$_2$ from toys in children. For determination of the potential oral exposure to TiO$_2$ via toys, three direct ingestion scenarios have been selected for further evaluation involving the products 1. lip gloss/ lipstick, 2. finger paint and 3. white colouring pencils.

Exposure estimation

Upper-bound air concentrations after TiO$_2$ release from toys were estimated for the four toy products considered relevant: casting kit, chalk, white colour pencil and powder paint. Realistic high air concentrations were also estimated for the more uncertain scenarios for casting kit and powder paint. Based on the assessment of the quality of the data and uncertainties the WoE for these estimations was considered weak, moderate, strong and weak, respectively.

Worst-case oral exposures were calculated for lip gloss/lipstick, finger paint and white colouring pencils. The WoE for these estimations was considered moderate, weak and weak, respectively. Aggregated exposure was considered for the three oral exposure scenarios.

Both oral and inhalation exposure can result in internal uptake of TiO$_2$ particles albeit at relatively low amounts. The WoE for low uptake after inhalation exposure is considered strong in view of high-quality data and high consistency. The WoE for oral uptake is considered moderate to strong.

Hazard characterisation

Regarding the toxicokinetics, it can be concluded that the systemic availability of TiO$_2$ after both oral and inhalation exposure is very low. For both inhalation and oral exposure, adverse effects of exposure to TiO$_2$ particles could be identified including direct effects such as lung and GIT oxidative stress and inflammation and indirect effects such as altering the immune system responses.

Although a threshold for TiO$_2$ size for induction of genotoxic effects cannot be established at the moment, it can be observed that in studies on TiO$_2$ in nanosize, the results (mainly *in vitro* studies) show higher probability of positive response than in studies on microsize or with sizes slightly above 100 nm. It is possible that a probability of a genotoxic effect diminishes as the size of TiO$_2$ increases, and the observed positive effects can depend on the presence of a nanofraction. The potential genotoxicity of pigmentary fine TiO$_2$, including the demonstration of the absence of a nanofraction, remains uncertain. Overall, based on the results of *in vitro* and *in vivo* genotoxicity studies, the SCHEER is of the opinion that the pigmentary fine TiO$_2$ grades can be considered to have no genotoxic potential after oral and inhalation exposure, provided the presence of a nanofraction can be excluded.

For inhalation exposure, the WoE of genotoxic hazard of TiO$_2$ is moderate, based on the high quality but relatively low consistency of the results. However, for oral exposure, the WoE of genotoxic hazard of TiO$_2$ is weak.

Although there is limited evidence in epidemiological studies for the induction of lung cancer in occupational settings, in combination with various animal studies the WoE is strong for a possible carcinogenic effect of TiO$_2$ in the lung after inhalation exposure. Re-evaluation of previous epidemiological studies indicate also for humans a carcinogenic risk

in the lung. From the available rodent studies and the adverse outcome pathway suggested, the mechanisms by which TiO_2 can induce lung tumours in rats after inhalation can operate via impaired clearance and persistent inflammation. The Point of Departure (PoD) for inhalation carcinogenicity can be based on a threshold for these effects. The short-term PoD was determined to be the No-Observed-Adverse-Effect Concentration (NOAEC) of 0.5 mg/m^3 air for ultrafine TiO_2 and 10 mg/m^3 air for fine TiO_2.

The available studies after oral exposure are not sufficient to draw conclusions on the potential carcinogenicity of TiO_2 particles. However, the induction of oxidative stress and inflammation in the GIT indicates a possible indirect or promoting effect of TiO_2 on tumour development. The WoE for tumour-promoting activity of TiO_2 particles in the GIT is moderate, whereas the WoE for tumour induction in the GIT is uncertain to weak. Considering the adverse outcome pathway, assumed to be similar to the inhalation adverse outcome pathway, and considering the weak WoE for genotoxic potential of a possible nanofraction after oral exposure, the SCHEER concludes that the PoD for oral exposure can also be based on a threshold for toxicity. The NOAEL was determined as 1,000 mg/kg bw per day for general repeated dose toxicity after oral exposure per day. The PoD for a single exposure was established to be 5,000 mg/kg bw.

The overall WoE for the inhalatory adverse effects and the NOAEC is considered strong, but for oral exposure the overall WoE for adverse effects is judged to be weak.

Risk characterisation

In the risk characterisation, conclusions are drawn based on the estimated Margins of Safety for children using toys containing TiO_2 (MoS, margin between the level of exposure considered without adverse effects and the estimated level of exposure) and the overall Weight of Evidence (WoE).

Risk characterisation for polymers

The application of TiO_2 as colouring agent for polymers used to produce toys is considered to pose no or negligible risk to children, as the potential release of the TiO_2 from the polymers is considered negligible to non-existent due to the fixation of the TiO_2 within the polymer matrix. However, when TiO_2 is not embedded within a polymer, TiO_2 release resulting in inhalation and/or oral exposure of children is possible.

Risk characterisation after inhalation exposure

Based on the MoS-values, it can be concluded that toys containing TiO_2 can be used with no or negligible risk (safely) in the realistic high- and upper-bound exposure scenarios considered, when the pigmentary TiO_2 does not contain ultrafine fractions. However, when an ultrafine fraction is assumed to be present, safe use is not indicated for exposure scenario 1 (casting kit, realistic high- and upper-bound estimate), scenario 2 (chalk, upper-bound estimate) and scenario 4 (powder paint, upper-bound estimate). White colour pencils (exposure scenario 3) can be used with no or negligible risk (safe) by children of different age groups even when an ultrafine fraction is present in the TiO_2 preparation. The WoE for the inhalation risk characterisation is strong for the hazard characterisation and, depending on the scenario, weak to strong for the exposure assessment.

When an ultrafine fraction is present, it cannot be concluded that casting kits, chalk, and powder paint can be used safely by children. This conclusion is based on the low MoS-values. The uncertainty of the exposure assessment (weak or moderate WoE) was addressed by using upper-bound exposure estimates for the determination of the MoS.

When it can be demonstrated with high certainty that no ultrafine fraction is present in TiO_2 preparations used in toys and toy materials, use with no or negligible risk (safe) for

all products with a TiO_2 content above 1% is indicated, based on the exposure estimations of this Opinion.

Risk characterisation after oral exposure

Based on the MoS-values only, it can be concluded that toys containing TiO_2 can be used with no or negligible risk in the worst-case oral exposure scenarios considered. However, the WoE is weak for the hazard characterisation and weak to moderate for the exposure assessment.

Although there is uncertainty on the hazard characterisation, the MoS for oral exposure for the pigmentary fine TiO_2 is sufficiently high to indicate safe use. When the absence of an ultrafine fraction can be demonstrated with an appropriate methodology, pigmentary TiO_2 can be considered to show safe use with no or negligible risk after oral exposure.

Final remarks

It should be recognised that the safety evaluation as presented is limited to the levels of TiO_2 in the toys used for the various evaluated exposure scenarios. Although the evaluated exposure scenarios have the highest possibility for TiO_2 exposure, possibly some toys containing TiO_2 may result in exposure for children that were not evaluated in this Opinion. In addition, aggregated exposure due to other sources of TiO_2 exposure, e.g., via food, cosmetics etc., is not considered.

2. MANDATE FROM THE EU COMMISSION SERVICES

This part is provided by the requesting Commission service.

2.1 Background

The Toy Safety Directive 2009/48/EC[1] prohibits the use of substances in toys if those substances are classified as carcinogenic, mutagenic or toxic for reproduction (CMR).[2, 3] Under certain conditions, however, the use of such substances may be permitted.

To permit the use of a CMR substance of category 2, the substance has to be evaluated by the relevant Scientific Committee and found to be safe, in particular in view of exposure. An additional condition is that the substance is not prohibited for use in consumer articles under REACH. [4, 5]

Titanium dioxide (CAS number 13463-67-7) in powder form containing 1% or more of particles with aerodynamic diameter ≤ 10 μm has been classified as carcinogenic

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[1] Directive 2009/48/EC of the European Parliament and of the Council of 18 June 2009 on the safety of toys. OJ L 170, 30.06.2009, p. 1.
https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1589882074178&uri=CELEX:02009L0048-20191118
[2] Annex II, Part III, point 3 of the Toy Safety Directive.
[3] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, p. 1.
https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1589288952589&uri=CELEX:32008R1272
[4] Annex II, Part III, point 5 (c) of the Toy Safety Directive.
[5] REACH: Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. OJ L 396, 30.12.2006, p. 1.
https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1589281141090&uri=CELEX:32006R1907

category 2 by inhalation.[6] Liquid mixtures containing 1% or more of titanium dioxide particles with aerodynamic diameter ≤ 10 μm have to be labelled with the warning that hazardous respirable droplets may be formed when sprayed, which should not be inhaled. Solid mixtures containing 1% or more of titanium dioxide have to be labelled with the warning that hazardous respirable dust may be formed when used, which should not be inhaled.[7]

The toy industry[8] reported that the vast majority of titanium dioxide placed on the market is in powder form and contains 1% or more particles with an aerodynamic diameter of ≤ 10 μm. The toy industry further signalled a wide use of titanium dioxide in toys and toy materials, including coatings, chalks and powder paints, clays and putties, and polymeric materials. The highest content of titanium dioxide has been indicated as ranging between 1% and 30%. The toy industry also provided a compilation on toxicology, exposure and risk assessment of titanium dioxide with regard to toys.

The writing instruments industry[9] reported the use of titanium dioxide in colour pencils and wax crayons (which can both be toys), in particular when white. It also reported the results of abrasive tests with colour pencils and wax crayons, including the number and mass of the dust particles observed after abrasion. Similarly, it reported abrasive tests on dried finger paint and with oven dried modelling clay. Finally, it also transmitted an occupational exposure study with titanium dioxide.

2.2 Terms of Reference

SCHEER is asked:

1. to review the available data on the use of titanium dioxide leading to inhalation exposure in particular in toys and toy materials

2. to evaluate whether the uses of titanium dioxide in toys and toy materials can be considered to be safe in light of the exposure identified, and in light of the classification of titanium dioxide as carcinogenic category 2 as outlined in the background above. Safe toys and toy materials should be indicated.

In replying to the above questions and in order to ensure coherence with other scientific bodies, SCHEER is invited to consult in particular the Scientific Committee on Consumer Safety (SCCS).

Timeline:
Preliminary Opinion – mid-2021
Final Opinion – autumn 2021

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[6] Commission Delegated Regulation (EU) 2020/217 of 4 October 2019 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures and correcting that Regulation. OJ L 44, 18.2.2020, p. 1.
https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=uriserv:OJ.L_.2020.044.01.0001.01.ENG&toc=OJ:L:2020:044:TOC
[7] Annex I, No (2) of the afore-mentioned Commission Delegated Regulation (EU) 2020/217.
[8] Toy Industries of Europe (TIE). See annexes 1-x to this mandate.
[9] European Writing Manufacturer's Association (EWIMA). See Annexes 2, 3, 4 and 5 to this mandate.

3. SCIENTIFIC OPINION

Background

Following the mandate from the European Commission, this scientific Opinion evaluates whether the uses of titanium dioxide (TiO_2) as colouring agent in toys and toy materials can be considered to be safe in light of the exposure identified, and in light of the classification of TiO_2 as carcinogenic category 2. Safe toys and toy materials, for which derogation is possible, should be indicated. This Opinion should be based on a review of the available data on the use of TiO_2 leading to inhalation exposure in particular for toys and toy materials.

The Toy Safety Directive 2009/48/EC prohibits the use of substances in toys if those substances are classified as carcinogenic, mutagenic or toxic for reproduction (CMR). Under certain conditions, however, the use of such substances may be permitted. To permit the use of a CMR substance of category 2, the substance has to be evaluated by the relevant Scientific Committee and found to be safe, in particular in view of exposure.

It should be noted that the CMR status of TiO_2 was challenged by industry. A recent judgement by the Court of Justice of the European Union (JUDGMENT OF THE GENERAL COURT (Ninth Chamber, Extended Composition), dated 23November 2022, annuled the Commission Delegated Regulation (EU 2020/217 of 4 October 2019)[10] in so far as it concerns the harmonised classification and labelling of titanium dioxide as a carcinogenic substance by inhalation in certain powder forms.

This SCHEER Opinion describes the risk assessment of the use of pigmentary TiO_2 in toys and/or toys materials irrespective of the legal classification of TiO_2 particles, and is based on the evaluation of scientific information available at the time of preparation of the Opinion.

Methodology

To address the terms of reference of this Opinion, scientific data on the toxicity of TiO_2 and information regarding approaches to derive NOAEL values were collected from available open literature, websites and from documents of other Scientific Committees and International Organisations (e.g. IARC (WHO), EPA (US), EFSA, SCCS, Health Canada). In addition, information on the use of TiO_2 in toys, provided by the Toys Industries of Europe (TIE), was evaluated and included in the Opinion where appropriate.

In its scientific work, the SCHEER relies on the Memorandum on Weight of Evidence (WoE), which describes how the level of quality and reliability of the conclusions and their uncertainties are reached (SCHEER, 2018). For each line of evidence, the criteria of relevance, validity, and reliability were applied to the information (e.g. references, reports) used. In the integration of the different lines of evidence, the strength of the overall weight of evidence depends on the consistency and the quality of the results. In the risk characterisation, conclusions were drawn based on the estimated Margins of Safety (MoS, margin between the level of exposure considered without adverse effects and the estimated level of exposure) for children using toys containing TiO_2 and the overall Weight of Evidence (WoE) (see Section 5.2 for a more extensive explanation of the SCHEER WoE-categories).

Throughout this Opinion, nanoscale/nanosized particles (1-100 nm) are indicated as ultrafine particles in line with conventions in inhalation toxicology. Microscale particles with an aerodynamic diameter above 0.1 μm are indicated as fine particles. However, when

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[10] CWS Powder Coatings and Others v Commission (europa.eu)

referring to studies performed with TiO_2 as nanomaterials, the Opinion retains the original wording of nanoparticle/nanomaterial/nanofraction.

Question 1

To review the available data on the use of titanium dioxide leading to inhalation exposure in particular in toys and toy materials

Application of TiO_2 in toys

According to the information on different white pigments containing TiO_2, provided by the toy industry, inhalable particles below a size of 10 μm (which is the size indicated in EU 2020/217 for a carcinogenic hazard) are present in the white pigments used in the toy production. A number of products contain TiO_2 pigments with a particle size <10 μm at a percentage above 1%, the threshold above which respirable TiO_2 is considered carcinogenic (EU 2020/2017). For these products, a specific risk assessment needs to be performed in view of the classification of TiO_2 particles as carcinogen category 2 by inhalation with a limitation to respirable TiO_2 particles. Based on the information as present in the literature and provided above, migration of TiO_2 when embedded in polymers is unlikely, so there is no or negligible risk for exposure to TiO_2 present in a polymer matrix

Particle size

Pigmentary fine TiO_2 should not contain a nanofraction. Limited industry data on pearlescent pigmentary TiO_2 show distributions of pigmentary TiO_2 with 10-90 percentile (by volume) ranges between 5 and 45 μm. It is noted that this pearlescent pigment is composed of mica coated with TiO_2 particles. These pearlescent TiO_2 coated particles can not be considered representative of the TiO_2 grades used in toy products. For a few other TiO_2 products used in toys, overall sizes ranged from 141nm to 39811nm. Although the information provided indicates that an ultrafine fraction would not be present to a significant degree for the pigmentary TiO_2 as used in toys and toy materials, the presence of an ultrafine fraction in the pigments cannot be excluded because measurement methods may not have evaluated constituent particles and agglomerates. The WoE is weak for the conclusion that there are no ultrafine particles present since this conclusion is based on limited data with medium consistency and medium quality. The data were provided by the toy industry, but robust study reports on the measurement methods of the particle size distribution of TiO_2 pigment used in toys were not available.

Inhalation and ingestion exposure potential

Regarding the toxicokinetics, it can be concluded that the systemic availability of TiO_2 both for oral and inhalation exposure is very low. The WoE is strong.

Inhalation exposure to TiO_2 may potentially occur with the use of products that can lead to the release of TiO_2 particles, such as powder products and spray products, and products for which wear and tear occurs. Therefore, when considering exposure to TiO_2, particular attention needs to be given to inhalation exposures arising from use of such products. On this basis, four exposure scenarios were selected that are intended to encompass the use of toys with highest potential for inhalation exposure, with respective toys at their maximum levels of TiO_2: casting kits (1.5% TiO_2), chalk (5% TiO_2), white colour pencils (51% TiO_2) and powder paint (25% TiO_2).

Regarding risks arising from oral exposure, recently EFSA expressed a concern for TiO_2 as food additive E171 after oral exposure in view of uncertainties regarding possible genotoxic effects. Based on this EFSA Opinion, the SCHEER concluded that there was a need to also assess the oral exposure to TiO_2 from toys in children. For determination of the potential oral exposure to TiO_2 via toys, three direct ingestion scenarios have been selected for further evaluation. The direct ingestion scenario is supposed to have the highest potential

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oral exposure compared to other exposures such as from mouthing and the mucocilliary pathway (ingestion following removal of particles from the lung). The selected products are lip gloss/lipstick (15% TiO_2), finger paint (30% TiO_2) and white colouring pencils (51% TiO_2), based on the likeliness of exposure when playing with these products as well as the relatively high percentage of TiO_2 in them.

Exposure estimation

Upper-bound air concentrations after TiO_2 release from toys were estimated for the four toy products considered relevant: 1. casting kit, 2. chalk, 3. white colour pencils and 4. powder paint. Realistic high air concentrations were also estimated for the more uncertain scenarios for casting kit and powder paint. When available, information on TiO_2 air concentrations provided by the toy industry was used. When such data were not available, information from the public literature were used as surrogate for TiO_2 particle air concentrations (e.g. air concentration after particle release from chalk/cosmetic particles). Based on the assessment of the quality of the data and uncertainties, the WoE for these estimations was considered to be weak for casting kit (scenario 1), moderate for chalk (scenario 2), strong for white colour pencils (scenario 3) and weak for powder paint (scenario 4), respectively. The air concentrations calculated based on the four exposure scenarios were used as input for the risk characterisation of the use of TiO_2 in toys.

Worst-case oral exposures were calculated for lip gloss/lipstick, finger paint and white colouring pencils based on default values as proposed in international reports. The WoE for these estimations was considered moderate (lip gloss/lipstick), weak (finger paint) and weak (white colouring pencils).

Aggregated oral exposure was considered for the three oral exposure scenarios. Concomitant oral and inhalation exposure is likely. However, the oral exposure resulting from the lung clearance and transport by the mucociliary escalator is rather low, and even orders of magnitude lower in view of the high oral exposures in the evaluated scenarios. Therefore, an aggregated oral exposure including the mucocilliary route was considered not relevant. In addition, the inhalatory and oral routes may result in exposure of different target organs. For inhalation, aggregation of different exposure sources is not relevant, since the exposures will not occur simultaneously, and the endpoint is a concentration-related effect (inflammation).

Both oral and inhalation exposure can result in systemic availability of TiO_2 particles albeit at relatively low amounts. The WoE that the uptake fraction after inhalation is small is considered strong in view of high-quality data and high consistency. The WoE for oral uptake is considered moderate to strong as there is some variation in the amount of Ti that can be detected in the body after oral exposure. In addition, there is considerable difference in the quality of the published results with respect to the characterisation of the TiO_2 materials used.

Question 2

To evaluate whether the uses of titanium dioxide in toys and toy materials can be considered to be safe in light of the exposure identified, and in light of the classification of titanium dioxide as carcinogenic category 2 as outlined in the background above. Safe toys and toy materials should be indicated.

Hazard characterisation

For both inhalation and oral exposure, adverse effects of exposure to TiO_2 particles could be identified including direct effects such as lung and GIT oxidative stress and inflammation and indirect effects such as altering the immune system responses.

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Although there is limited evidence in epidemiological studies for the induction of lung cancer in occupational settings, recent re-evaluation of epidemiological studies applying more sophisticated statistics revealed a Healthy Worker Survivor Effect (HWSE) for occupational exposure. These results, in combination with various animal studies, clearly indicate a possible carcinogenic effect of TiO_2 in the lung after inhalation exposure. The WoE is considered strong. The re-evaluation of previous epidemiological studies indicates also for humans a carcinogenic risk in the lung. From the available rodent studies and the adverse outcome pathway suggested, the mechanisms by which TiO_2 can induce lung tumours in rats after inhalation can operate via impaired clearance and persistent inflammation. Whether the carcinogenic effect is due to a specific effect of (ultra)fine TiO_2 particles or due to a general carcinogenic effect of particles in the lung is as yet unknown. Therefore, also considering the moderate WoE for genotoxic potential of a possible nanofraction after inhalation exposure (see below), the SCHEER concludes that the PoD for inhalation carcinogenicity can be based on a threshold for these indirect effects as proposed by the SCCS (2020). The short-term PoD was selected based on Bermudez *et al.* (2002, 2004): the NOAEC was determined to be 0.5 mg/m^3 for ultrafine TiO_2 and 10 mg/m^3 for fine TiO_2.

The available studies after oral exposure are not sufficient to draw conclusions on the potential carcinogenicity of TiO_2 particles. The different results in the studies available might indicate that there is a matrix effect of the exposure vehicle on the outcome. However, the induction of oxidative stress and inflammation in the GIT indicates a possible indirect or promoting effect of TiO_2 on tumour development. The WoE for tumour-promoting activity of TiO_2 particles in the GIT is moderate, whereas the WoE for tumour induction in the GIT is uncertain to weak. Considering the adverse outcome pathway, assumed to be similar to the inhalation adverse outcome pathway and considering the weak WoE for genotoxic potential of a possible nanofraction after oral exposure (see below), the SCHEER concludes that the PoD for oral exposure can also be based on a threshold for toxicity. The NOAEL was determined as 1,000 mg/kg bw per day for general repeated dose toxicity after oral exposure per day (Warheit *et al.,* 2015). The PoD for a single exposure was established to be 5,000 mg/kg bw.

There may be a risk for genotoxicity due to TiO_2 exposure both after inhalation and oral uptake. A substantial proportion of genotoxicity studies on ultrafine TiO_2 indicates a genotoxic potential (both chromosomal aberrations/MN test and comet assay). In contrast, a majority of studies on chromosomal aberrations/MN test (basic mutagenic endpoints) are negative for fine TiO_2. However, for fine TiO_2, the comet assay was observed to be positive in many studies. In most positive genotoxic studies with fine TiO_2 particles, either a nanofraction was present or could not be excluded. There is some evidence for internalisation of TiO_2 ultrafine particles in the nucleus and mitochondria.

The *in vivo* genotoxic effects were observed in very limited number of the evaluated studies. The relative contributions of the postulated modes of action to the genotoxicity elicited by TiO_2 (ultrafine) particles are unknown (neither primary not secondary mechanisms can be excluded) and there is uncertainty as to whether a threshold dose or particles size cut-off point for any mode of action could be established. No clear relationship is observed between the physicochemical properties of TiO_2 particles and the outcome of either *in vitro* or *in vivo* genotoxicity studies.

Although a threshold for TiO_2 size for induction of genotoxic effects cannot be established at the moment, it can be observed that in studies on TiO_2 in nanosize the results (mainly *in vitro* studies) show higher probability of positive response than in studies on microsize or with sizes slightly above 100 nm. It is possible that a probability of a genotoxic effect diminishes as the size of TiO_2 increases, and the observed positive effects can depend on the presence of a nanofraction. The potential genotoxicity of pigmentary fine TiO_2, including the demonstration of the absence of a nanofraction, remains uncertain. Overall,

based on the results of *in vitro* and *in vivo* genotoxicity studies, the SCHEER is of the opinion that the pigmentary fine TiO_2 grades can be considered to have no genotoxic potential after oral and inhalation exposure, provided the presence of a nanofraction can be excluded. For inhalation exposure, the WoE for a genotoxic hazard of TiO_2 is moderate, because although the studies were of high quality, the results were inconsistent. However, for oral exposure, the data are scarce and the WoE for a genotoxic hazard of TiO_2 is weak.

The overall WoE for the inhalatory adverse effects and the NOAECs is considered strong, but for oral exposure, the overall WoE for adverse effects is judged to be weak.

Risk characterisation for polymers

The application of TiO_2 as a colouring agent for polymers used to produce toys is considered to pose no or negligible risk to children, as the potential release of the TiO_2 from the polymers is considered negligible to non-existent due to the fixation of the TiO_2 within the polymer matrix. Potential exposure is only possible when pieces of the toy break off due to mouthing (see oral exposure below).

Risk characterisation after inhalation exposure

For the risk characterisation after inhalation exposure, the air concentration of the rat-NOAEC as obtained in the inhalation studies by Bermudez *et al.* (2002, 2004) was extrapolated to a human equivalent concentration (HEC) using a dosimetric adjustment factor (DAF). This HEC for no effect was compared to the air concentrations determined for the four inhalation exposure scenarios evaluated, resulting in MoS-values for the various exposure scenarios.

Based on the determined MoS-values, it can be concluded that toys containing TiO_2 can be used with no or negligible risk (safely) in the realistic high- and upper-bound exposure scenarios considered, when the pigmentary TiO_2 does not contain ultrafine fractions. However, if an ultrafine fraction is assumed to be present, safe use is not indicated for exposure scenario 1 (casting kit, realistic high- and upper-bound estimate), scenario 2 (chalk, upper-bound estimate) and scenario 4 (powder paint, upper-bound estimate).

The WoE for the inhalation risk characterisation is strong for the hazard characterisation and, depending on the scenario, is weak to strong for the exposure assessment. The main uncertainty in the hazard characterisation is connected to the relatively low consistency of the genotoxicity results. Pigmentary TiO_2 grades can be considered to have no genotoxic potential after inhalation exposure, provided the presence of a nanofraction can be excluded. For inhalation exposure, the WoE of genotoxic hazard of TiO_2 is moderate, because although the studies were of high quality, the results were inconsistent. It remains uncertain whether this will affect the threshold approach followed.

The weight of evidence for the exposure estimations varies from weak (casting kit, powder paint) to moderate (chalk) and strong (white colour pencils). The high uncertainty in the casting kit and powder paint exposure estimates is addressed by a conservative approach (realistic high- and upper-bound estimation). The evaluations were performed with the following TiO_2 concentrations in the toys (casting kit 1.5%, chalk 5%, white pencils 51%, and powder paints 25%), therefore these levels should be considered the highest levels to be used in the designated toys for which safe use is indicated.

For the white colour pencils (scenario 3), a weak WoE was observed for particle size distribution of the TiO_2 pigment used, but a strong WoE for exposure and for the hazard characterisation. Overall white colour pencils can be used with no or negligible risk (safe) by children of different age groups even when an ultrafine fraction is present in the TiO_2 preparation.

When an ultrafine fraction is present, it cannot be concluded that casting kits, chalk, or powder paint can be used safely by children. This conclusion is based on the low MoS-

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values. The uncertainty of the exposure assessment (weak or moderate WoE) was addressed by using upper-bound exposure estimates for the determination of the MoS.

When it can be demonstrated with high certainty that no ultrafine fraction is present in TiO_2 preparations used in toys and toy materials, use with no or negligible risk (safe) for all products with a TiO_2 content above 1% is indicated based on the exposure estimations of this Opinion. It should be realised that the safety evaluation as presented is limited to the levels of TiO2 as present in the toys used for the various evaluated exposure scenarios.

For the safe use of pigmentary TiO_2 particles in toys, it is essential that the number size distribution of the particles, including both constituent particles and agglomerates/aggregates, is known. In addition, it should be demonstrated plausibly that no or a negligible ultrafine fraction is present in the TiO_2 preparations.

For upper-bound estimates, considered as worst-case scenarios, the MoS-values for different children age groups are as presented in Table 3.1. Based on the calculated human exposure concentrations (HEC), for inhalation exposure to toys, a margin of safety (MoS) of at least 25 can be considered to pose no or negligible risk (safe).

Table 3.1: MoS* calculated for the 4 inhalation scenarios at upper bound exposure to ultrafine TiO₂ (NOAEC = 0.5 mg/m³)

Scenario	Duration (min)	Children of 23 months	Children of 3 years	Children of 6 years
		+	+	+
1 Casting kit	10	**2.3**	**2.5**	**2.5**
2 Chalk	45	**6.5**	**6.9**	**6.7**
3 White colour pencil	45	27	29	29
4 Powder paint	10	**2.9**	**3.1**	**3.0**

* + = corrected for human elimination; a MoS >= 25 is considered safe

This upper-bound exposure for ultrafine TiO_2 in several exposure scenarios results in a MoS that is below 25 when calculated, including elimination from the lung: for scenario 1 casting kits, scenario 2 chalk and for scenario 4 powder paint for all age groups. So, the inhalation exposures to ultrafine TiO_2 released form casting kits, chalk, and powder paint can not be considered safe.

For white pencils for all age groups the MoS is above 25, and the use of ultrafine TiO_2 can be considered to pose no or negligible risk regarding inhalation exposure based on the upper-bound exposure estimates.

The upper-bound exposure for fine TiO_2, shown in Table 3.2, results in lowest MoS for the casting kit to be 51, for the chalk in a lowest MoS of 137, for the white colour pencils a lowest MoS of 589, and for the powder paint in a lowest MoS of 61, all scenarios were for the age of 23 months and the risk was calculated including elimination.

For the evaluated uses of fine TiO_2 in casting kits, chalk, white colour pencils and powder paint the MoS show safe use with no or negligible risk after inhalation exposure based on the upper-bound exposure estimates.

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Table 3.2: MoS[*] calculated for the 4 inhalation scenarios at upper-bound exposure to fine TiO₂ (NOAEC = 10 mg/m³)

Scenario	Duration (min)	Children of 23 months	Children of 3 years	Children of 6 years
		+	+	+
1 Casting kit	10	51	54	55
2 Chalk	45	137	146	141
3 White colour pencil	45	589	626	606
4 Powder paint	10	61	65	63

[*] + = corrected for human elimination; a MoS >= 25 is considered safe

Risk characterisation after oral exposure

Based on the MoS-values only, it can be concluded that toys containing fine TiO₂ can be used with no or negligible risk in the worst-case oral exposure scenarios considered. However, the WoE is weak for the hazard characterisation and weak to moderate for the exposure assessment. The weak WoE in the hazard characterisation is connected to uncertainties regarding immunotoxic, genotoxic and carcinogenic activity. Pigmentary fine TiO₂ grades can be considered to have no genotoxic potential after oral exposure, provided the presence of a nanofraction can be excluded. For oral exposure, the WoE of genotoxic hazard of fine TiO₂ is weak. The WoE for the exposure estimations is weak for the finger paint and white colour pencils scenarios and moderate for the lipstick scenario. The high uncertainty in the exposure estimates is addressed by a worst-case approach.

Although there is a weak WoE on the hazard characterisation, the MoS for oral exposure for the pigmentary fine TiO₂ is sufficiently high to indicate safe use (Table 3.3). When the absence of an ultrafine fraction can be demonstrated with an appropriate methodology, pigmentary fine TiO₂ in toys can be considered to be safe for use with no or negligible risk after oral exposure.

Table 3.3: MoS[*] calculated for the three selected oral exposure scenarios (pigment grade TiO₂)

Scenario	MoS
1. Finger paint	2564
2. White colouring pencil	1818
3. Lipstick	7692

[*] A MoS >= 100 is considered to pose no or negligible risk

Final remarks

It should be realised that the safety evaluation as presented is limited to the levels of pigmentary fine TiO₂ in the toys used for the various evaluated exposure scenarios. Pigmentary fine TiO₂ should not contain a nanofraction to have no or negligible risk when used in children's toys.

Although the evaluated exposure scenarios have the highest possibility for TiO_2 exposure, it should be noted that possibly some toys containing pigmentary fine TiO_2 may result in exposures for children that were not evaluated in this Opinion.

In addition, no aggregated exposure considering other sources of TiO_2 exposure, e.g., food, cosmetics etc., was considered.

An overview of the conclusions is shown in Table 3.4.

Table 3.43: Summary of conclusions

	Fine particles	**Ultrafine particles**
Inhalation		
Casting kit	safe	safe use not determined conclusively
Chalk	safe	safe use not determined conclusively
White colour pencil	safe	safe
Powder paint	safe	safe use not determined conclusively
Oral		
Finger paint	safe	safe use not determined conclusively
White colour pencil	safe	safe use not determined conclusively
Lipstick/ lip gloss	safe	safe use not determined conclusively

4. MINORITY OPINIONS

None

5. DATA AND METHODOLOGIES

5.1 Data/Evidence

The SCHEER, on request of Commission services, provides scientific Opinions on questions concerning health, environmental and emerging risks. The scientific assessments carried out should always be based on scientifically accepted approaches, and be transparent regarding the data, methods and assumptions that are used in the risk assessment process. They should identify uncertainties and use harmonised terminology, where possible, based on internationally accepted terms. In its scientific work, the SCHEER relies on the Memorandum on Weight of Evidence (WoE) and uncertainties (SCHEER, 2018), i.e. the search for relevant information and data for the SCHEER comprises the identification, collection and selection of possible sources of evidence in order to perform a risk assessment and/or to answer the specific questions being asked. For each line of evidence, the criteria of validity, reliability and relevance need to be applied and the overall quality has to be assessed. In the integration of the different lines of evidence, the strength of the overall evidence depends on the consistency and the quality of the information used (*e.g.* original peer-reviewed publications, reviews, reports, dossiers).

5.2 Methodologies

To address the terms of reference of this Opinion, scientific data on the toxicity of TiO_2 and information regarding approaches to derive NOAEL values were collected from available open literature, websites and from documents of other Scientific Committees and International Organisations (e.g. IARC (WHO), EPA (US), EFSA, SCCS, Health Canada). In addition, information on the use of TiO_2 in toys, provided by the Toys Industries of Europe (TIE), was evaluated and included in the Opinion where appropriate.

The Commission library service performed a literature search for publications between January 2015 and March 2021. The search terms and results are listed in Tables 5.1 and 5.2. This search resulted in 152 published articles. In addition, the SCHEER made use of reports by other organisations on this topic (EFSA and SCCS), as well as on information provided by the Commission. Additional literature provided by the working group members was considered and information provided by the Toys Industries of Europe (TIE), was evaluated.

The COVID pandemic in 2021 resulted in a delay of finalising the Opinion, so, relevant literature published after the literature search of 2021 was identified and collected by members of the Working Group.

Each document and line of evidence is assessed for relevance, validity and reliability on a 0-3 scale and then the overall WoE is assessed by combining the scores and considering the consistency of the results from the different lines of evidence.

As the information from the industry contained only relevant information, the submitted information was only assessed for validity and reliability.

Table 5.1: Results from PubMed search

Key words including MeSH terms	No of entries
Titanium dioxide AND toy OR toy materials	3
Titanium dioxide AND pigment grade AND toy OR toy materials	0
Titanium dioxide AND inhalation AND toxicokinetics	4
Titanium dioxide AND inhalation AND toy OR toy material	0
Titanium dioxide AND composition AND toy OR toy material	5
Titanium dioxide AND migration OR release AND toy materials	1
Titanium dioxide AND exposure AND inhalation AND toy OR toy materials	3
Titanium dioxide AND exposure AND toy OR toy materials	5
Titanium dioxide AND pigment grade AND particle size	18
Titanium dioxide AND modelling clay	10
Titanium dioxide AND photo degradation OR photo deterioration AND paint	73

Table 5.2: Results from Find-eR and Science Direct search

Key words including MeSH terms	No of entries
Titanium dioxide AND toy OR toy materials	4
Titanium dioxide AND pigment grade AND toy OR toy materials	0
Titanium dioxide AND inhalation AND toxicokinetics	6
Titanium dioxide AND inhalation AND toy OR toy material	
Titanium dioxide AND composition AND toy OR toy material	2
Titanium dioxide AND migration OR release AND toy materials	3
Titanium dioxide AND exposure AND inhalation AND toy OR toy materials	7
Titanium dioxide AND exposure AND toy OR toy materials	
Titanium dioxide AND pigment grade AND particle size	4
Titanium dioxide AND modelling clay	0
Titanium dioxide AND photo degradation OR photo deterioration AND paint	4

In its scientific work, the SCHEER relies on the Memorandum on Weight of Evidence (WoE) and uncertainties (SCHEER, 2018), *i.e.* the search for relevant information and data for the SCHEER comprises the identification, collection and selection of possible sources of evidence in order to perform a risk assessment and/or to answer the specific questions being asked. For each line of evidence, the literature/information used to support the conclusions is evaluated for the criteria of validity, reliability and relevance. Integrative assessment means that the results from all relevant individual lines of evidence are compiled into an overall assessment, taking into account their reliability, validity and relevance. The integration of the different lines of evidence may demand an element of expert judgement. The WoE depends on the consistency and the quality of the results. Consistency is defined as the agreement in the results of the analysis between all the lines of evidence; but also as the extent to which contributions of different pieces or lines of evidence to answering the specified question are compatible (EFSA Scientific Committee, 2017). Quality is defined as the combined result of the judgement on relevance, reliability and validity. The overall quality has to be assessed and is expressed as presented below. The SCHEER Memorandum (SCHEER, 2018) classifies results and conclusions of the analysis for human and environmental risks as follows:

- Strong weight of evidence: Coherent evidence from a primary line of evidence (human, animal, environment) and one or more other lines of evidence (in particular mode/mechanistic studies) in the absence of conflicting evidence from one of the other lines of evidence (no important data gaps)

- Moderate weight of evidence: good evidence from a primary line of evidence but evidence from several other lines is missing (important data gaps)

- Weak weight of evidence: weak evidence from the primary lines of evidence (severe data gaps)

- Uncertain weight of evidence: due to conflicting information from different lines of evidence that cannot be explained in scientific terms

- Weighing of evidence not possible: No suitable evidence available

6. ASSESSMENT

6.1 Introduction

This chapter describes the assessment of the risk for children caused by potential exposure to titanium dioxide (TiO_2) released from toys. In addition to the physicochemical characterisation of TiO_2, the basis for the risk evaluation is the potential exposure and the hazards that might occur after exposure to TiO_2 released form toys. The evaluation is focused on the risk due to release of TiO_2 from toys resulting in mainly inhalation exposure and where appropriate oral exposure. The relevance of dermal exposure is addressed as well.

As reviewed by Braakhuis *et al.* (2021), titanium dioxide (TiO_2) is a natural mineral widely used in pigments and paints for providing white colouring. In its natural form, three crystal structures are distinguished, rutile, anatase and brookite. The desired light-scattering effect (e.g. white colour) of TiO_2 particles occurs in the particle size range of 200–300 nm. A smaller particle size (nanosized, <100 nm) results in a transparent opaque colouring. The inhalation of titanium dioxide is generally considered to pose the most severe risk. As particle size determines penetration and deposition in the lung, potentially resulting in lung damage, it is of high importance to have a precise determination of the particle size distribution of the pigmentary titanium dioxide used in toys.

The European Union has published a delegated regulation (EC, 2020/217) (EC, 2020) regarding the suspected carcinogenicity (category 2) of TiO_2 powder after inhalation exposure. This regulation designates TiO_2 as carcinogenic and thus limits the use of TiO_2 in powder form when resulting in a content of 1% or more of particles with aerodynamic diameter ≤ 10 μm. Products containing TiO_2 in powder form with more than 1% particles with an aerodynamic diameter ≤ 10 μm shall be accompanied by specific labelling containing a warning for hazardous respirable droplets and/or respirable dust (EUH211 and EUH212, respectively). Liquid and solid mixtures are not classified, but specific warning statements and labels need to be applied to those that contain more than 1% of TiO_2[11].

In light of the classification under CLP and due to the TSD's CMR rules, the above-classified TiO_2 will not be permitted for use in toys if its concentration exceeds 1% in toy materials unless the specific requirements for a derogation can be met. The requirements for derogation are complete containment of the substances in concentrations below levels indicated in EU legal acts referred to in Section 2 of Appendix B for the classification of mixtures containing these substances, the substances and mixtures are inaccessible to children in any form, or a decision in accordance with Article 46(3) has been taken to permit the substance or mixture and its use. The latter decision may be taken when the substance has been evaluated by a relevant Scientific Committee and found to be safe, when there are no suitable alternative substances or mixtures, or the substance is not prohibited for use in consumer articles under Regulation (EC) No 1907/2006, Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

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[11] It should be noted that the CMR status of TiO_2 was challenged by industry. A recent judgement by the Court of Justice of the European Union (JUDGMENT OF THE GENERAL COURT (Ninth Chamber, Extended Composition), dated 23rd November 2022[1], annuled the Commission Delegated Regulation (EU 2020/217 of 4 October 2019) in so far as it concerns the harmonised classification and labelling of titanium dioxide as a carcinogenic substance by inhalation in certain powder forms.

6.2 Chemical and physical specification

6.2.1 Chemical identity

Chemical Formula: TiO_2

IUPAC ID: Titanium dioxide, Titanium (IV) oxide

MDL Number: MFCD00011269

EC No.: 236-675-5

CAS No: 13463-67-7

6.2.2 Physical form

Natural titanium dioxide (CAS number: 13463-67-7) consists of different crystalline forms, of which the most common are rutile (tetragonal), anatase (tetragonal) and brookite (orthorhombic), each of them with their own CAS numbers as shown in Table 6.1.

6.2.3 Molecular weight
Molecular weight of TiO_2 is 79.88.

6.2.4 Purity, composition and substance codes
TiO_2 (CAS No. 13463-67-7) is a solid, white and odourless powder, with the following composition: titanium 59.93% and oxygen 40.55%.

6.2.5 Impurities / accompanying contaminants
TiO_2 minerals can contain impurities such as iron, chromium, vanadium or zirconium that confer a spectrum of different colours. The TiO_2 materials are produced according to USP 31 specifications, in high purity, with concentration of the active material ≥99.0 %.

Table 6.1: TiO_2 physical forms and CAS-codes

Form	CAS No	EC No	Reference to ECHA substance infocard
Rutile	1317-80-2	215-282-2	https://echa.europa.eu/substance-information/-/substanceinfo/100.013.894
Anatase	1317-70-0	215-280-1	https://echa.europa.eu/substance-information/-/substanceinfo/100.013.892
Brookite	12188-41-9	not available	https://echa.europa.eu/substance-information/-/substanceinfo/100.289.655?_disssubsinfo_WAR_disssubsinfoportlet_backURL=https%3A%2F%2Fecha.europa.eu%2Fhome%3Fp_id%3Ddisssimplesearchhomepage_WAR_disssearchportlet%26p_p_lifecycle%3D0%26p_p_state%3Dnormal%26p_p_mode%3Dview%26_disssimplesearchhomepage_WAR_disssearchportlet_sessionCriteriaId%3D

6.2.6 Solubility
All TiO_2 particles are insoluble in water, organic solvents, hydrochloric acid and dilute sulfuric acid. They may be slowly soluble in hot concentrated sulfuric acid, hydrochloric acid, hydrofluoric acid and nitric acid (Cho *et al.*, 2013; MacNicoll *et al.*, 2015).

6.2.7 Partition coefficient (Log Pow)

Log Pow: Not applicable for TiO_2, as it is insoluble in water and organic solvents.

6.2.8 Additional physical and chemical specifications

An overview of pigmentary TiO_2 as provided by titanium dioxide manufacturers is presented in Table 6.2 (TIE, 2020a). However, according to the information provided by the industry, this table contains general information on TiO_2 pigment, but is not specific to TiO_2 as used in toys.

Table 6.2: Identity and physicochemical properties of titanium dioxide

Substance	titanium dioxide
Molecular formula	TiO_2
CAS number	13463-67-7
EINECS number	215-280-1
Molecular weight	79.8
State at room temperature	solid, crystalline, white, odourless inorganic substance
Melting point	anatase: 1560 °C, rutile: 1843 °C, brookite: 1825 °C ca. 3000 °C
Boiling point	anatase: 3.9, rutile: 4.26, brookite: 4.17
Relative density	not soluble in water
Solubility	

6.2.9 Particle shape, particle size and distribution

Particle shape

The TiO_2 particle shape is different depending on the crystalline forms. In nature, rutile crystals may vary in shape between long acicular (needle-like) crystals to a short blocky configuration, due to the morphology being dominated by an (open) tetragonal prismatic form, while the anatase crystals may exhibit a (closed) tetragonal bipyramidal form (Barnard and Curtiss, 2005). However, anatase particles could show an almost spherical shape with a certain level of agglomeration (Pal *et al.,* 2007).

Depending on the physicochemistry of the environment in the used media and particle concentration, TiO_2 nanoparticles may show various degrees of aggregation which can also influence particle size determination (Domingos *et al.*, 2009). Also, for powder TiO_2 preparations issued from referenced, synthesized materials, raw materials (additives) and extracted materials from manufactured products such as children's paint, various parameters may affect particle sizes (Bouzakher-Ghomrasni *et al.*, 2021). The particles may be present in the form of heterogeneous structures in particle size, size distribution, morphology (shape) and physical properties (density, specific surface area and porosity) Both primary particle size and aggregate sizes may vary both within one preparation and between various preparations (JRC, 2014).

Particle size and distribution

The particle size of TiO_2 and the particle size distribution directly impact its performance in numerous applications, necessitating the measurement and control of this important property. The size and shape of powders influence flow and compaction properties. Larger, more spherical particles will typically flow more easily than smaller or high-aspect ratio particles. In general, smaller particles may dissolve more quickly and lead to higher suspension viscosities than larger ones.

In the case of TiO_2, the existence of two categories of powders, micronic particles (diameter of the particle varying between a value of 500 nm and 50 µm) and nanometric

particles (diameter of the particle less than 500 nm), have been identified. The high value of 500 nm was based on the fact that particles up to 500 nm can be easily taken up by cells (SCENIHR 2009, Bruinink *et al.* 2015). This large interval can be attributed to the presence of agglomerates (Fatah and Sanchez-Calvo, 2004). In the recommendation for the definition of a nanomaterial (EC 2011/696/EU) a size between 1 nm and 100 nm is considered to define a nanomaterial. In the 2022 revision of this recommendation[12], again the size of 1 nm to 100 nm is indicated as the size range for a nanomaterial.

Throughout this Opinion, nanoscale/nanosized particles (1-100 nm) will be indicated as ultrafine particles in line with conventions in inhalation toxicology. Microscale particles with an aerodynamic diameter above 0.1 µm will be indicated as fine particles[13].

It should be considered that TiO_2 pigments, similar to food grade TiO_2 E171, may contain a nanoscale fraction (ultrafine fraction) that may need specific considerations in view of their toxicokinetics and potential toxicity. For food grade TiO_2 (E171), the observed ultrafine fraction varied between 10% up to even 64% depending on the methods used including de-agglomeration of large structures (Weir *et al.*, 2012, Peters *et al.*, 2014, Dudefoi *et al.*, 2017, Verleysen *et al.*, 2020, EFSA FAF Panel, 2021). TiO_2 is produced in two main forms, pigment grade and ultrafine (nanomaterial). Ultrafine TiO_2 is composed of constituent particles which are <100 nm in size. The constituent particles naturally form aggregates and agglomerates which are larger than 100 nm. (Motzkus *et al.,* 2013)

The TiO_2 used in all toy products is pigment grade, with no deliberate use of ultrafine TiO_2.

For four preparations of TiO_2 used in toys and toy material, information was received regarding the particle size distribution of the pigmentary TiO_2 as determined by laser diffraction (TIE, 2020a).

1. SunPURO® Pearl Gold C84-6118, Lot Z70KR7262, Particle size distribution, median (D50) 21.4 µm (with a D10 value of 9.9 µm and D90 value of 43.1 µm).

2. SunPURO® Pearl Silver C80-1608, Lot Z92KR9202, Particle size distribution, median (D50) 21.9 µm (with a D10 value of 9.8 µm and D90 value of 43.6 µm).

3. 1.17733 Timiron Synwhite 40 (Cosmetic Pigment), Particle size distribution, median size D_{50} 16.0 – 23.0 µm with 80% between 5.0 – 40.0 µm.

4. 1.17771 Colorona® SynRussian Gold, (Cosmetic Pigment), Particle size distribution, median size D_{50} 14.0 – 20.0 µm with 80% between 5.0 – 40.0 µm.

It is noted that these four products are pearlescent pigments composed of mica with, amongst others, a TiO_2 coating. Therefore, these products can not be considered representative of the TiO_2 pigmentary grades used in toy products. For two additional products used in toys limited data on TiO_2 particle size were provided, one product with an average particle size of 200 nm (by electron microscopy), and one product with an average particle size between 447 nm – 478 nm with a range of 150 nm (0.03vol%) to 2135nm (0.01vol%). At the public consultation additional information was provided on Ti-Pure™ Titanium Dioxide Pigment (MSDS provided by Chemours) with an overall size between 0.2 µm – 4 µm, with a D_{10} of 0.274 µm, D_{16} of 0.32 µm, D_{50} of 0.541 µm, D_{84} of 0.96 µm, and a D_{90} of 1.151 µm. The measurement range was 0.00vol% at 0.126 µm and 0.05vol% at 0.141 µm for the lower end, and 0.01vol% at 39.8 µm and 0.00vol% at 44.6 µm at the high end. The equipment used was a Malvern Mastersizer 2000 with a measuring range of 0.02 µm to 2000 µm (Malvern Panalytical Ltd, Malvern, UK). However, in a recent

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[12] COMMISSION RECOMMENDATION of 10 June 2022 on the definition of nanomaterial. Brussels 10.6.2022 C(2022) 3689 final. Official Journal European Union C 229/1– C 229/5, 14.6.2022.
[13] SCHEER is aware that fine particles are defined as particles between 0.1 and 2.5 µm and particles above 2.5 µm are considered coarse particles. For simplicity and unequivocal interpretation in this opinion only the terminology of ultrafine and fine particles is used as indicated in the text.

report also the presence of a nanofraction in marketed children's paint was indicated (Bouzakher-Ghomrasni *et al.*, 2021). The presence of a nanofraction was further confirmed by limited additional data on D50 particle sizes and the particle content below 100nm, of several samples of different TiO_2 preparations, provided by the Titanium Dioxide Manufacturers Association at the public consultation.

Different analytical methods may be used for the characterisation of TiO_2 particle size and size distribution, as shown for the characterisation of five different ultrafine preparations including Transmission Electron Microscopy (TEM), Scanning Mobility Particle Sizer (SMPS), and Aerodynamic Particle Sizer (APS) (Motzkus *et al.*, 2013). With regard to the dustiness, the results for the evaluated ultrafine particles showed a strong presence of agglomerates/aggregates of constituent particles and a significant presence of emitted airborne ultrafine particles with a diameter below 100 nm (composed of isolated constituent particles and small aggregates/agglomerates formed from a few constituent particles): the proportion of these particles varies from 0 to 44 % in the measurement range 14-360 nm depending on the types of powders and corrections of measurements. An extensive report on measurement techniques to determine the size of a number of TiO_2 nanomaterials was published by the JRC (JRC, 2014). Furthermore, it should be noted that different methodologies of measurements may result in different outcomes for the sizes measured as demonstrated by Domingo *et al.* (2009) for ultrafine particles measured using six different methods. Also, EM measurements may result in uncertainty on particle sizes of nanomaterials as noted for the presence of nanomaterials in food. This uncertainty was ascribed to the combined influence of sampling, sample preparation prior to imaging and the image analysis, the main influence being the sampling step (Dudkiewicz *et al.,* 2015). Especially when evaluating particles not in their pristine form as manufactured but as present in products, the sampling method is therefore highly important.

6.2.10 Homogeneity and Stability

Based on the information on the size distribution, there can be a considerable variation in the homogeneity of TiO_2 powders. The homogeneity and the stability are reported for TiO_2 at various intervals of time and temperature (https://echa.europa.eu/registration-dossier/-/registered-dossier/15560/7/9/3). Rutile is the thermodynamically stable form of titanium dioxide; anatase and brookite are metastable; anatase rapidly transforms to rutile above 700°C. (Zhang and Banfield, 1998).

With regard to the crystallite size, a similar size for anatase and brookite is reported, but a larger value for rutile crystallite size (Allen *et al.*, 2018).

However, although titanium dioxide can exist in three forms, only the anatase and rutile crystalline structures are found in manufactured products for which the risk of exposure is of interest for toxicological studies (Rouxel *et al.*, 2017).

6.2.11 Conclusions

Pigmentary fine TiO_2 should not contain a nanofraction. Particulate $TiO_2,$ composed of constituent particles with a mean particle size in the range of 0.2 to 0.3 µm diameter, results in light scattering with a white colour effect. Limited industry data show distributions for a few fine pigmentary TiO_2 used in toys with product particle sizes ranging from the lowest, being 141nm, to the largest, being 39811nm. Although the information provided indicates that a nanofraction would not be present to a significant degree for the pigmentary TiO_2 as used in toys and toy materials, the presence of an ultrafine fraction in the pigments cannot be excluded because measurement methods may not have evaluated constituent particles and agglomerates.

Based on the information provided by the toy industry on different white pigments containing TiO_2, a fraction with a size below 10 µm (as indicated in EU 2020/217 for a carcinogenic hazard) is present in the white pigments.

The WoE for the particle sizes of pigmentary fine TiO₂ used in toys and the absence of an ultrafine fraction is considered weak, since this conclusion is based on limited data with medium consistency and medium quality. The data are provided by the toy industry while robust study reports on the measurement methods of the particle size distribution of TiO₂ pigment used in toys were not available.

6.3. Application of TiO₂ in toys

Titanium oxide pigment is an important product made from titanium minerals, with microcrystalline TiO₂ for white pigment being produced in the largest volumes. Due to its extremely high refractive index (as rutile), TiO₂ is the main opacifying pigment used in paint (50%+ of global production) and other products such as plastics (30%), and paper (5%), for both white and a range of colours.

Titanium dioxide can be used in toys in a number of ways, including as a pigment in craft materials (e.g. chalks, pencils, etc.), as a pigment in paints applied to toys, and in polymeric toy materials (e.g. acrylonitril-butadiene-styrene (ABS), polyethylene, polypropylene, polyvinyl chloride, etc.). A survey of TIE (TIE, 2020a) identified a number of toy products that contain TiO₂ (see Table 6.3).

Table 6.3: Toy products containing TiO₂ (TIE, 2020a)

Toy or toy material
Applied coatings and printings, dried
Casting kits
Chalks, including pastels
Clays and putties
Colouring pencils
Doughs
Dry paint tablets
Face paints
Finger paints, liquid
Glue, dried
Lip gloss, lipstick
Nail varnish
Paper
Polymeric materials (including synthetic textiles)
Powder paints
Solvent-based paints, liquid
Water-based paints, liquid (excluding finger paints)
Wax crayons

Source – Toy Industries of Europe (personal communication)

6.3.1 Function and uses of TiO₂ in toys

Besides the function of colouring agent, TiO₂ in different categories of toys also provides additional functionalities:

1. In paints and coatings: TiO₂ provides opacity and durability, while helping to ensure the longevity of the paint and protection of the painted surface.
2. In plastics, adhesives and rubber: TiO₂ can help minimise the brittleness, fading and cracking that can occur in plastics and other materials as a result of light exposure.
3. In paper: TiO₂ is used to coat paper, making it whiter, brighter and more opaque.

TiO₂ pigments used by members of the Toy Industries of Europe are known by several different trade names e.g. SunPURO® Gold C84-6118, SunPURO® Pearl Silver C80-1608, Timiron® Synwhite 40 and Colorona® SynRussian Gold (TIE, 2020a), see chapter 6.2.9.

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6.3.2 Titanium content in toy materials

In the reports submitted by the toys industry (TIE, 2020a, TIE, 2020b), different toy polymeric materials were analysed for TiO_2 content. These materials are mainly used in plastics, adhesives and rubber (Category 2 in 6.3.1). The content in a number of polymeric materials is shown in Table 6.4 and varies between 1 to 10 % (of the total material).

Table 6.4: Uses and concentrations of TiO_2 in polymeric materials (TIE, 2020b)

Toy polymeric materials	Highest TiO2 content (or range) in %
ABS (acrylonitrile-butadiene-styrene)	1 to 10
PE (polyethylene)	3
PP (polypropylene)	3
PVC (polyvinyl chloride)	2 to 10
PS (polystyrene), including HIPS (high impact polystyrene), MIPS (medium impact polystyrene) and GPPS (general purpose polystyrene)	1 to 10
SBS (styrene butadiene styrene)	3 to 10
SEBS (styrene ethylene butylene styrene)	3 to 10
TPE (thermoplastic elastomer)	1 to 10
Latex balloons	10

A survey among toy companies organised within TIE identified a number of toy products that contain TiO_2 (Table 6.5).

Table 6.5: TiO_2 content in different toys or toy materials (TIE, 2020b)

Toy or toy material	Highest TiO2 content in %
Polymeric material (including synthetic textiles)	10
Applied coatings and printings (dried form)	60
Paper	0.5
Pencil cores	23 for coloured ones, 51 for white ones
Wax Crayons	18
Chalks (including pastels)	5
Dry paint tablets	13
Water based paints (liquid) excluding finger paints	30
Finger paints (liquid)	30
Glue (dried form)	4
Solvent based paints (liquid)	30
Powder paints	25
Casting kits	1.5
Face paints	20
Nail varnish	8
Lip gloss, lipstick	15
Clays and putties	3
Doughs	2
Mouldable/coloured sand	1

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6.3.3 Migration/ release of TiO₂ from toy materials

According to the TIE-report (TIE 2020a), no studies were identified that have directly assessed the migration of TiO₂ from toy products to humans, but there have been several evaluations of the potential for migration of TiO₂ from food packaging materials. In particular, the EFSA Panel on Food Contact Materials, Enzymes and Processing Aids evaluated the safety of TiO₂ used as a colourant/filler (up to 25% w/w) in polymers used as food contact materials (EFSA CEP Panel, 2019). The assessment concluded that the added particles did not migrate, that they resisted release by abrasion, and did not transfer into a simulant for solid/dry foods. As such, the added TiO₂ particles did not constitute a toxicological concern regarding exposure via food (EFSA CEP Panel, 2019). Other studies of the potential for TiO₂ to migrate from food contact materials have identified that some ultrafine TiO₂ particles may migrate, but the amounts concerned are consistent with TiO₂ having a low potential for migration (< 0.05 mg/kg food; EFSA CEP Panel, 2019). In particular, Tang *et al.* (2020) assessed the migration potential of TiO₂ from polylactic acid (PLA) and found that migration of ultrafine TiO₂ from PLA into a food stimulant solution (50% ethanol) was 0.43 mg/kg; similarly, Yang *et al.* (2019) found the maximum migration of ultrafine TiO₂ from PLA was 0.54 mg/kg. Lin *et al.* (2014) assessed the migration potential of titanium from ultrafine TiO₂-polyethylene and found that the migration of titanium was 0.5 µg/kg into a 50% ethanol solution at 25°C. Bott *et al.* (2014) assessed the migration of titanium from low-density polyethylene containing titanium nitride into a 95% ethanol solution and found no measurable titanium.

6.3.4 Conclusions

According to the information on different white pigments containing TiO₂, which was provided by the toy industry, particles below a size of 10 µm (which is the size indicated in EU 2020/217 for a carcinogenic hazard) are present in the white pigments. As presented in section 6.3.2, a number of products contain TiO₂ pigments that contain particles <10 µm at a percentage above 1% of the limit indicated in EU 2020/217. For these products, a specific risk assessment needs to be performed in view of the classification of TiO₂ particles as carcinogen category 2 by inhalation with a limitation to respirable titanium dioxide particles. Based on the information provided above, migration of TiO₂ when embedded in polymers is unlikely. Based on the information in the public literature, the WoE for no or limited TiO₂ release from polymers is strong.

6.4   Exposure assessment

6.4.1 General introduction to TiO₂ exposure assessment from toys

To assess the potential exposure to TiO₂ from toy products, it is necessary to identify which toys contain TiO₂ and how much, to identify the most highly exposed/sensitive population sub-groups, and to develop relevant exposure scenarios related to those population sub-groups.

The Toy Safety Directive (EC, 2009) defines toys as '…products designed or intended, whether or not exclusively, for use in play by children under 14 years of age…'. Accordingly, considering the safety of toys with regard to exposure to chemical substances, the exposure of children is of primary concern. Children can be exposed to chemicals that are released from toys in three principal ways:

• *Dermal exposure*: can occur via direct handling of the toy product, splashes of a product onto the skin, skin contact with residues, and from the deposition of particles from an airborne substance. The amount and concentration of the substance, the area of skin

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exposed, and the duration and frequency of exposure can influence the amount of dermal exposure.

• *Inhalation exposure*: this occurs when compounds are released from a product in vapour form and/or as respirable suspended particulate matter (<10 µm aerodynamic diameter) and subsequently are inhaled. If the exposure is of an intermittent and/or short duration, it may be appropriate to determine exposure over short event periods.

• *Oral exposure*: this occurs when a substance in a product is ingested, for example, due to hand-to-mouth transfer, chewing, licking, and/or sucking of a product or powder/dust generated through product use. Oral exposure is of particular relevance to children because of their tendency to engage in hand-to-mouth behaviour and/or mouthing behaviour. Migration characteristics of the substance in the product matrix, substance release by the product, solubility, and amounts typically used are important determinants that, together with concentration and contact parameters, are used to estimate oral exposure. It should be noted that also inhaled particles may be ultimately ingested after removal from the airways of the lung by the so-called mucociliary escalator, in which inhaled particles are removed together with mucus from the respiratory tract by the villi on ciliated cells and will be ultimately swallowed and end up in the GI tract.

When estimating exposures to a substance present in toys, it is important to consider the different phases of activity during which the toys are used; these activities may include:

- • preparatory activity, such as handling and mixing of liquids and powders,
- • application/use of products, including handling of finished articles,
- • post-use or post-application exposure, e.g. solvent exposure from paints after use, and
- • removal or cleaning leading to user exposure.

Furthermore, exposure may be through occasional/single or regular/repeated use and may range from a short duration of a few minutes to continuous exposure over 24 hours. Where exposure to a substance occurs by different routes and/or from different sources, the contribution of each route/source to the aggregate exposure also needs to be assessed. Figure 6.1 provides an overview of the different exposure pathways through which children may be exposed to chemical substances in toys. An additional arrow (in red) is introduced between inhalation exposure and gastrointestinal absorption oral exposure – ingestion to include the mucociliary escalator that removes particles from the airways of the respiratory tract.

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Figure 6.1: Overview of different exposure pathways/ scenarios through which children may be exposed to chemical substances in toys *(adapted from Van Engelen et al., 2008 and SCHER, 2016, TIE, 2020a)*
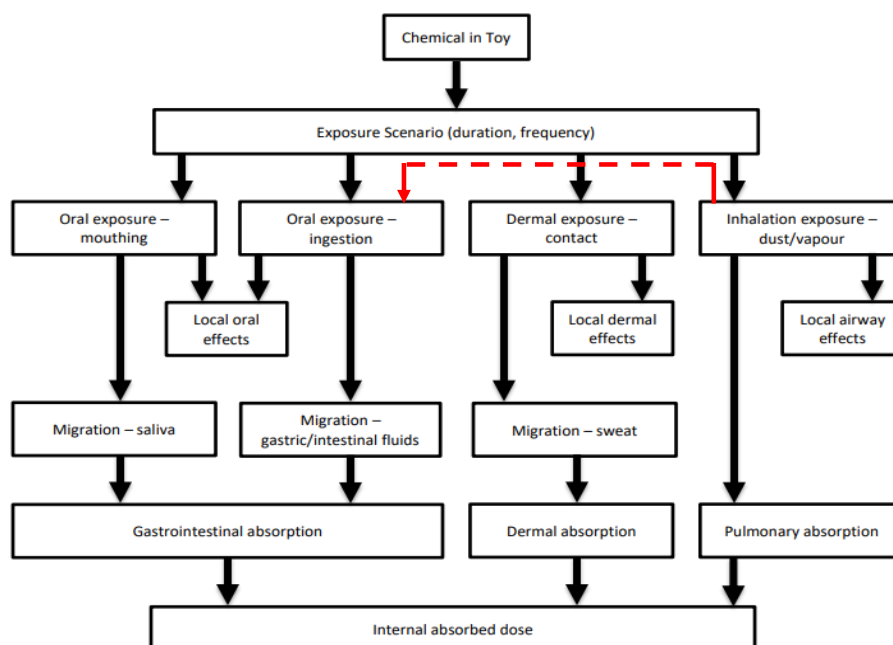
Titanium dioxide is used in toy products in a variety of ways, including as a pigment in paints and polymers, in paper, and in art and craft materials. Given that these products are specifically intended to be used by children, they may be a source of children's exposure of TiO_2, in addition to exposure to TiO_2 in food.

Inhalation exposure to TiO_2 may potentially occur with the use of products that can lead to the release of TiO_2 particles, such as powder products and spray products (e.g. chalk and paint sprays). Therefore, when considering exposure to TiO_2, particular attention needs to be given to inhalation exposures arising from use of such products.

Regarding risks arising from dermal and oral exposure, the ECHA Risk Assessment Committee found that there was no concern as to dermal or oral carcinogenicity (ECHA, 2017). However, recently (May 2021), EFSA expressed a concern for TiO_2 (as food additive designated E171) oral exposure in view of uncertainties regarding possible genotoxic effects (EFSA, 2021). Based on this conclusion, there is a need to assess the oral exposure to TiO_2 from toys in children.

In this Opinion, additional possible exposures to TiO_2 from other sources were not considered as the evaluation was strictly limited to toys and toy materials.

6.4.2 Exposure assessment for TiO_2 present in toys

6.4.2.1 Introduction

Exposure of children to certain elements from toys depends on the toy characteristics like composition of the toy material (physicochemical properties of the chemicals), its surface, volume and its intended use, as well as the playing behaviour and the physiological characteristics of a child, the latter two both changing with age. Exposure scenarios therefore need to reflect the intended and foreseeable use of a toy at specific age groups of children (SCHER, 2016).

The toy industry of Europe (TIE; TIE, 2020a) has provided an assessment of children's exposure to TiO_2 that was based on ECHA guidance on consumer exposure assessment (ECHA, 2016) and RIVM guidance on the assessment of chemical safety of toys (Van Engelen *et al.*, 2008).

SCHEER follows the selection of exposure scenarios proposed by TIE. However, the SCHEER does not agree with the TIE exposure calculations, e.g. the way the use amount was determined for some scenarios and how extrapolation was performed in cases where no specific data was available for the evaluated toys. Therefore, the SCHEER recalculated the respective air concentrations for the selected exposure scenarios as indicated in the calculations below.

Given that at the time no concerns regarding carcinogenicity of TiO_2 had been identified via the dermal and oral routes of exposure (ECHA, 2017), the TIE exposure assessment focuses solely on exposure via the inhalation route. However, in view of the recent EFSA 2021 Opinion on genotoxicity after oral exposure to TiO_2 as E171 food additive, and the uncertainties indicated therein, the oral exposure to TiO_2 in toys is evaluated in the current Opinion.

6.4.2.2 Migration/ release of TiO_2 from toy materials

According to the TIE-report (TIE 2020a), no studies were identified that have directly assessed the migration of TiO_2 from toy products to humans. However, there have been several evaluations of the potential for migration of TiO_2 from food packaging materials. In particular, the EFSA Panel on Food Contact Materials, Enzymes and Processing Aids evaluated the safety of TiO_2 used as a colourant/filler (up to 25% w/w) in polymers used as food contact materials (EFSA CEP, 2019). The assessment concluded that the added particles did not migrate, that they resisted release by abrasion, and did not transfer into a simulant for solid/dry foods. As such, the added TiO_2 particles did not constitute a toxicological concern regarding exposure via food (EFSA CEP, 2019).

Other studies of the potential for TiO_2 to migrate from food contact materials have identified that some ultrafine TiO_2 particles may migrate, but the amounts concerned are consistent with TiO_2 having a low potential for migration (< 0.05 mg/kg food; EFSA CEP, 2019). For ultrafine TiO_2, release was studied for textiles and paints, although the data reporting was in most cases insufficient to be used for an exposure assessment under REACH (Mackevica and Hansen, 2015). Tang *et al.* (2020) assessed the migration potential of TiO_2 from polylactic acid (PLA) and found that migration of ultrafine TiO_2 from PLA into a food stimulant solution (50% ethanol) was 0.43 mg/kg; similarly, Yang *et al.* (2019) found the maximum migration of ultrafine TiO_2 from PLA was 0.54 mg/kg. Lin *et al.* (2014) assessed the migration potential of titanium from ultrafine TiO_2-polyethylene and found that the migration of titanium was 0.5 µg/kg into a 50% ethanol solution at 25°C. Bott *et al.* (2014) assessed the migration of titanium from low density polyethylene containing titanium nitride into a 95% ethanol solution and found no measurable titanium.

When TiO_2 is fixed in a polymer matrix, release of TiO_2 from this matrix is considered unlikely. Potential exposure is only possible when pieces of the toy break off due to mouthing (see oral exposure below).

6.4.2.3 Exposure scenarios – inhalation

Inhalation via evaporation
Toys may release chemicals into the air via evaporation, such as the solvent in a felt pen. To be available for inhalation after evaporation, the chemical has to be volatile. Since TiO_2 is not volatile, this scenario is not relevant for exposure to TiO_2.

Inhalation via spray/powder/dust

Some toys may release considerable amounts of dust, such as plaster mix (for example, when beating out a brush), chalk and crayons. Other toys may release chemicals into the air via a spraying system. Contrary to evaporating chemicals, chemicals in sprays or dust do not necessarily need to be volatile to be available for inhalation. This exposure scenario therefore is relevant for TiO_2 present in toys.

A survey among toy companies organised within TIE identified a number of toy products that contain TiO_2. It was not indicated if the survey could be taken as representative for the entire toy industry. The initial qualitative assessment by TIE (see Table 6.6) of the potential for exposure to particles containing TiO_2 was made for each of the identified toy products, based on the following criteria:

- presence of TiO_2,
- physical form of product (e.g. liquid, paste, powder, solid, etc.),
- product usage form (e.g. powder, spray, solid) and potential for generation of TiO_2 particles during product use, and
- exposure scenario (e.g. duration, frequency, etc.).

Table 6.6: Potential for inhalation exposure to TiO_2 from powders used in toys and toy materials (TIE, 2020b)

Toy or toy material	Maximum TiO₂ content (%)	Possible inhalation exposure to TiO₂
Applied coatings and printings, dried	60	No
Casting kits	1.5	Yes
Chalks, including pastels	5	Yes
Clays and putties	3	No
Colouring pencils	Coloured pencils: 23 White pencils: 51	Yes
Doughs	2	No
Dry paint tablets	13	Yes
Face paints	20	No
Finger paints, liquid	30	No
Glue, dried	4	No
Lip gloss, lipstick	15	No
Nail varnish	8	No
Paper	0.5	No
Polymeric materials (including synthetic textiles)	10	No
Powder paints	25	Yes
Solvent-based paints, liquid	30	No
Water-based paints, liquid (excluding finger paints)	30	No
Wax crayons	18	No

Information based on survey of the Toy Industries of Europe members

The release of powders in the air from various TiO_2 uses can be considered to cause the highest risk for children playing with toys containing TiO_2, while considerable TiO_2 release from TiO_2-containing plastics or paints used for toys is less likely. Therefore, the inhalation exposure scenarios are limited to those applications that may result in dust generation and subsequent inhalation of TiO_2 particles. On this basis, four scenarios were selected that are intended to encompass the toys with highest potential for exposure (all toys presented above, except for "dry paint tablets" that were assumed to be covered by the more relevant powder paint) (TIE report, 2020a).

Measured data for TiO_2 air concentrations were provided by TIE for colour pencils, wax crayons, finger paint and modelling clay (see Annex I).

An overview of the selected scenarios is presented in Table 6.7.

Table 6.7: Scenarios to assess toys that may represent an upper bound of exposure to TiO₂ by inhalation

Scenario	Toy	Weight fraction TiO$_2$	Physical state
1	casting kit	0.015	powder
2	chalk	0.05	compressed powder
3	pencil	0.51	compressed and formulated powder
4	powder paint	0.25	powder

6.4.2.4 Exposure modelling – inhalation

For assessing inhalation exposure, the most important product-dependent parameter is the air concentration of the target substance. In addition, the deposited fraction of particles in the lung is important. Not all particles or droplets reach the lower areas of the lungs (the alveolar region) after inhalation. This depends on the size of the particles or droplets that can be distinguished in three identifiable fractions depending on the Mass Median Aerodynamic Diameter (MMAD), the inhalable fraction (MMAD<100 µm), the thoracic fraction (MMAD<10 µm), and the respirable fraction (MMAD<5; cut off 10 µm) (CEN, EN 481 1993, Brown *et al.*, 2013, SCCS, 2021a). Therefore, to assess the exposure from inhalation, the particle size distribution of the spray or dust must be known and considered together with the other exposure parameters.

Considering the respirable fraction with a size <10 µm, the fraction of product released into the air with particles or droplets below 10 µm is considered as the relevant exposure concentration in the air.

Instead of calculating the air concentrations that are relevant for exposure, they can be directly measured. Such measurement data have been provided by TIE for several white colour pencils (TIE report, 2020a, Appendix II). For wax crayons, specific measurements were provided with releases below the ones for the colouring pencils. These are therefore assumed to be covered by the scenario for pencils. For finger paints, specific measurements have been provided, but these were investigated in an abrasion scenario and not in a use scenario and are therefore considered not relevant (see Annex I for measurement results provided by TIE). For other toy products, potential air concentrations may be extrapolated from air concentrations measured after dissipation of a cosmetic powder in an experimental chamber (Rasmussen *et al.*, 2019) and other experiments for different kinds of chalk (Goel *et al.*, 2015). These results were chosen for the exposure calculations provided below.

The following considerations were used for the extrapolation of measured air concentrations to scenarios, for which no data were available: Air concentrations of TiO$_2$ particles < 10 µm are dependent on the amount of product dispersed in the air (a), on the weight fraction of TiO$_2$ in the dispersed product (wf) and the volume of air in which the product is distributed (V_{air}). Assuming homogeneous distribution, the air concentration of TiO$_2$ < 10 µm (C_{air}) can then be calculated according to *Formula 1*.

Formula 1: C$_{air}$: concentration of TiO2 in the air

$$C_{air} = \frac{a * wf}{V_{air}}$$

a = amount of product dispersed in the air
wf = weight fraction of TiO$_2$ in product

V$_{air}$ = volume of air in which TiO$_2$ is distributed/released

Formula 1 can be transformed by applying the rule of proportion to determine the air concentration predicted for the selected scenarios (C$_{air\_scen}$) based on the experimentally determined air concentration (C$_{air\_meas}$), the measurement conditions (V$_{air\_meas}$, a$_{meas}$, wf$_{meas}$) and the assumptions for the selected scenarios (*i.e.,* V$_{air\_scen}$, a$_{scen}$. wfscen):

Formula 2A: C$_{air}$ concentration of TiO$_2$ for exposure scenario with measured data.

$$\frac{a_{meas} * wf_{meas}}{V_{air\_meas} * C_{air\_meas}} = \frac{a_{scen} * wf_{scen}}{V_{air\_scen} * C_{air\_scen}}$$

a$_{meas}$ = amount of product measured in the air
wf$_{meas}$ = measured weight fraction of TiO$_2$ in product
V$_{air\_meas}$ = measured volume of air in which TiO$_2$ is distributed/released

C$_{air\_meas}$ = measured concentration of TiO$_2$ in air
a$_{scen}$ = amount of product measured in the air for a specific scenario
wf$_{scen}$ = measured weight fraction of TiO$_2$ in product for a specific scenario
V$_{air\_scen}$ = measured volume of air in which TiO$_2$ is distributed/released for a specific scenario

C$_{air\_scen}$ = measured concentration of TiO$_2$ in air for specific scenario

Formula 2A is transformed into Formula 2B for the calculation of *C$_{air\_scen}$.*

Formula 2B:

$$C_{air\_scen} = C_{air\_meas} * \frac{V_{air\_meas}}{V_{air\_scen}} * \frac{a_{scen}}{a_{meas}} * \frac{wf_{scen}}{wf_{meas}}$$

In addition, the relevant size fraction in the air is TiO$_2$ particles < 10 µm (aerodynamic diameter), hence the PM$_{10}$ fraction is most relevant. In case that C$_{air\_meas}$ relates to PM$_{10}$, the C$_{air\_scen}$ PM$_{10}$ fraction released from a product can be calculated accordingly.

Thus, based on the different parameters that determine the air concentration of TiO$_2$ particles < 10 µm adjustment factors can be derived when measurement data are not available. For this, the chosen scenario has to be compared to the experimental setup for which measurements are available, and the differing parameters identified and adjusted. Whether an adjustment factor is necessary thus depends on the measurement conditions of the experiment selected for input into the different scenarios.
For scenario 3 (emission from pencils), measurements of TiO$_2$ particles < 10 µm emission from white pencils were available (TIE 2020a and various reports TÜV Rheinland, 2020), so only the air volume had to be adjusted with the adjustment factor V$_{air\_meas}$ /V$_{air\_scen}$. For scenario 2 (emission from chalk), data on the emission of PM$_{10}$ particles from chalk under

a normal use scenario were available (Goel *et al.,* 2015). Since the experiment did not include TiO_2 containing chalks, in addition to the adjustment factor for V, an adjustment factor for the weight fraction wf of TiO_2 in PM_{10} was used, with the assumption that the weight fraction of TiO_2 in PM_{10} after release is the same as the weight fraction of the TiO_2 in the solid chalk. Scenarios 1 (casting kit) and 4 (powder paint) rely on data from the release of cosmetic talcum (hydrous magnesium silicate) powder in a chamber experiment, with PM_{10} particles measured as inhalable fraction (Rasmussen *et al.*, 2019). Therefore, in addition to the adjustment factors for V and wf, the amount distributed in the chamber had to be adjusted to the use conditions of casting kit and powder paint, respectively (see Annex II).

The measurement conditions relevant for the adjustment factors are summarised in Table 6.8. For cases where no adjustment was done (e.g., in the case of weight fractions, if the same product was investigated and the TiO_2-PM_{10} was measured directly) it was denoted in the Table as "n.a", meaning that no correction for this value was needed.

The calculations aim at determining an upper-bound air concentration for the different scenarios. Therefore, for C_{air_meas}, always the highest values of all measurements were used in the calculations.

Table 6.8: Description of experimental studies providing measurements of air concentrations used for scenario adjustment according to *Formula 2*

Reference	Product investigated	Used for scenario	Cair_meas (µg/m3)	Vmeas (m³)	ameas (mg)	wfmeas (-)
Rasmussen *et al.*, 2019	cosmetic powders, talcum	1, 4	8420 (PM_{10})	0.77	700	1 (PM_{10})
Goel *et al.*, 2015	chalk (4 brands)	2	170 (PM_{10})	10*	n.a.	1 (PM_{10})
TÜV Rheinland, 2020 (TIE 2020a)	white colour pencil (4 brands)	3	20	1	n.a.	n.a.

*whole room was 136.8 m^3, but dust samples taken at 2 m distance from source, and room height of 2.5 m results in volume of 10 from 2 m x 2 m x 2.5 m) n.a = not applicable/no adjustment needed

As relevant air volume in all scenarios (V_{scen}), the direct breathing zone is considered with a volume of 2 m^3. Therefore, all of the product that was released was considered to be distributed in this volume. The product amount (a_{scen}) was chosen specific to the toy use.

For the assessments for scenarios 1 and 4, no release rates of TiO_2 from the toys were available. In addition, product use data were only available from product websites. In view of the lack of data, the SCHEER has made several assumptions to assess an upper-bound exposure, which may be very conservative for scenarios 1 and 4. Therefore, another scenario was calculated that is still considered a high estimate, but seems more realistic (realistic high). For both scenarios it was assumed that up to 1% of the amount of the product used can be released as PM_{10} during the mixing phase. Based on marketed products in 2021 (information from product websites), product use amounts were assumed as 500 and 1,000 g for scenario 1 (casting kits) for realistic high and upper-bound estimates, respectively, and 2 or 50 g for scenario 4 (powder paints) for realistic high andupper bound, respectively. The a_{scen} presented in Table 6.9 results from reducing this product amount to the 1% assumed to be released.

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Table 6.9 Scenario parameters used for adjustment according to *Formula 2*

Scenario	Toy	Adjusted from	Uncertainties	a_{scen} (mg) realistic high	a_{scen} (mg) upper bound	wf_{scen}
1	casting kit	talcum powder	measurement with talcum powder, airborne TiO_2 unknown	5,000	10,000	0.015
2	chalk	chalk	measurements with chalk, 4 different chalks tested	n.a.	n.a.	0.05
3	white colour pencil	white colour pencil	measurements of TiO_2 for one type of pencil (white drawing pencil)	n.a.	n.a.	n.a.
4	powder paint	talcum powder	measurement with talcum powder, airborne TiO_2 unknown	20	500	0.25

n.a..: no adjustment needed

The air concentrations calculated with these parameters are presented below (Table 6.10, detailed calculations are provided in Annex III). In order to assess uncertainties, two different product amounts were used for scenarios 1 and 4 to mimic a "realistic high" case, and to provide an "upper bound" (*i.e.,* a highly conservative case indicating the maximum possible air concentration when using the highest values available). For scenarios 2 and 3, only the "upper bound" values were used.

6.4.2.5 Conclusions on potential release of TiO_2 into the air

Table 6.10 shows the air concentrations calculated for the four selected-use scenarios.

Table 6.10: Calculated air concentrations

Scenario	Toy	Air conc. PM_{10}-TiO_2 "realistic high" ($\mu g/m^3$)	Air conc. PM_{10}-TiO_2 "upper bound" ($\mu g/m^3$)
1	casting kit	347	695
2	chalk	-	42.5
3	white colour pencil	-	10
4	powder paint	23.2	579

The weight of evidence for the various inhalation exposure scenarios is as follows:

As described in Table 6.9, the uncertainty related to scenario 3 (pencil use) is very small, because 5 different pencils had been tested in a specific, relevant use scenario and the emissions had been measured at a distance of 3 cm and 50 cm directly as $TiO_2 < 10$ µm. Based on the available and measured data, the WoE for the exposure scenario 3 due to the use of pencils is strong.

For the calculation of scenario 2 (chalk), the uncertainty is somewhat higher, because the calculations were based on an experiment with chalk where only particles in the air ($PM_{2.5}$, PM_{10} and total suspended particulates) were measured. However, the assumption that the same weight fraction of TiO_2 is present in the air as it is in the chalk itself is quite plausible. Based on the measured data on particles released from chalk, the WoE for the extrapolation for the release of TiO_2 particles from chalk can be considered moderate as

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the measurements used for the calculations in scenario 2 did not determine the emission of TiO_2 itself.

Large uncertainties are associated with the scenarios 1 and 4, because those are based on an experiment with cosmetic talcum powder (hydrous magnesium silicate), not with the materials of the respective toys (gypsum or powder paint). In addition, no information was available on the dustiness of the cosmetic products used for the measurements. Therefore, based on considerations regarding usability, it was assumed that 1% of the amount used can be airborne and that the dustiness of the toys is comparable to that of talcum. Large uncertainties are associated with this assumption, but a 1% release is considered very conservative, so it is nevertheless plausible that the upper bound could not be exceeded in reality. In view of the uncertainties for the emissions in scenario 1 (casting kit) and scenario 4 (powder paint), the WoE is considered weak.

6.4.2.6 Exposure scenarios –oral

Indirect ingestion

As indicated above, a majority of the particles that can be inhaled and reach the thoracic parts of the lung are removed from the lung by the mucociliary escalator into the pharynx where the mucus, including the particles, can be swallowed. This mucociliary clearance from the lung will eventually result in exposure and possible uptake via the gastro-intestinal tract. Inhalation exposure is expressed as TiO_2 air concentration in $\mu g/m^3$, and, as deposition is a fraction of the exposure dose, lung deposition and entry into the mucociliary escalator will likely be in the μg range as well. In contrast, oral exposure is determined by mg possibly released from the toy product. The contribution of the oral uptake due to the mucociliary escalator transport to the mouth can be considered to be very low to negligible compared to direct oral uptake and is therefore not further considered in the oral exposure scenarios.

Direct ingestion

Direct ingestion of toy and toy material can be assumed to occur mainly in children under 3 years of age due to the oral exploratory behaviour that is natural at this age (Van Engelen and Prud'homme de Lodder, 2004). Toys intended for children of this age group are regulated such that they should not contain small detachable parts that may pose a choking hazard. These parts should therefore also not be accessible for ingestion. However, some liquid toys used by children under 36 months of age such as finger paint are easily swallowed. The swallowing of finger paints is specifically discouraged by addition of an embittering agent to the finger paints according to the European standard EN 71-7:2014+A3:2020 (CEN, 2014). Toys that consist of dry, brittle, powder-like or pliable material, such as chalk crayons, plaster or modelling clay may also be ingested, for example they can be bitten off or via hand-mouth contact. In addition, some toys may have a layer of paint or other coating, or textile fibres that may easily be scraped off and swallowed. Ingestion of scraped-off material is relevant for toys intended to be placed in the mouth, such as whistles used by older children (Van Engelen *et al.*, 2008).

Mouthing

Similar to the direct ingestion scenario described above, mouthing of toys can be assumed to occur mainly by children under 36 months of age. The mouthing may result in scraping or biting on the toy with a possible release of pieces of the toys including the TiO_2 pigment, which may result in an indirect exposure. In fact, some toys available on the market are specifically designed to be mouthed, such as teething rings. However, children mouthed on a broad range of items, including toys and other items not intended to be mouthed (De Groot *et al.*, 1998; DTI, 2002; Juberg *et al.*, 2001; Reed *et al.*, 1999; Smith and Norris, 2003; Tulve *et al.*, 2002). Although the dimensions of some toys may be such that they cannot be placed in the mouth, ridges can still be sucked on. In addition, some toys

intended for children over 3 years of age are intended to be placed in the mouth. The mouthing scenario can be relevant for substances present in toys.

In Table 6.11 an overview is given of which toys or toy materials may have the potential for oral TiO_2 exposure in children and by which scenario (direct ingestion or mouthing).

Table 6.11 presents TiO_2 pigment levels as present in toys for which mouthing needs to be considered as exposure route. For a number of products, a direct ingestion was considered not possible as the TiO_2 pigment would be embedded within the matrix of the toys. However, oral uptake by scraping of material or biting on the products with release of pieces of the toys and thus oral uptake including the TiO_2 pigment, which may or may not be freely available, remains possible. From this table, scenarios have been made in order to explore the contribution of oral exposure to the total TiO_2 exposure (via oral and inhalation). Since the *direct ingestion* scenario is supposed to have the highest potential oral exposure (Van Engelen *et al.,* 2008), three direct ingestion scenarios have been selected for further calculation. The selected products are lip gloss/lipstick, finger paint and white colouring pencils, based on the likeliness of exposure when playing with these products as well as the relatively high percentage of TiO_2 in these products. For the exposure scenarios indicated above, it should be realised that the exposure will not be limited to children up to three years of age, but also older children might be exposed due to direct ingestion or mouthing.

Table 6.11: Potential for oral exposure to TiO_2 in toys and toy materials

Toy or toy material	Concentration TiO_2 (%)	Direct ingestion	Mouthing
coating	60	no	yes
casting kit	1,5	yes	no
chalks	5	yes	no
clays/ putties	3	yes	no
pencils coloured	23	yes	yes
pencils white	**51**	**yes**	yes
doughs	2	yes	no
dry paint tablets	13	no	yes
face paints	20	yes	no
finger paint	**30**	**yes**	no
glue	4	no	no
lipgloss/lipstick	**15**	**yes**	no
nail varnish	8	no	no
paper	0.5	no	no
polymeric materials	10	no	yes
powder paints	25	yes	no
solvent-based paints	30	no	no
water based paints	30	no	no
wax crayons	18	no	yes

The concentration of TiO_2 in various toys was provided by TIE (TIE 2020b).

6.4.2.7 Exposure modelling- oral

Direct ingestion

The amount of TiO_2 ingested can be calculated as presented in Formula 3 (Van Engelen *et al.*, 2008):

Formula 3:

$$D = a_{product} * wf_{product}/ bw$$

with
D = dose [mg/kg bw]
$a_{product}$ = amount of toy product (material) swallowed [kg]
$wf_{product}$ = weight fraction of the chemical in the toy product (material) [mg/kg]
bw = body weight of the exposed person [kg]

The parameter values needed for this calculation are:
$a_{product}$: amount of toy material swallowed, which depends on whether the toy is made of dry or liquid, pliable or otherwise sticky material, or whether the ingested material is from scraping off a toy layer.

$wf_{product}$: fraction of the chemical in the toy material. This depends entirely on the material the toy consists of and no default values can be given, some data are presented in Table 6.11. The total amount of chemical migrated from the toy (material) can be used as an upper bound, if composition data of the material are not available. The amount of migrated chemical depends entirely on the chemical-material combination and should be assessed with methods described in chapter 6.3 and 6.4.

bw: body weight of the exposed child. Mean, standard deviation and 25th percentile default values for body weight of Dutch children from 1.5 months to 17.5 years have been given in the general fact sheet of ConsExpo (Te Biesebeek *et al.,* 2014):

The mean body weight of the lower 25th percentile of the weight of children of 3.5-4.5 years of age was used in the calculations as being 15 kg. For the use of finger paint also an exposure was calculated for a younger child weighing approximately 10 kg.

Selected scenarios for oral exposure:

Finger paint

The oral exposure to TiO_2 from finger paint is estimated via the hand-mouth contact scenario as described in the ConsExpo Children's Toys Fact Sheet (Bremmer and Van Veen, 2002). In this "hand to mouth contact" scenario, the ingestion rate [in cm[3] /min] is the most important parameter.

In Van Engelen *et al.* (2008), additional exposure characteristics for chemicals in finger paint intended for children < 3 year are given (van Engelen *et al.*, 2008). For all liquid toys such as finger paint, it is assumed that these are directly ingested. For finger paint and other products that stick to the skin, a default value of 30 mg/min has been derived (Bremmer and Van Veen, 2002). It was further assumed that children play with finger paint for 45 minutes. Total amount swallowed is then 30 mg/min x 45 min = 1350 mg. This amount may be an overestimation and, therefore, it was proposed in the Chemicals in Toys report by Van Engelen *et al.* (2008) to use a value of 400 mg as a default, with the comment that this value is a rough estimate and needs further research.

However, the estimated total intake by Van Engelen *et al.* (2008) does not apply. Since 2014 as in EN 71-7:2014 (CEN, 2014), an obligation was included to add an embittering agent to finger paints to limit and prevent uptake of finger paint by direct ingestion. It is likely that uptake of finger paint due to direct ingestion will be rather limited, as the bitter

taste will result in avoiding oral uptake. More recent estimations for the possible uptake of finger paints containing an embittering agent propose an exposure frequency of 18 times per year for children 2 years of age (CEN/TC 52-WG5 N1682 20201127). SCHEER uses this proposal for estimating the oral exposure to pigmentary TiO_2 for children 3.5 to 4.5 years of age. In view of the low frequency of exposure, SCHEER estimated both the effects of an acute and subchronic exposure.

For the current assessment, the amount ingested of 400 mg per event is still used as proposed by Van Engelen *et al.* (2008). Based on the concentration data provided by the TIE (2020a), finger paint can contain a maximum level of 30% TiO_2.

- Single acute event:
  Uptake of 400 mg with 30% TiO_2 content results in an exposure of 120 mg, translating for a 15 kg child into a single acute exposure of 8 mg TiO_2/ kg bw.

- Semi-chronic multiple events:
  Uptake of 400 mg with 30% TiO_2 content results in an exposure of 120 mg TiO_2/event, for 18 events per year this results in 2160 mg per year, resulting in 2160/365= 5.9 mg /day, resulting in a dose of 0.39 mg TiO_2/ kg bw/ day for a 15 kg child.

As there is also a foreseeable use of finger paint for children below the age of 3, also an exposure calculation and risk assessment were performed for a child of 10 kg. The single acute event exposure for finger paint is 12 mg/TiO_2/ kg bw for a 10 kg child. For the semi-chronic multiple events, the dose is 5.9/10 = 0.59 mg TiO_2/kg bw.day.

White colouring pencils

Exposure to TiO_2 from white colouring pencils is estimated via the direct ingestion scenario, as children may put pencils into the mouth and ingest small parts of the pencil point. It is estimated that 8 mg of pencil is ingested (see Figure 6.2 below).

Figure 6.2 Example of 8 mg scraped material; taken from Van Engelen *et al.*, 2008. The size of the bucket is 3x3 cm²

According to the concentration data provided by TIE (2020a), white colouring pencils will contain a maximum level of 51% TiO_2 (see Table 6.13). The intake will be 51% x 8 mg product per event = 4.1 mg TiO_2 per event. For worst-case use, it is considered that the event occurs twice a day, therefore, the total exposure would be 8.2 mg TiO_2/day.

For ingestion of scraped-off toy material from toys intended to be mouthed by children over 3 years of age, the bodyweight of a child of approximately 3-4 years of age will be used. The 25th percentile of Dutch children 3.5 and 4.5 years of age is 14.1 and 16.3 kg, respectively. A default value of 15 kg is proposed for the exposure calculation per kg body weight.

The amount of TiO_2 ingested for two events per day via white colouring pencils is therefore 8.2 mg/day for a 15 kg child = 0.55 mg TiO_2/kg bw.day.

Lip gloss/lipstick

For lip gloss/lipsticks, oral exposure is estimated in a different way. The amount of lipstick applied to the lips is 0.9 mg/kg bw/day (SCCS 2019, 2021c). It is assumed that the whole amount applied to the lips is swallowed and ingested.

Based on the concentration data provided by TIE (2020a) (see also table 6.11), lip gloss/lipstick will contain a maximum level of 15% TiO_2. The bioavailability of TiO_2 from the lipstick is unknown, and is therefore assumed to be 100%.

The daily intake will be 15% x 0.9 mg product/kg bw.day = 0.135 mg TiO_2/kg bw.day.

The exposure of a child of 4.5 years is 0.135 mg TiO_2/kg bw.day x 15 kg bw = 2.0 mg TiO_2/day.

For this case, it is assumed that children of 4.5 years use lipstick/lip gloss on a regular/daily basis. This is a worst-case scenario as Ficheux and co-authors have reported that only 24% of 0–15-year-old girls use lipstick with a frequency of 0.47 times per day (Ficheux *et al.*, 2015).

Mouthing

In general, TiO_2 used as colouring agent in plastics and/or in paints applied to toy surfaces show little or no migration (see section 6.3.3, EFSA CEP, 2019). Therefore, the potential exposure to TiO_2 by mouthing is considered to be lower than uptake from direct ingestion, as calculated for different toy materials in the previous section. Therefore, exposure to TiO_2 via mouthing of toys will not be further evaluated in the current Opinion.

6.4.2.8 Conclusion on oral exposure

To determine the potential oral exposure to TiO_2 via toys, three direct ingestion scenarios were selected for further calculation. The direct ingestion scenario is supposed to have the highest potential oral exposure (Van Engelen *et al.*, 2008). The selected products are lip gloss/lipstick, finger paint and white colouring pencils based on the likeliness of exposure when playing with these products, as well as on the relatively high percentage of TiO_2 in these products. Calculated exposure values of the selected products for a 15kg child are:

- Finger paint: 120 mg TiO_2/event (8 mg TiO_2/kg bw/event) for a single acute exposure and 5.9 mg TiO_2/per day (0.39 mg TiO2/ kg bw/ day) fo a semi-chronic exposure
- White colouring pencil: 8.2 mg TiO_2/day (0.55 mg TiO_2/kg bw.day)
- Lipstick: 2.0 mg TiO_2/day (for a child of 4.5 years weighting 15 kg) (0.135 mg TiO_2/kg bw.day)

The weight of evidence (WoE) for the oral exposure scenarios is as follows:

Great uncertainty related to the finger paint scenario results in a weak WoE (low quality, low consistency) for the oral exposure calculation. A value of 400 mg for finger paint has been used as a default (defined in Van Engelen *et al.,* 2008) which is a very rough

estimate. In addition, since it is not completely clear what the frequency of oral exposure is due to the addition of an embittering agent in the finger paint, two different scenarios are calculated for oral exposure: the single acute exposure as well as the semi-chronic exposure. Another assumption is that all finger paint contains TiO_2, not only the white colour paint.

For white colouring pencils, the uncertainty related to the indirect ingestion scenario is also great, resulting in a weak WoE (low quality, low consistency). The amount ingested (8 mg) is again based on the amount of scraped-off material from a pencil (Van Engelen *et al.,* 2008), which is a very rough estimation. Furthermore, for a worst-case scenario it is considered that the ingestion of pencil material occurs twice a day.

For lipstick/lipgloss, there is somewhat less uncertainty, resulting in a moderate WoE for the oral exposure calculation (medium quality, low consistency). The amount of lipstick/ lipgloss applied to the lips is taken from a recent SCCS Opinion and seems to be a rather accurate estimation. On the other hand, the assumption that 100% of the lipstick/ lipgloss is ingested is a worst-case estimate. For this case, it is assumed that children of 4.5 years use lipstick/lip gloss on a regular/daily basis. Ficheux and co-authors have reported that only 24% of 0–15-year-old girls use lipstick with a frequency of 0.47 times per day (Ficheux *et al.,* 2015). In reality, the number of girls using lipstick/ lipgloss at this age is most probably lower.

6.5   Toxicokinetics

6.5.1. Inhalation exposure

6.5.1.1. Introduction
The human respiratory tract can be divided into three main regions based on size, structure, and function, namely, 1: the nose, the pharynx and larynx region, 2: the tracheobronchial region and 3: the alveolar or pulmonary region. These three areas translate for the exposure to an airborne aerosol into the inhalable fraction, the thoracic fraction, and the respirable fraction (CEN, EN 481 1993, Brown *et al.*, 2013, SCCS, 2021b).

The fraction comprising droplets/particles with a Mass Median Aerodynamic Diameter (MMAD) of ≤100 µm is generally regarded as inhalable. The inhalable fraction represents particles that enter the respiratory system via the nose or mouth (total dust). The thoracic fraction of the total dust is that portion of the inhalable particles that pass the larynx and penetrate the conducting airways and the bronchial region of the lung (conventionally these are the particles with a MMAD ≤10 µm). The respirable fraction is the portion of inhalable particles that enter the deepest part of the lung, the non-ciliated alveoli (conventionally these are the particles with a MMAD ≤4-5 µm).
As described in section 6.2.9, TiO_2 particles in different batches and uses have a wide range of sizes, ranging from about 40µm down to less than 100nm. The region of deposition of different inhaled TiO_2 particles will therefore depend on their size (see figures 6.3 and 6.4).
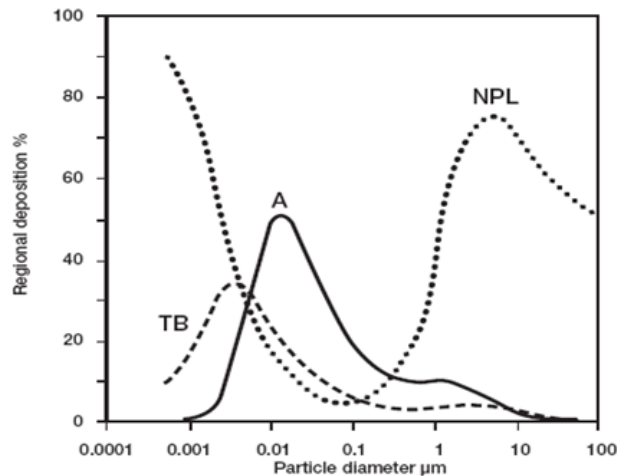
Figure 6.3: Deposition of particles in the human respiratory tract according to ICRP (1994).
A = alveolar region, TB = tracheobronchal region, NPL = nasal, pharynx and larynx region.
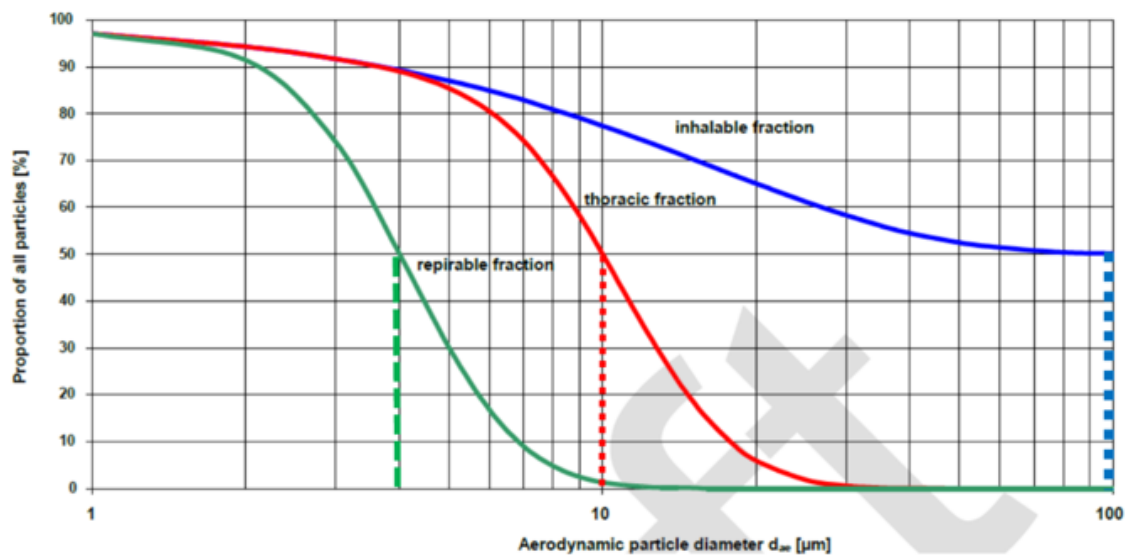
Figure 6.4: Separation curves for inhalable, thoracic and respirable fractions in accordance with EN 481:1993 (SCCS, 2020)

6.5.1.2 Inhalation exposure

Following inhalation, TiO$_2$ particles are retained in the respiratory tract, of which the smaller particles could eventually reach lower respiratory tract regions, including the conducting airways, the bronchial region and the non-ciliated alveoli (see Figures 6.3 and 6.4 above). From the conducting airways and the bronchial region, particles are removed from the lung by the mucociliary escalator into the oral cavity, from which they can be swallowed and excreted into feces.

Ultrafine particles that reach the alveolar lumen and interstitial tissues have also been documented to be predominantly cleared by alveolar macrophage toward the mucociliary escalator and be excreted into feces as well (Pujalté *et al.*, 2017). A fraction of the inhaled dose appeared to be transferred into the systemic circulation and to reach secondary organs, such as the liver, kidneys and spleen, which contained detectable levels of TiO$_2$

(Kreyling *et al.*, 2017a, Pujalté *et al.*, 2017, Gaté *et al.*, 2017). Kreyling *et al.* (2017a) indicated that 4% of the dose after respiratory exposure can be taken up into the systemic circulation. However, the systemic exposure might be due from migration from the lung or absorption by gastrointestinal tract.

6.5.1.3 Modelling

Different models are available to estimate the total and regional lung deposition of aerosol droplets and/or dry particles. Examples include the Human Respiratory Tract Model (HRTM) (International Commission on Radiological Protection - ICRP, 1994, 2002), the NCRP model (National Council on Radiation Protection and Measurement), the IDEAL model (Inhalation, Deposition and Exhalation of Aerosols in/from the Lung) or the MPPD model (Multiple-Path Particle Dosimetry).

The ICRP human respiratory tract model is used to estimate particle penetration through the extrathoracic (ET) airways. The ICRP predictive equations for ET deposition are based on experimental measurements in humans.

The Multiple Path Particle Deposition (MPPD) model (Anjivel and Asgharian 1995, Cassee *et al.* 2002, RIVM 2002) allows the direct extrapolation of laboratory animal data to human exposure and is capable of estimating specific doses deposited at various sites of the respiratory tract. For deriving the human equivalent concentration (HEC) at inhalation, a dosimetric adjustment factor (DAF) was used to convert the rat 6-h NOAEC of 0.5 mg/m^3 (from Bermudez *et al.*, 2004) to a 24-h HEC based on species-specific information on deposition, pulmonary surface area, and breathing volume. The DAF was calculated as the ratio of the steady state load/lung surface area of the rat and the steady state load/lung surface area of humans. Deposition per pulmonary surface area is the key dose metric for inflammatory effects. The DAF was calculated using the MPPD v3.04[14] to estimate the pulmonary deposition fraction to the human and rat lungs. This DAF is also known as the regional deposited dose ratio (RDDR) (EPA US, 1994).

6.5.2. Oral exposure

6.5.2.1. Introduction

The absorption of TiO$_2$ particles from the gastrointestinal tract (GIT) is influenced by their size. The absorption is higher for smaller particles than for larger ones as demonstrated for TiO$_2$-NPs, which can be absorbed through the lymphoid tissues. (Cho *et al.*, 2013, Rollerova *et al.*, 2015). In addition, part of the oral exposure is due to particles originating from the respiratory tract due to the removal of particles from the lung by the mucocilliary escalator.

6.5.2.2. ADME

The absorption of different sized TiO$_2$ particles (148, 36, 28 nm) in an *ex vivo* porcine buccal model showed that all investigated particles could permeate the mucosa layer and enter the oral epithelium (Teubl *et al.*, 2015). Penetration depth varied with particle size, with smaller particles penetrating deeper. Mucosal penetration of the TiO$_2$ NP was also demonstrated *in vitro,* using reconstructed normal human buccal mucosa (Konstantinova *et al.*, 2017). These two models demonstrated that ultrafine TiO$_2$ particles can also enter the buccal mucosa under physiological conditions, which included digestive enzymes e.g., mucins, and relevant pH levels. Two studies in humans indeed indicate that absorption of TiO$_2$ form the GI-tract is possible (Bockmann *et al.*, 2000, Pele *et al.*, 2015).

For ADME, extensive *in vivo* studies have been performed with ultrafine TiO$_2$ particles. Kreyling *et al.*, (2017b) performed a toxicokinetic study in rats, where pure anatase TiO$_2$

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[14] https://www.ara.com/mppd/

ultrafine particles with a median aggregate/agglomerate in the form of $[^{48}V]TiO_2NP$ (size of 7088 ± 11 nm in aqueous suspension, polydispersity index (PDI) 0.18 ± 0.04) was administered by intraesophageal instillation. Rats were sacrificed 1, 4, 24 h and 7 days after gavage to assess the transfer of radioactivity from the gastrointestinal tract into the systemic circulation and different tissues (such as liver, lungs, kidney, spleen, brain, uterus, skeleton). This toxicokinetic study showed that a small proportion of the applied radioactivity (very low oral bioavailability of 0.6%) was detected in the blood and lymph and internal organs like liver, spleen and kidneys (Kreyling *et al.*, 2017b). Other reports also indicate a low systemic absorption of orally administered TiO$_2$ (Jani *et al.*, 1990 Geraets *et al.*, 2014).

After a very low oral absorption, TiO$_2$ NPs distribute into the liver, spleen, and lymph (Jani *et al.*, 1990, Geraets *et al.*, 2014, Kreyling *et al.*, 2017b). Geraets *et al.* (2014) indicated organ levels in only a few of the orally treated animals just above the limit of detection (LOD) with an overall estimation of 0.02% of the exposure dose recovered in all organs measured. Kreyling *et al.* (2017b) observed that approximately 0.6% of the administered dose passed the gastro-intestinal-barrier after one hour and about 0.05% were still distributed in the body after 7 days (Kreyling *et al.* 2017b). Transport of TiO$_2$ particles in systemic circulation and further transition through barriers, especially the placental and blood-brain barrier has been described (Jani *et al.*, 1990 Geraets *et al.*, 2014, Rollerova *et al.*, 2015, Kreyling *et al.*, 2017b, Aengenheister *et al.*, 2019). These finding have been confirmed by other authors, where rats were intravenously administered by TiO$_2$-NPs. After systemic exposure, it has been shown that TiO$_2$-NPs mainly accumulated in liver and spleen and could be retained for over 90 days in these tissues due to the phagocytosis by macrophages (Geraets *et al.*, 2014; Kreyling *et al.*, 2017c). The excretion route of TiO$_2$-NPs through urine was higher than that of feces, indicating that renal excretion was the main excretion pathway of TiO$_2$-NPs (Xie *et al.*, 2015). In a recent evaluation by Health Canada (2022) systemic bioavailability of food grade TiO$_2$ after oral exposure was estimated to be in the order of 0.001% of the exposure dose (Health Canada, 2022).

Studies in humans revealed a low oral bioavailability (Winkler *et al.*, 2018). Bockmann *et al.* (2000) performed a toxicokinetic study in man where male subjects ingested anatase particles at doses of 23 and 46 mg in gelatin capsules (mean particle size of 160 nm) or as a powder (mean particle size of 380 nm). Blood was obtained at different times over 24 h. The authors found peak TiO$_2$ concentrations around 8–12 h after the intake of 160-nm anatase at the dose of 23 mg in the blood that reached 0.04–0.05 µg/ml.
Jones *et al.* (2015) exposed volunteers to a lower 5-mg/kg single oral dose of different TiO$_2$, (anatase with a size of 15 nm, rutile with a size of 100 nm). Judging by the blood collected over a 4-day period, the authors found that the administered TiO$_2$ particles were not systemically absorbed.

6.5.3. PBPK Modelling

Bachler *et al.* (2015) developed a physiologically based pharmacokinetic (PBPK) model for oral and dermal administration of TiO$_2$ (with a range of particle size from 15 to 150 nm). The applicability of the PBPK model was evaluated by comparing organ titanium levels to three independent *in vivo* studies. To assess the potential of the PBPK model to be extrapolated to other particle sizes, species and routes of administration, and data of various particle sizes (from mice and rats and after intravenous, oral and dermal administration) was used for the evaluation.

6.5.4 Conclusions

Both oral and inhalation exposure can result in systemic availability of TiO$_2$ particles, albeit at very low amounts. Estimations range from less than 0.5% of the exposure dose, (Geraets et al, 2014, Kreyling et al, 2017b, EFSA 2021), to approximately in the order of 0.001% (Health Canada 2022). The WoE that the uptake fraction after inhalation is small

is considered strong in view of high-quality data and high consistency. The WoE for very limited oral uptake is considered moderate to strong as there is some variation in the amount of Ti that can be detected in the body after oral exposure, and measurements were at or very near the LOD. In addition, there is considerable difference in the quality of the published results with respect to the characterisation of the TiO_2 materials used (medium quality of studies, medium to high consistency in findings). It should be noted that studies on toxicokinetics usually use highly dispersed TiO_2 solutions that may differ in their exposure compared to TiO_2 originating from toys, similar as the difference in exposure to food grade TiO_2 with respect to the food composition in which the TiO_2 is applied (Health Canada 2022).

6.6   Toxicological evaluation

6.6.1. Introduction on TiO₂ toxicity

Titanium dioxide (TiO_2) has a long history of use and has been evaluated for possible adverse effects including specific effects of the ultrafine TiO_2 materials and/or the ultrafine fraction that might be present in pigmentary TiO_2 preparations (Schins and Knaapen, 2007, Shi *et al.*, 2013, SCCS, 2014, Warheit *et al.*, 2015, Shakeel *et al.*, 2016, Heringa *et al.*, 2016, Kawasaki, 2017, Winkler *et al.*, 2018, Saber *et al.*, 2019, Baranowska-Wójcik *et al.*, 2020, Carriere *et al.* 2020, EFSA 2016, 2021, Shabbir *et al.*, 2021). Most of these reviews were considering the adverse effects of ultrafine TiO_2. For ultrafine particles when embedded in paints the resulting toxicity seems low (Smulders *et al.*, 2015b, Mittal *et al.*, 2021).

6.6.2. Inhalation hazard of pigmentary TiO₂

The assessment committee of the European Chemical Agency has classified Titanium dioxide as a suspected human carcinogen (category 2) upon inhalation (see footnote 11). The classification is based upon occupational exposure to TiO_2 particles and is supported by the induction of lung tumours in rats after high-dose chronic inhalation exposure, representing an overload situation with decreased and retarded lung clearance. Lung overload was evaluated to occur at doses of 200 – 300 cm^2 of total particle surface area (Tran *et al.* 2000). In a 13-week inhalation study with ultrafine TiO_2, particle persistence indicative for overload was observed at a dose of 10 mg/m^3 (Bermudez *et al.*, 2004). A single intratracheal TiO_2 particle administration presented an overload dose at 5 mg/kg body weight (Warheit *et al.* 2007). A volumetric dose estimated for lung overload was 10 µL/kg (Pauluhn 2011) and 4.2 µL/kg (Pauluhn 2014). Relier *et al.* (2017) in an extensive study in rats with repeated intratracheal (3 times) P25 TiO_2 ultrafine particles presented an overload dose at 10 mg/kg body weight. The overload doses resulted in an altered clearance, persistent lung inflammation and delayed lung DNA damage, and biodistribution to the liver with induction of markers of genotoxicity (Relier *et al.* 2017). Thomson *et al.* (2016) predicted a NOAEC of 2.4 mg/m^3 for overload of the macrophage compartment in rats using specific values for fine TiO_2. A no effect level was estimated at 0.5 mg/kg. Many different types of ultrafine TiO_2 particles have been investigated and biopersistence of the particles may be a determining factor (Relier *et al.*, 2017).

The biological mechanism of the induced lung TiO_2 toxicity, *i.e.*, lung carcinogenesis remains unclear (Braakhuis *et al.*, 2021), however a particle overload situation results in chronic inflammation and cell proliferation. Subacute (4 weeks) inhalation exposure to pigmentary TiO_2 (rutile, fine) at an extremely high aerosol concentration (250 mg/m^3) produced sustained pulmonary inflammatory reactions, e.g. an elevated number of neutrophils in bronchoalveolar lavage fluid (BALF) (Warheit *et al.*, 1997). In later studies, large species differences in long-term pulmonary response to inhalation of pigmentary

TiO$_2$ particles have been reported, with rats being the most sensitive, compared to mice and hamsters (Bermudez *et al.*, 2002, Warheit *et al.*, 2016).

A dose-related increase in lung burden was observed in female CDF/CrlBR rats exposed sub-chronically for 6 hours per day, 5 days per week to pigmentary TiO$_2$ (rutile) for 10, 50 or 250 mg/m^3 daily for 13 weeks. The lung burden decreased with post-exposure time accompanied by an increase in the burden in lymph nodes. In the low-dose group, the level decreased to 15% of the initial burden, while the high-dose group decreased to 75% of initial dose. Total protein concentration in BALF was used as a marker of toxicity, and it was only significantly increased at the highest dose and sustained up to 52 weeks' post exposure. Cell turnover, *i.e.*, labeling index, was significantly increased at week 4 and sustained at the highest dose up to week 52. Markers of inflammation or lung lesion were not induced at the lowest concentration (10 mg/m^3) and showed a concentration-related increase at higher concentrations (Bermudez *et al.*, 2002). Similar results were observed using an ultrafine TiO$_2$ preparation (Bermudez *et al.*, 2004) with a no-adverse effect level of 0.5 mg/m^3. In this study, exposure was to ultrafine P25 with an average constituent particle size of 21 nm, which is comprised of uncoated ultrafine particles of a mixture of 80% rutile and 20% anatase forms of TiO$_2$.

Based upon data from studies in rats, an adverse outcome pathway (AOP) has been developed for the toxicity of TiO$_2$ particles. At high concentration chronic inhalation exposure, particles will accumulate in the lung, overwhelming the clearing capacity, which can be considered as the initiating event (IE). This results in continuous recruitment of neutrophils and persistent inflammation resulting in Key Event 3 (KE3). The persistent inflammation and/or cellular interaction with the surface of the TiO$_2$ particles results in reactive oxygen species (ROS) being generated (KE1), which can induce oxidative stress (KE2) if the antioxidant capacity of the lung is exceeded. The generation of ROS and induction of oxidative stress promote the inflammation response (KE3). Inflammation (KE3) and oxidative stress (KE2) can become persistent upon chronic exposure to TiO$_2$ and can induce persistent epithelial injury (KE4). This leads to regenerative cell proliferation (KE6) and hyperplasia (KE7). The initiating event, impaired clearance, may not be relevant in humans due to the difference in lung physiology between rat and human, which may not result in a lung particle overload in humans, and thus not result in a persistent inflammation as occurs in rats (Braakhuis *et al.*, 2021). However, repeated lung inflammation by TiO$_2$ due to repeated short-term inhalation exposures may also be considered a risk resulting in (sub)chronic inflammation.

General endpoints for the toxicity of inhaled TiO$_2$ particles are acute airway irritation, pulmonary inflammation, as for example assessed by analysis of bronchoalveolar lavage (BAL) cell composition, and DNA damage evaluated by the comet assay. Several rodent studies have investigated the toxicity of TiO$_2$ by inhalation (see below). Various dose metrics have been used to express the TiO$_2$ particles exposure – e.g. aerosol concentration, lung mass burden (mg/lung) and particle surface area burden (cm^2/lung). In addition to the dose, it is also important to adjust for deposition efficiency. Another problem in comparing the different studies is that the particles (anatase and rutile) are not well characterised and the size is not well-described, e.g., pigmentary TiO$_2$.

Titanium dioxide is a poorly soluble low-toxicity (PSLT) material frequently used as a negative control in inhalation toxicology studies on dust particles. In a chronic toxicity study using nose-only exposure in male Wistar rats for 13 weeks (0.70 mg/m^3 - <5 µm, 2762 particles/cm^3), bronchial alveolar lavage was assessed up to 9 months post exposure. The % of eosinophils was significantly increased only at day 90 and the expression of TNF-alpha and IL-1β at day 45 was increased. However, these values were not statistically different from that measured in the control animals (Bernstein *et al.*, 2020).

Analysis of the data from Tran *et al.* (1999) on pulmonary inflammation induced by TiO$_2$ showed that the level of inflammation could be explained when the lung burden was

expressed as total particle surface area and the dose response analysis indicated the presence of a response threshold at approximately 200-300 cm^2 of lung burden. A piecewise linear regression analysis provided evidence of a response threshold at 0.0134 m^2/rat lung (CI 0.0109-0.0145) (Dankovic *et al.*, 2007). Based on these estimates in rats, human occupational exposures thresholds were calculated for pulmonary inflammation after a 45-yr working lifetime as 1.0 mg/m³ and 0.11 mg/m³ for fine (MMAD 2.1 μm) and ultrafine (MMAD 0.8 μm) TiO_2 particles respectively (Dankovic *et al.*, 2007).

Based upon a systemic literature review and using a reconstructed Hill-model based dose-response profile, Liao *et al.* (2008) estimated the median effective surface area-based TiO_2 lung burden (EC50) using rat data and surface based TiO_2 from pooled fine and ultrafine particles. The authors determined an EC50 for inflammatory effect, using an elevated level of PMN as marker, of 0.11 m^2/g wet lung weight, and an EC50 for lung tumour formation was calculated as 1.15 m^2/g wet lung weight.

Based upon the data from Lee *et al.* (1985) and neutrophil inflammation in rat lungs, a Lowest-Observed-Adverse-Effect Level (LOAEC) of 10 mg/m³ was estimated for pigmentary TiO_2. The test material was titanium dioxide particles with a spherical configuration and a 1.5 to 1.7-μm aerodynamic mass median diameter (MMD). Approximately 84% of the dust particles were of respirable size (<13 μm MMD). This is quite similar to the predicted NOAEC of 2.4 mg/m³ for overload of the macrophage compartment (Thompson *et al.*, 2016).

Most of the published studies use intratracheal administration of a NP suspension rather than inhalation of an aerosol, the latter being more relevant for humans. However, the intratracheal installation provides more certainty on the administered dose and thus exposure levels in the study. A single Intra-tracheal administration of anatase TiO_2 particles to SD rats increased markers of oxidative stress, as determined by malondialdehyde production and the fluorescent DHP assay, in lung tissues as well as in the extra-pulmonary organs of the liver and kidney, followed by a decrease in the markers of oxidative stress superoxide dismutase and GSH-Px. DNA damage was shown in all three tissues by the comet assay and by the activation of the p13K/AKT/Foxo3a signalling pathway and induction of the DNA repair pathway GADD45a/ChK/and XRCC1. The TiO_2 was retained in the tissues up to 7 days, and the effect was observed only at the highest dose level 1 g/kg bw (Han *et al.*, 2020a).

DNA damage was measured by the comet assay following intratracheal instillation of four ultrafine TiO_2 materials (anatase 12-50nm, 16-28nm, tube length 40-500nm and cube 11-27nm) in BAL cells, lung and liver tissue of C57BL/6j mice, additionally to pulmonary toxicity and inflammation. Specific surface area, crystal phase and shape of TiO_2 are important predictors for the pulmonary effects of TiO_2. Neutrophil influx in BAL fluid correlates closely with pulmonary phase response in terms of increased serum amyloid A3 (Saa3) level. SAA generated in the lungs enters systemic circulation and can contribute to the pathogenesis of cardiovascular diseases (Danielsen *et al.*, 2020).

Following a single intratracheal dose of TiO_2 to C57BL/6j mice, TiO_2 nano tubes (162 μg) induced lymphocytes and macrophages infiltration, as well as proteinosis. The expression of pulmonary Saa3mRNA was only significantly induced at the highest dose of ultrafine TiO_2 material. The expression of Saa3 was also induced in the liver. There were only few significant increases in DNA strand break levels. However, DNA strand break levels were also observed to be statistically significantly decreased for some of the TiO_2 material, especially in the lung tissue, at different time points. The observed changes were in general not dose-dependent and considered as chance findings. A multiple regression analysis of Saa3 expression levels and the different physiochemical properties showed that Mass dose, BET and tube shape had a significant influence on the expression of Saa3 (Danielsen *et al.*, 2020).

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In BALB/c mouse models for asthma TiO$_2$-NP exposure (0.8mg/kg by oropharyngeal aspiration) increased airway hyperreactivity (AHR), oxidative damage, and the BAL cell count (Hussain *et al.*, 2011; Kim *et al.*, 2017). In addition, a NLRP3 induction of the inflammasome pathway causing airway inflammation and hyper reactivity and an increased caspase activity was noted (Kim *et al.*, 2017). Adjuvant activity was also reported for both *in vitro* dendritic cell activation and *in vivo,* in a murine inhalation allergy model with anatase TiO$_2$ NP having higher adjuvant activity than rutile NP (Vandebriel *et al.*, 2018). The results suggested that TiO$_2$ NP may aggravate the situation for children with asthma symptoms.

6.6.3 Oral hazard of TiO$_2$ pigment

6.6.3.1 Introduction

In March 2021, EFSA adopted its update on the safety assessment of titanium dioxide used and approved as food additive E171 (EFSA, 2021) following the principles of the EFSA Guidance on Nanotechnologies applicable at that time (EFSA Scientific Committee Guidance, 2018) based on the characterisation of TiO$_2$ used as E171 (EFSA FAF Panel, 2019). In this evaluation, potential toxicity was noted regarding immunotoxicity, inflammation induction, and neurotoxicity for TiO$_2$ ultrafine particles present in the TiO$_2$ (E171) food colouring agent, and a potential to induce aberrant crypt foci for E171 as food colouring agent, indicating adverse effects. In addition, a concern could not be ruled out for the genotoxic effects, as for TiO$_2$ particles, the potential was noted to induce DNA strand breaks and chromosomal damage, but not gene mutations. No clear correlation was observed between the physico-chemical properties of TiO$_2$ particles and the outcome of either *in vitro* or *in vivo* genotoxicity assays. The EFSA Panel concluded that E171 can no longer be considered as safe when used as a food additive. This conclusion was based on all the evidence available, the concern for genotoxicity that could not be ruled out, and the many uncertainties observed. The EFSA scientific Opinion (EFSA, 2021) provided important information that supported the risk assessment of the oral exposure to TiO$_2$ pigment as used in children's toys. Additional information will be added where appropriate.

It should be noted that TiO$_2$ pigments, similar to food additive TiO$_2$ E171 (EFSA FAF Panel, 2019), may contain an ultrafine fraction that may need specific considerations in view of their toxicokinetics and potential toxicity (Weir *et al.*, 2012, Peters *et al.*, 2014, Dudefoi *et al.*, 2017).

6.6.3.2 EFSA 2021 evaluation E171

The EFSA Panel concluded that the available information in the literature did not indicate adverse effects with either E171 up to a dose of 1,000 mg/kg bw per day for general toxicity or with TiO$_2$ NP > 30 nm up to the highest dose tested of 100 mg/kg bw per day for reproductive and neurotoxic effects. Also, in a newly performed extended one-generation reproduction toxicity (EOGRT) study (performed as a follow-up of the re-evaluation of E171 (EFSA ANS Panel, 2016), there were no indications of general toxicity, no effect on thyroid or sex hormone levels, no effect on reproductive function and fertility in either male or female rats. Furthermore, no effects were observed on pre- and postnatal development. No effects on neurofunctional endpoints in F1 offspring were observed either. Concerning immunotoxicity, a marginal but statistically significant decrease in antigen-induced IgM levels (-9%) in males of the F1 Cohort 3 only was noted, with no apparent dose-response. However, the Panel could not conclude on immunotoxicity due to methodological shortcomings in the design of the immunotoxicity evaluation of the EOGRT study. In a satellite group of that study, E171 at doses up to 1,000 mg/kg bw per day did not induce aberrant crypt foci (ACF) in the colon. The EFSA Panel considered that there was uncertainty regarding the extent of the internal exposure to ultrafine TiO$_2$ particles (present in E171) across the range of tested doses (EFSA, 2021). The uptake from the GIT of TiO$_2$ particles is low (probably <0.5%); however, they may accumulate in

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the body due to their long half-life (up to 450 days for the ultrafine TiO2 particles) (EFSA, 2021).

6.6.3.3 TiO$_2$ pigment after oral exposure

TiO$_2$ pigment was studied in both a 90- and 28-day oral repeated dose response study by Warheit *et al.* (2015) with a NOAEL of 1,000 mg/kg bw.day (rutile type pigment grade alumina coated TiO$_2$ particles with D_{50} = 145 nm, containing 21% nanoparticles by number) and 24,000 mg/kg bw.day (rutile-type, uncoated, pigment-grade TiO$_2$ test particles with D_{50} = 173 nm by number), respectively, based on a lack of TiO$_2$ particle-related adverse effects on any in-life, clinical pathology, or anatomic/microscopic pathology parameters. Warheit also performed an OECD acute oral toxicity study with female rats dosed with a single oral dose of surface-treated rutile/anatase nanoscale TiO$_2$ particles (D_{50} = 73 nm by number). Under the conditions of this study, the oral LD50 for the test substance was >5,000 mg/kg bw (Warheit *et al.*, 2015). However, for TiO$_2$ as E171 used in food as coloring agent several data gaps were identified limiting the possibility to perform an adequate risk assessment (Winkler *et al.*, 2018). Food-grade TiO$_2$ particles were found not to be totally inert upon oral intake, while observations that TiO$_2$ particles cause at least some adverse reactions in experimental animals might give cause for concern (Winkler *et al.*, 2018). Some of these data gaps were addressed by the EFSA evaluation of 2021, but some issues remain uncertain (EFSA, 2021). A NOAEL was considered for E171 up to a dose of 1,000 mg/kg bw per day or with TiO$_2$ NP > 30 nm up to the highest dose tested of 100 mg/kg bw per day (EFSA, 2021). For immune effects, reported results are variable (EFSA, 2021). Regarding interaction of TiO2 particles in the GIT uptake of TiO$_2$ by M cells in Peyer's Patches and other immune cells in Peyer's Patches was reported (Bettini *et al.*, 2017, Riedle *et al.*, 2020). Brand *et al.* (2020) evaluated adverse outcome pathways (AOP) after oral exposure to TiO$_2$ and concluded that TiO$_2$ can trigger a number of key events in the liver and intestine mainly related to ROS generation, induction of oxidative stress and inflammation. Recently it was considered that TiO$_2$ is able to alter various aspects of the intestinal barrier function, composed of microbiota, mucus layer, epithelium, and immune system inducing a low-grade intestinal inflammation that may be or not be associated with preneoplastic lesions (Barreau *et al.*, 2021).

6.6.3.4 Other aspects of oral TiO$_2$ exposure

Indications for immune stimulation were also noted after *in vitro* cell exposure and other routes of TiO$_2$ NP exposure. In a study using murine, bone-marrow derived macrophages TiO$_2$ augmented proinflammatory IL-1β secretion (Riedle *et al.*, 2017). Dermal application of TiO$_2$ NPs enhanced lymph node reactivity in a local lymph node model for skin sensitisation using DNCB as model sensitiser (Smulders *et al.*, 2015a). Adjuvant activity was also reported for both *in vitro* dendritic cell activation and *in vivo*. In a murine inhalation allergy model with anatase, NP have higher adjuvant activity than rutile NP (Hussain *et al.* 2011, Vandebriel *et al.*, 2018). Combined, these findings may indicate the possibility for immune activation by TiO$_2$ in the GIT. This effect may have implications for children with diseases, such as was suggested for inflammatory bowel disease (IBD) as discussed in Brand *et al.* (2020).

In a recent paper, the effect of several food additives including TiO$_2$ on gut biota was evaluated (Gerasimidis *et al.*, 2020). The additives were individually added to faecal samples of young healthy adults and incubated *in vitro*. TiO$_2$ had only a limited effect in decreasing the amount of C. Leptum bacteria in the microbiome, whereas other additives had strong effects on the microbiome. However, also minor effects might be considered to contribute to the microbial dysbiosis as seen in inflammatory bowel disease (Gerasimidis *et al.*, 2020, Chassaing *et al.*, 2015).

For both oral and inhalation exposure, it was demonstrated that both photocatalytic and food grade TiO$_2$ NPs, can generate low levels of reactive oxygen species (ROS), specifically

hydroxyl radicals and superoxides in the dark (Jayaram *et al.*, 2017). Also, after intraperitoneal injection of TiO$_2$ NPs, thus bypassing the GI tract, oxidative stress was reported in the liver for Kupffer cells and hepatocytes, and in the brain neurons (Valentini *et al.*, 2018, 2019; Brand *et al.*, 2020). Oral exposure in mice and rats showed an effect on the testis that was attributed to oxidative stress (Elnagar *et al.*, 2018). The induction of oxidative stress and accompanying radical formation is considered a possible mode of action for the DNA damaging activity of TiO$_2$ (EFSA, 2021).

6.6.4 Conclusions on TiO$_2$ toxicity

For both inhalation and oral exposure adverse effects of exposure to TiO$_2$ particles could be identified, including direct effects such as lung and GIT oxidative stress and inflammation and indirect effects such as altering the immune system responses. However, it should be noted that similar to studies on toxicokinetics usually highly dispersed TiO$_2$ solutions are used for toxicity studies, that may differ in their exposure compared to TiO$_2$ originating from toys, similar as the difference in exposure to food grade TiO$_2$ with respect to the food composition in which the TiO$_2$ is applied (Health Canada 2022).

Overall, a large variety of different TiO$_2$ preparations was used in the various inhalation studies. The physical chemical properties of TiO$_2$ are influencing agglomeration of the particles and the toxicological properties *in vitro* and *in vivo*. The grouping of ultrafine TiO$_2$ particles for hazard assessment is challenging due to the large variation in physiochemical properties (SCCS, 2014). Based on inflammation responses for ultrafine TiO$_2$ (P$_{25}$) in a 90-day repeated dose toxicity inhalation study, the no-observed-adverse-effect concentration (NOAEC) was found to be 0.5 mg/m^3, whereas for fine TiO$_2$ a NOAEC of 10 mg/m^3, was observed (Bermudez *et al.*, 2002, 2004). The best estimates of a human exposure threshold for pulmonary inflammation (in occupational setting) are 1.0 mg/m³ and 0.11 mg/m³ for fine (MMAD 2.1 µm) and ultrafine (MMAD 0.8 µm) TiO$_2$ particles, respectively (Dankovic *et al.*, 2007).

A NOAEL was determined for general toxicity after oral exposure at 1,000 mg/kg bw per day in a 90-day repeated dose study, after oral gavage of 5 different types of TiO$_2$ particles (Warheit *et al.*, 2015, EFSA, 2021). However, several uncertainties remain regarding possible immunotoxic, genotoxic and carcinogenic activity after oral exposure for which use of TiO$_2$ particles as food additive could not be considered safe. The oral LD50 for the TiO$_2$ was found to be > 5,000 mg/kg bw.

The toxicological and AOP data of fine and ultrafine TiO$_2$ are both considered highly consistent and of medium to high quality for both routes of exposure. For oral exposure, however, uncertainties remain regarding immunotoxic, genotoxic and carcinogenic activity (see Sections 6.6.5 and 6.6.6), diminishing the reliability and consistency of the NOAEL. Therefore, the overall WoE for the inhalatory NOAEC is considered strong, but for oral exposure the overall WoE for adverse effects is judged to be weak.

6.6.5 Carcinogenicity

6.6.5.1 Inhalation exposure

Several *in vivo* studies in experimental animals have identified the tumourigenic activity of TiO$_2$ after inhalation exposure (Lee *et al.*, 1985; Muhle *et al.*, 1991, Heinrich *et al.*, 1995, Pott and Roller, 2005, Mohrfeld *et al.*, 2006). Human epidemiological data are limited and do not show any significantly elevated risk of lung cancer in association with TiO$_2$ exposure (Bofetta *et al.*, 2004; Thomson *et al.*, 2016; IARC, 2010). For instance, in a pooled European study of TiO$_2$ workers, no evidence of an association between respirable TiO$_2$ exposure and lung cancer mortality was observed despite the excess of lung cancer mortality among male TiO$_2$ workers as compared with the general population (Bofetta *et al.*, 2004).

However, Canu *et al.*, (2020) observed a positive relationship between TiO_2 exposure and lung cancer mortality in a cohort of 833 French TiO_2 workers, despite a limited statistical power in some models and the heavy use of imputation to complete smoking status. Subsequently, Canu *et al.* (2022), applied the g-computation algorithm formula, to reanalyse a subset of the pooled European cohort of TiO_2 workers. The g-computation algorithm formula approach is recommended for statistical analysis of cohort data in the presence of time-varying confounders affected by prior exposure, typical of the Healthy Worker Survivor Effect (HWSE) (Brown *et al.*, 2017). The original pooled European cohort included workers who had been employed at least 1 month in 1 of 11 TiO_2 production factories in six European countries. The reanalysis restricted the study to four countries (Finland, France, Italy and the UK), for which data were still available (7341 workers) and ethical approvals obtained. The factories produced mainly pigment-grade TiO2. A HWSE was observed, and it was shown that the HWSE can hide an exposure–response relationship. A positive association between lagged cumulative exposure to TiO2 and lung cancer mortality was found.

IARC classified TiO_2 as "possibly carcinogenic to humans (group 2B)" in 2010 (IARC, 2010), followed by NIOSH in 2011 indicating concerns that ultrafine TiO_2 might be carcinogenic in occupational settings (NIOSH, 2011). From the available rodent studies, the mechanisms by which TiO_2 can induce lung tumours in rats after inhalation is considered to be via impaired clearance and persistent inflammation. This is consistent with the KEs in the AOP as suggested recently by Nymark *et al.* (2021) and Braakhuis *et al.* (2021). Probably, the suggested AOP is operative in rats upon long-term exposure to high concentrations (>10 mg/m^3 per day for 2 years) of TiO_2.

Even though the Muhle *et al.* (1991) study only used one concentration of pigmentary TiO_2 (5 mg/m^3) and was included as a negative control in a 24-month carcinogenicity study, the results are considered relevant for the evaluation of pigmentary TiO_2.

6.6.5.2 Oral exposure

For oral exposure, there is uncertainty whether TiO_2 can induce intestinal tumours in rats and mice.

During the re-evaluation of E171 in 2016, the EFSA ANS Panel evaluated a carcinogenicity study in mice and rats (NCI, 1979), performed with TiO_2 mixed with the diet. The ANS Panel concluded that the study indicated that TiO_2 was not carcinogenic in rats and mice. However, in the recently updated evaluation (2021), the EFSA Panel considered that this study was not appropriate to ascertain the absence of a potential to elicit chronic toxicity and carcinogenicity by ultrafine TiO_2 particles.

There are 3 available repeated dose studies in which aberrant crypt foci formation was investigated. From the Extended One-Generation Reproductive Toxicity Study (EOGRT) study (OECD TG 443) (scoring 4 for nanoscale considerations (NSC) with score 1 having the highest and score 4 the lowest reliability/relevance), the EFSA Panel considered that oral exposure of rats to E171 at doses up to 1,000 mg/kg bw per day did not induce aberrant crypt foci (ACF) in the colon (EFSA, 2021). From the study by Bettini *et al.* (2017) (scoring 1 for NSC), the Panel considered that E171 at a dose of 10 mg/kg bw per day may induce ACF per se. In addition, E171 enhanced ACF formation after pretreatment with a genotoxic carcinogen (*i.e.*, dimethylhydrazine - DMH) in rats (Bettini *et al.*, 2017). From the study by Blevins *et al.* (2019) (scoring 3 for NSC), the EFSA Panel noted that no changes in the number of ACF or aberrant crypt (ABC) were observed due to E171 exposure alone. However, limitations in the pathological examination of ABC and ACF (limited sampled colon area; technical issues with fixation) precluded a conclusion by the Panel on any potential for ABC and ACF formation. Dietary E171, with or without treatment with DMH, had no effect on the length of the colonic glands examined or the number of goblet cells/unit. In the Bettini *et al.* (2017) study, the food grade TiO_2 (E171) was administered

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by oral gavage in water, while in the Blevins _et al._ (2019) study, the TiO$_2$ (E171) was dosed as a food ingredient which may result in different physicochemical aspects of the TiO$_2$ in the GIT (e.g. agglomeration, attachment of biological molecules et cetera). Furthermore, in recent studies (reported as abstract and poster at the Society of Toxicology, USA, meeting of 2023) no indications for abnormality of colonic crypts were noted after 28 or 90 days oral exposure to 6nm sized anatase TiO$_2$ primary particles with doses up to 1,000 mg/kg body weight per day, (Ogawa _et al._, 2023).

E171 was found to promote the development of tumours in a murine colon cancer model in which colorectal tumours were chemically induced by azoxymethane /dextran sodium sulphate (Urrutia-Ortega _et al._, 2016). In a follow-up study it was shown that immune related genes and signalling genes involved in colorectal cancer and other types of cancers were modulated, with a majority of the innate and adaptive immune system genes down-regulated (Proquin _et al.,_ 2018).

Brand _et al._ (2020) suggested that some of the key events (KEs)in the postulated AOPs for liver alterations and intestinal tumours can be induced by TiO$_2$ after oral exposure in both rats and mice (e.g., intestinal uptake, ROS generation, oxidative stress, inflammation, and hyperplasia). Braakhuis _et al._ (2021) also identified a molecular initiating event (MIE), cellular uptake, and a number of early KEs after oral TiO$_2$ exposure in a postulated AOP such as ROS generation, oxidative stress and inflammation, although there was insufficient information on later events in the postulated AOP. In addition, more recently, AOPs for possible adverse outcomes were proposed for colorectal cancer, liver injury, reproductive toxicity, cardiac and kidney damage, as well as hematological effects (Rolo _et al._, 2022). These recents overviews have so far identified the presence of MIEs and KEs that fit the proposed AOPs. However, definitive experimental evidence for the final outcomes (including tumorigenicity) of these proposed AOPs is not yet available. Most of the available evidence supporting the AOPs relate to nanosized TiO$_2$, and the influence of particle size within these AOPs is not known.

6.6.5.3 Dermal exposure

Both noncoated (ncTiO$_2$) and coated (alumina and stearic acid) spindle-shaped ultrafine titanium dioxide have been studied in the two-stage mouse skin carcinogenicity (DMBA initiation) studies with CD1 mice (Furukawa _et al._, 2011) and no promoter activity was found. Furthermore, no promoter activity was found for ncTiO$_2$ (rutile, mean particulate diameter 20 nm) and silicon-coated TiO$_2$ (rutile, mean particulate diameter 35 nm) in another study using a transgenic mouse strain (rasH2) and their wild-type counterparts CB6F1 mice and CD1 mice, as well as in Hras128 rats and their wild-type counterparts SD rats (Sagawa _et al._, 2012). TiO$_2$ NPs (uncoated, rutile, size 20 nm) were not shown to induce or promote skin carcinogenesis in dermal UV-B initiated skin carcinogenesis promotion study on Sprague-Dawley rats (wild-type and transgenic Hras128) (Xu _et al._, 2011).

6.6.5.4 In vitro studies

In the cell transformation assay (CTA) _in vitro_, statistically significant increases in the frequency of morphologically transformed BALB/c 3T3 cells (mouse embryo fibroblasts) were observed after treatment with ultrafine TiO$_2$ particles (P25) (Stoccoro _et al._, 2017). With the same cell line, transformed colonies (foci type III) were observed with rutile TiO$_2$ (micro- and nanosized), whereas no effects were observed with anatase TiO$_2$ (micro- and nanosized) (Uboldi _et al._, 2016). For anatase TiO$_2$, an effect on colony formation (_i.e._ increase) was observed in human embryonic kidney cells and in mouse embryonic fibroblasts (NIH/3T3) (Demir _et al._, 2015) and in human bronchial epithelial cells (BEAS-2B) (Vales _et al._, 2015). The CTAs provide information on initial steps of carcinogenesis that may include both genotoxic and non-genotoxic events. However, results from CTAs alone are considered to be of limited relevance for the assessment of carcinogenicity.

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6.6.5.5 Conclusions on carcinogenicity

Although there is limited evidence in epidemiological studies for the induction of lung cancer in occupational settings, recent re-evaluation of epidemiological studies applying more sophisticated statistics revealed a HWSE for occupational exposure. These results in combination with various animal studies clearly indicate a possible carcinogenic effect of TiO_2 in the lung after inhalation exposure. The WoE is considered strong.

In addition, the AOP of lung tumour induction after inhalation of TiO_2 was recently described. Whether the carcinogenic effect is due to a specific effect of (ultra)fine TiO_2 particles or due to a general carcinogenic effect of particles in the lung is as yet unknown. It is now being discussed whether to classify inhaled Poorly Soluble Low Toxicity (PSLT) particles as a carcinogenic hazard, of which TiO_2 is considered to be an example (Borm and Driscoll, 2019).

The available studies after oral exposure are not sufficient to draw conclusions on the potential carcinogenicity of TiO_2 particles. However, the induction of oxidative stress and inflammation in the GIT indicates a possible indirect or promoting effect of TiO_2 on tumour development. The WoE for tumour-promoting activity of TiO_2 particles in the GIT is moderate, whereas the WoE for tumour induction in the GIT is uncertain to weak. An indication for induction of ACF in the colon of animals was observed after exposure to food grade TiO_2 (E171) in drinking water (Bettini *et al.*, 2017 - dispersed in drinking water at a human relevant dose (~10 milligrams per kilogram of body weight per day; mg/kg bw.day) for 100 days). The relevance of the study for conclusions on carcinogenicity, however, is limited. It is still debated whether ACF is an early expression of a pre-neoplastic lesion (Health Canada, 2022, Appendix G). In addition, two subsequent studies with food grade TiO_2 in diet (at much higher doses) did not confirm the effects (Blevins *et al.*, 2019; Han *et al.*, 2020b). Also, the NTP 1993 study with Unitane O-220 (most probably similar to food grade TiO_2) was negative. The different results in these studies might indicate that there is a matrix effect of the exposure vehicle on the outcome.

A carcinogenic effect, either tumour initiating or promoting activity of TiO_2, could not be established for dermal exposure.

6.6.6 Mutagenicity / genotoxicity

For background data see the following Annexes:
A-IV.1 Summary of selected genotoxicity papers
A-IV.2 Uptake of TiO_2 by cells
A-IV.3 DNA binding of TiO_2

6.6.6.1 Potential genotoxic hazard of ultrafine TiO_2 particles after inhalation exposure

In their recent Opinion, the EFSA Panel (EFSA, 2021) concluded, after combining the available lines of evidence on different routes of exposure, that TiO_2 nanoparticles have the potential to induce DNA strand breaks and chromosomal damage, but not gene mutations. There are several *in vivo* studies which indicate increased level of DNA damage by the comet assay in lung, liver or blood cells after intratracheal instillation of P25 TiO_2 ultrafine particles (Relier *et al.*, 2017) or rutile TiO_2 ultrafineparticles (Wallin *et al.*, 2017). The interaction between TiO_2 particles and liver DNA extracted from rats after intranasal administration indicated that DNA binding was observed with the TiO_2 ultrafine particles, but not with microforms of TiO_2 (Jin *et al.*, 2013).

6.6.6.2 Potential genotoxic hazard of ultrafine TiO_2 particles after oral exposure

For pigment grade (n=3) and nanoscale (n=3) TiO_2 particles, negative results were obtained after a single oral gavage according to OECD protocols for evaluating *in vivo* micronucleus induction (Donner *et al.*, 2016). However, it was noted by the authors that the exposure to target tissues was likely negligible, as no significant increases in TiO_2 over

controls were measured in blood (48 or 72 h) or liver (72 h) following exposures to 2,000 mg/kg bw TiO_2 that was evaluated for one pigmentary and one nanoscale TiO_2. Therefore, this negative outcome cannot be considered relevant, as exposure of the evaluated target cells could not be demonstrated. Several *in vivo* studies using repeated oral dosing of ultrafine TiO_2 (anatase or rutile forms) provided positive results for chromosomal aberration or micronucleus tests in bone marrow cells in mice (Chakrabarti *et al.*, 2019, Shukla *et al.*, 2014; Sycheva *et al.*, 2011; Grissa *et al.*, 2015; Manivannan *et al.*, 2020) indicating a potential clastogenic mode of action. In the comet assay after oral exposure to ultrafine TiO_2, there are studies with both positive results (Sycheva *et al.*, 2011; Shukla *et al.*, 2014; Grissa *et al.*, 2015; Shi *et al.*, 2015; Manivannan *et al.*, 2020; Murugadoss *et al.*, 2020) and negative results (Bettini *et al.*, 2017; Martins *et al.*, 2017; Jensen *et al.*, 2019). EFSA expressed a concern for E171 TiO_2 as colouring agent for oral use in view of uncertainties regarding possible genotoxic effects (EFSA, 2021).

6.6.6.3 Effect of coating of TiO_2 nanoparticles on genotoxicity

It is well known that the coating of particles can influence their behaviour in biological fluids and no general conclusion can be drawn on the genotoxicity of coated TiO_2 ultrafine particles as a whole, as has been clearly demonstrated for nanoparticles (Charles *et al.*, 2018). For example, Saber *et al.* (2012) found that coated TiO_2 induced DNA damage by the comet assay after TiO_2 instillation in lung lining fluid cells whereas uncoated TiO_2 particles did not. For the safety assessment of coated TiO_2 ultrafine particles, a case-by-case evaluation would be needed. If information on a specific TiO_2 ultrafine particle is incomplete or lacking (*i.e.,* uptake study, micronucleus and mammalian gene mutation test), a worst-case scenario should be assumed – *i.e.,* that the potential positive genotoxic effect of ultrafine TiO_2 is not diminished and even under some conditions could be enhanced.

In vitro, a number of studies demonstrated a modified genotoxic activity when a coating is present on ultrafine TiO_2:

Comet assay:
- in BEAS-2B cells nanosized SiO_2-coated rutile TiO_2 was a less effective inducer of cell toxicity and DNA damage than nanosized anatase or fine rutile (Falck *et al.*, 2009).
- in rat hepatocytes, negatively charged coated rutile TiO_2 (NRCWE03) decreased DNA damage, but on the other hand positively coating (NRCWE02) slightly increased the DNA damaging effect of NRCWE01 (Kermanizadeh *et al.*, 2012).
- in Chinese hamster lung fibroblast cells exposed to the polyacrylate coated ultrafine TiO_2 showed decreased cyto- and genotoxic responses (Hamzeh and Sunahara, 2013).
- in BEAS-2B cells a weak genotoxic effect of the tested TiO_2 (NM-100 anatase, 50–150 nm, uncoated; NM-101 anatase, 5–8 nm, coated; and NM-103 rutile, 20–28 nm, coated) was observed with an induction of oxidised bases for all three materials; of which NM-100 was the most potent (Di Bucchianico *et al.*, 2017).
- in human renal proximal tubule epithelial cells HK-2 positive charge coating (NRCWE02) increased DNA damage comparing to NRCWE01 (no coating) (Kermanizadeh *et al.*, 2013).

Micronucleus test:
- in PBL coated rutile TiO_2 NM-103 (dimethicone) and NM-104 (glycerine) induced the more pronounced effects, comparing to uncoated NM-102 anatase and not active uncoated NM-105 (rutile-anatase) (Tavares *et al.*, 2014).

Micronucleus and Comet assay:
- in A549 cells both coated and uncoated TiO$_2$ were positive, though in some cases the effect after exposure to coated NPs was less pronounced (Stoccoro *et al.*, 2017).

In vivo, Wallin *et al.* (2017) showed in mice that both NRCWE-001 (unmodified rutile TiO$_2$ negative surface charge), and NRCWE-002 (positively charged TiO$_2$) induced increased levels of DNA strand breaks in lung tissue at all doses 1- and 28-days post-exposure and NRCWE-002 at the lowand middle dose 3 days post-exposure. The DNA strand break levels were statistically significantly different for NRCWE-001 and -002 for liver and for BAL cells, but no consistent pattern was observed. After intravenous administration, TiO$_2$ nanoparticle aggregates were observed in the cells and cell nucleus of the liver (Louro *et al.*, 2014). However, a genotoxic effect was not observed using several genotoxicity endpoints in the LacZ plasmid-based transgenic mouse model (micronuclei in peripheral blood reticulocytes, DNA strand breaks by comet assays and gene mutations in spleen and liver).

6.6.6.4 Modes of action for genotoxicity of ultrafine TiO$_2$ particles

There is evidence that several modes of actions for genotoxicity may operate in parallel:

- Uptake of ultrafine TiO2 particles to nucleus was demonstrated by several authors (Jugan *et al.,* 2012; Ahlinder *et al.*, 2013, Shukla *et al.*, 2013, Bettini *et al.*, 2017, Jain *et al.,* 2017, Murugadoss 2020, Kazimirova *et al.*, 2020 and others) but mostly with uncoated ultrafine particles (Annex A-IV.2).
- Direct interaction of ultrafine TiO$_2$ particles with DNA is demonstrated in a number of *in vitro* studies (Zhu *et al*, 2007; Zhang *et al.* 2014; Patel *et al.*, 2016, 2017; Alsudir and Lai 2017; Ali *et al.*, 2018; Hekmat *et al.*, 2013, 2020), which show the affinity of ultrafine TiO$_2$ particles to intercalate or bind with DNA *in vitro*. The precise nature of these interactions, *i.e.,* whether involving covalent or non-covalent binding, has not yet been established (Annex IV Table A-IV.3).
- Zhu *et al* (2007) reported that DNA with P=O and C-O-P groups could be attached onto the surface of TiO$_2$ by chemical adsorption. The type of binding depends on various conditions, e.g. concentration and treating time (Zhu, 2007).
- Two studies (Li *et al.*, 2010; Jin *et al.*, 2013) have indicated that, when administered via intraperitoneal or intranasal routes, ultrafine TiO$_2$ particles (anatase) in high concentrations end up in the liver of the test animals. Anatase ultrafine TiO$_2$ particles were observed to bind to DNA whereas microrutile (fine) TiO$_2$ particles did not (Jin *et al.* 2013). However, the assertion from these studies that TiO$_2$ particles then go further into the nucleus and interact/bind with DNA *in vivo* is questionable. Results from *in vitro* and *in vivo* studies of interaction between ultrafine TiO$_2$ particles and DNA resulted in spectrally contradicting effects indicating hyperchromicity *in vitro* and hypochromicity after exposure *in vivo*. Furthermore, it cannot be excluded that the reported interaction between DNA and ultrafine TiO$_2$ particles determined after *in vivo* exposure using physico-chemical analysis might also be due to the sample preparation method used in these studies (e.g., during the extraction of DNA, co-extraction might have been occurred).
- Direct formation of reactive (oxygen) species, due to intrinsic properties of ultrafine TiO$_2$ particles, might result in chronic inflammation. Reactive (oxygen) species formation can also occur via interference of ultrafine TiO$_2$ particles with mitochondrial function (Braakhuis *et al.*, 2021).
- Additionally, there are indications that ultrafine TiO$_2$ particles may:

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- o induce epigenetic modifications affecting the expression of genes involved in the maintenance of genome function (e.g. downregulation of some genes involved in DNA repair pathways) (Pogribna *et al.*, 2020),
- o interact with proteins involved in the control of chromosome segregation and the spindle apparatus (Magdolenova *et al.*, 2014).

In conclusion, direct binding of ultrafine TiO$_2$ particles to DNA was demonstrated in several *in vitro* studies, e.g. by adsorption, electrostatic attraction, van der Waals and hydrogen bonds, and by covalent bonds (the latter not convincingly described), and suggested in two *in vivo* studies. For micro-particles (fine fraction), it was shown that there was no such interaction with DNA. The relative contributions of the modes of action mentioned above to the genotoxicity elicited by ultrafine TiO$_2$ articles are unknown and there is uncertainty as to whether a threshold dose for any mode of action could be established. Even assuming that all modes of action would be indirect, the available data do not allow identification of a threshold dose.

6.6.6.5 Potential genotoxic hazard of TiO$_2$ non-ultrafine forms

The available data on *in vitro* and *in vivo* genotoxicity of non-ultrafine forms of TiO$_2$ show varying outcomes. In one study on mammalian cell gene mutations (thymidine kinase (Tk) locus in mouse lymphoma cells), the result using micro-TiO$_2$ (no further information available) can be assessed as equivocal (Demir *et al.*, 2017). In the studies assessed on *in vitro* micronucleus test using different cell lines (human peripheral blood mononuclear cells, HEK293, NIH/3T3, SHE, BEAS-2B, HCT116), various non-ultrafine forms of TiO$_2$ have been tested with negative results (Andreoli *et al.*, 2018; Demir *et al.*,2015; Uboldi *et al.*, 2016; Rahman *et al.*, 2002; Falck *et al.*, 2009; Guichard *et al.*, 2012) and some with positive results (Rahman *et al.*, 2002; Proquin *et al.*, 2017).

Studies on *in vitro* comet assay gave negative results on BEAS-2B (Gurr *et al.*, 2005), or HEK293 cells (Demir *et al.*, 2015) as well as positive results using Caco-2 (Proquin *et al.* 2017; Brown *et al.*, 2019; Andreoli *et al.*, 2018; Murugadoss *et al.*, 2020), HepG2 (Brown *et al.*, 2019), HCT116 (Proquin *et al.*, 2017), A549 (Karlsson *et al.*, 2009), BEAS-2B (Gurr *et al.*, 2005 ; Falck *et al.*, 2009 ; Zijno *et al.*, 2020), polymorphonuclear blood cells (PMBC) (Proquin *et al.*, 2017, Murugadoss *et al.*, 2020) or SHE cells (Guichard *et al.*, 2012). In the cell transformation assay on Balb/c 3T3 cells, rutile TiO$_2$ (size of 250-600 nm) induced significant increase in number of transformed colonies (Uboldi *et al.*, 2016). These studies include results obtained with E171 that is known to contain both an ultrafine and non-ultrafine (fine, pigmentary) fraction of TiO$_2$ (EFSA, 2021).

For pigment grade (n=3) TiO$_2$ particles, negative results were obtained after a single oral gavage according to OECD protocols for evaluating *in vivo* micronucleus induction (Donner *et al.*, 2016). However, target cell exposure to TiO$_2$ *in vivo* could not be established. *In vivo* testing in mice exposed orally to 40 or 200 mg/kg of TiO$_2$ microparticles resulted in positive bone marrow micronucleus (Sycheva *et al.*, 2011). For TiO$_2$ anatase particles, anatase (Unitane® 0-220, particle size > 100 nm) in B6C3F1 mice treated by the intraperitoneal route, an equivocal effect was observed in a micronucleus test in bone marrow cells (Shelby *et al.*, 1993). In the *in vivo* comet assay in Wistar rats treated by gavage with E171, no increased level of DNA damage was indicated in the cells isolated from the Peyer' patches (Bettini *et al.*, 2017). Similarly, no increased DNA damage was observed in liver and lung cells in rats treated with E171 by gavage (Jensen *et al.*, 2019). However, studies on mice show induction of DNA breaks after exposure to TiO$_2$ microparticles in bone marrow (Sycheva *et al.*, 2011) and BAL cells (Saber *et al.*, 2012). In the *in vivo* study after intranasal application of TiO$_2$ (rutile, <5 µm) no DNA binding of TiO$_2$ was detected in the liver cells (Jin *et al.*, 2013).

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In conclusion, for non ultrafine TiO$_2$ grades > 100 nm the results indicates both negative and positive outcomes *in vitro* and *in vivo* regarding genotoxicity. However, in these positive studies either a nanofraction was present, or information on the absence of such a nanofraction was not presented.

6.6.6.6 Conclusions on genotoxicity

1. The possible oral exposure conditions of children to TiO$_2$ particles are quite specific – generally this is not exposure with diet but rather as a result of biting, chewing and/or licking, *i.e.,* after suspension in saliva. So, food matrix effects can rather be excluded. As discussed in Section 6.2.11, limited industry data show size ranges for only a few pigmentary TiO$_2$ products as used in toys with size ranges between 141nm to 39811nm. Although the information provided indicates that an ultrafine fraction would not be present to a significant degree for the pigmentary TiO$_2$ as used in toys and toy materials, the information on particle sizes and particle size distribution of pigmentary TiO$_2$ used in toys, has been very limited. Only for 3 TiO$_2$ preparations information on size was provided. The WoE for the particle sizes of pigmentary TiO$_2$ used in toys, and the absence of an ultrafine fraction is considered weak.

2. As discussed in Section 6.6.5.5, an indication for induction of ACF in the colon of animals was observed after exposure to food grade TiO$_2$ (E171) in drinking water (Bettini, 2017 - dispersed in drinking water at a human relevant dose (~10 milligrams per kilogram of body weight per day, mg/kg bw/d, for 100 days). The relevance of the study for conclusions on carcinogenicity, however, is limited. It is still debated whether ACF is an early expression of a pre-neoplastic lesion (the predictive value of ACF as a biomarker of colorectal cancer in rodents and humans is controversial; for a comprehensive discussion on ACF as a potential biomarker of colorectal cancer, see Appendix G of the State of the Science of Titanium Dioxide (TiO$_2$) as a Food Additive by Food Directorate, Health Canada, June 2022). In addition, two subsequent studies (Blevins *et al.* 2019; Han *et al.* 2020b) with food grade TiO$_2$ in diet (at much higher doses) did not confirm the effects. Also, the NCI (1979) study with TiO$_2$ preparation Unitane O-220 was negative. The different results in these studies might indicate that there is a matrix effect of the exposure vehicle on the outcome. In Section 6.6.5.5. it was concluded that the available studies after oral exposure are not sufficient to draw conclusions on the potential carcinogenicity of TiO$_2$ particles. However, the induction of oxidative stress and inflammation in the GIT indicates a possible indirect or promoting effect of TiO$_2$ on tumour development. The WoE for tumour-promoting activity of TiO$_2$ particles in the GIT is moderate, whereas the WoE for tumour induction in the GIT is uncertain to weak.

3. SCHEER performed analysis of genotoxic effects of a broad range of TiO$_2$ (ultrafine and fine, coated and uncoated particles) based on *in vitro* and *in vivo* endpoints. This is an important difference compared to the analysis of Health Canada (2022) and Food Standards Australia/New Zealand (2022) who did not consider *in vitro* and *in vivo* studies on non-food-grade TiO$_2$ with a mean diameter of < 100 nm as being relevant for the hazard characterization of food-grade TiO$_2$.

4. SCHEER considered using sonicated TiO$_2$ particles suspensions as relevant for genotoxicity assessment. Health Canada (2022) and Food Standards Australia/New Zealand (2022) considered studies where sonication was used as being of less importance or not relevant.

5. SCHEER agrees with Health Canada (2022) conclusions on the *in vivo* studies with TiO$_2$ nanoparticles on the observed induction of DNA and chromosome damage as presented in Table 10 of the Health Canada report (page 145). Health Canada did not include

these studies in their final evaluation, as this evaluation was limited to E171, *i.e.,* TiO$_2$ used as food additive. SCHEER evaluated studies using various types of TiO$_2$, including nano (ultrafine) TiO$_2$.

6. SCHEER analysis of *in vitro* studies, especially on DNA damage (comet assay and chromosomal aberrations), with TiO$_2$ NPs reveals many studies indicating a genotoxic potential. The genotoxic potential was also demonstrated using various different methods (e.g. H2AX, gene alterations, sister chromatid exchange). The SCHEER analysis identifies more studies on genotoxic effects as relevant compared to the studies as indicated in the Health Canada report.

7. Direct binding of TiO$_2$ with DNA was demonstrated in several *in vitro* studies and in two *in vivo* studies for ultrafine TiO$_2$ particles. For micro-particles (fine fraction), it was shown that there was no such interaction with DNA.

8. Detailed analysis by SCHEER of test results of *in vitro* genotoxicity studies with TiO$_2$-NPs (similar to the nanoparticles present in E171) as well as other TiO$_2$ grades with a mean diameter of > 100 nm indicates both negative and positive outcomes in different cell lines. For most of the positive studies, the presence of a nanofraction was demonstrated or information on the absence of a nanofraction was not presented. For a number of studies, positive genotoxicity in the comet assay was observed, for which the presence of a nanofraction was considered unlikely, although information on the presence or absence of a nanofraction was not presented.

9. Detailed analysis by SCHEER of test results of *in vivo* genotoxicity studies with TiO$_2$-NPs (similar to the nanoparticles present in E171) as well as other TiO$_2$ grades with a mean diameter of > 100 nm indicates, in the majority of cases, negative or inconclusive results in animals after exposure via different routes. One paper showed positive response (on comet assay) for DNA damage after intratracheal installation of ultrafine and fine TiO$_2$ in mice. However, in the fine TiO$_2$, a nanofraction is likely to be present (Saber *et al.,* 2012).

10. A gene mutation effect was not demonstrated although a genotoxic effect based on DNA damage by TiO$_2$ in both ultrafine and non-ultrafine forms was demonstrated in several *in vitro* or *in vivo* studies (as concluded also by EFSA FAF Panel, 2021). In a weight of evidence approach Kirkland *et al.*, (2022) concluded that TiO$_2$ did not have a gene mutation effect. However, DNA damaging effects observed in *in vitro* comet assay studies were excluded in their evaluation. More robust *in vitro* and *in vivo* genotoxicity studies were considered to be needed for definitive conclusions (Kirkland *et al.,* 2022).

11. SCHEER in this Opinion and previously Elespuru *et al.* (2018, 2022) noted that generally many genotoxicity studies did not meet a number of quality criteria of a valid test. Therefore, there exists uncertainty in the outcomes of these studies.

12. Overall, there may be a risk for genotoxicity due to TiO$_2$ exposure both after inhalation and oral uptake. A substantial proportion of genotoxicity studies on ultrafine TiO$_2$ indicates a genotoxic potential (both chromosomal aberrations/MN test and comet assay). In contrast, a majority of studies on chromosomal aberrations/MN test (basic mutagenic endpoints) are negative for fine TiO$_2$. However, for fine TiO2, the comet assay was observed to be positive in many studies. In most positive genotoxic studies with fine TiO$_2$ particles either a nanofraction was present or could not be excluded. The *in vivo* genotoxic effects were observed in very limited number of the studies. The relative contributions of the postulated modes of action to the genotoxicity elicited by

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TiO₂ (ultrafine) particles are unknown (neither primary not secondary mechanisms can be excluded) and there is uncertainty as to whether a threshold is present whether expressed as dose or particles size for any mode of action. No clear correlation is observed between the physicochemical properties of TiO₂ particles, such as crystalline form, size and content of constituent particles, shape and agglomeration state, and the outcome of either *in vitro* or *in vivo* genotoxicity studies. There is some evidence for internalisation of TiO₂ ultrafine particles in the nucleus and mitochondria. An overview of the evaluated studies is presented in Annex IV, A-IV.2.

13. Considering that there are several uncertainties regarding the genotoxicity of TiO₂ particles (existence of both positive and negative results with different endpoints, varying quality of the studies in terms of the characterisation of the TiO₂ used in studies) the WoE for genotoxicity is weak to moderate. For inhalation exposure, the WoE of genotoxic hazard of TiO₂ is moderate, based on the high quality, but relatively low consistency of the results. However, for oral exposure, the WoE of genotoxic hazard of TiO₂ is weak.

Although a threshold for TiO₂ size for induction of genotoxic effects cannot be established at the moment, it can be observed that in studies on TiO₂ in nanosize the results (mainly *in vitro* studies) show higher probability of positive response than in studies on microsize or with sizes slightly above 100 nm. It is possible that a probability of a genotoxic effect diminishes as the size of TiO₂ increases, and the observed positive effects can depend on the presence of a nanofraction. The potential genotoxicity of pigmentary fine TiO₂, including the demonstration of the absence of a nanofraction, remains uncertain. Overall, based on the results of *in vitro* and *in vivo* genotoxicity studies, the SCHEER is of the opinion that the pigmentary fine TiO₂ grades can be considered to have no genotoxic potential after oral and inhalation exposure, provided the presence of a nanofraction can be excluded.

6.7 Risk assessment

6.7.1 Introduction

Following the mandate from the European Commission, this scientific Opinion evaluates whether the uses of titanium dioxide in toys and toy materials can be considered to be safe in light of the exposure identified, and in light of the classification of titanium dioxide as carcinogenic category 2. Safe toys and toy materials, for which derogation is possible, should be indicated. The risk assessment below is based on the exposure to TiO₂ after possible release from toys, for which information was provided by TIE (TIE, 2020a and b). It follows the inhalation and oral exposure assessment in Sections 6.3 and 6.4 and the Points of Departure (PoD),) for toxic effects, identified in Sections 6.5 and 6.6., based on the dose response and toxicological information as presented in the open literature. In the risk characterisation, conclusions are drawn based on the estimated Margins of Safety (MoS) for children of 23 months, 6 and 8 years and on the overall Weight of Evidence (WoE). First, relevant previous hazard and risk assessments are summarised to provide context for the SCHEER assessment.

6.7.2. Previous risk assessments of TiO₂

SCCS, 2020 (inhalation exposure to cosmetics)

The SCCS is of the opinion that the CMR2 classification of TiO₂ cannot be disputed because of an official body's conclusion on its classification and subsequent inclusion in the CLP regulation by the Commission. In the absence of a conclusive evidence to suggest otherwise, the position therefore remains that the carcinogenic effects observed in animals

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are also possible in humans. Although the detailed mode of action is still unclear, an inflammatory process and indirect genotoxic effect by ROS production seems to be the major mechanism to explain the effects induced by TiO_2. It is considered that this mode of action is principally due to the biopersistence and poor solubility of the TiO_2 particles. However, a genotoxic effect by direct interaction with DNA cannot be excluded since TiO_2 was found in the cell nucleus in various *in vitro* and *in vivo* studies.

According to the SCCS, the margin of safety (MoS) should be calculated based on the toxicological point of departure derived from the Bermudez *et al.* (2004) study, using 0.5 mg/m^3 as the NOAEC.

The Bermudez *et al.* (2004) study used P_{25}, which is comprised of uncoated ultrafine particles of a mixture of 80% rutile and 20% anatase forms of TiO_2. The SCCS considered it relevant for the assessment of pigmentary TiO_2 materials in cosmetics because these contain a significant fraction of ultrafine-scale particles that are most important to consider in the estimation of inhalation exposure of the alveolar region of the lungs.

For estimating spray / powder exposure, the SCCS calculated the human deposition value (after time adjustment to chronic exposure) using the MPPD software (v3.4). A dosimetric adjustment factor (DAF) was used to convert the time-adjusted point of departure to a continuous-exposure human equivalent concentration (HEC) based on species-specific information on deposition, pulmonary surface area, and breathing volume. The DAF is used to extrapolate the deposition of TiO_2 particles in the rat lung, to the possible deposition of TiO_2 particles in the human lung. The SCCS considers it important to take the fractional deposition into account because of the concerns for the ultrafine-scale fraction reaching the alveoli. In the SCCS Opinion, the relevant dose metric is the deposition in the pulmonary region (pulmonary deposited dose) and not the inhalable fraction.

A Margin of Safety is calculated from the TiO_2 pulmonary deposited dose based on the NOAEC and the measured pulmonary deposited doses, for which a MoS above 25 can be considered safe for both hairdressers and consumers. This was based on 'a factor of 2.5 (toxicodynamic difference between rats and humans) and a factor of 10 for interindividual variability among consumers and hairdressers' using the TiO_2 containing sprays for the safety calculation. In the opinion of SCCS, hairdressers are also consumers and this explains the factor of 10 for these workers. In the SCCS's opinion, the use of pigmentary TiO_2 in a typical hair styling aerosol spray product is safe up to a maximum concentration of 1.4 % for general consumers, and 1.1 % for hairdressers (SCCS, 2020).

ANSES, 2020 (inhalation exposure)

ANSES derived occupational and general population exposure limits for the ultrafine form of TiO_2. It was concluded that TiO_2-NP is a weak genotoxic substance, with effects appearing only at high doses but showing a dose-response in a number of positive studies. Carcinogenic effects, as evidenced by lung tumours, appear only at high concentrations, associated with altered clearance and inflammatory response. ANSES concluded that based on the available data, a threshold approach is considered to be the most relevant approach to derive the reference values. The Bermudez *et al.* (2004) study with rats was selected as the key study for the establishment of the TiO_2-NP reference values. The NOAEC of 0.5 mg/m^3 was chosen as the point of departure and converted to a human equivalent concentration. The reference value of 0.80 $\mu g/m^3$ was then calculated applying assessment factors for inter and intra-species variability, for subchronic to chronic transposition and for the inadequacy of the database.

TIE (2020a) (inhalation exposure)

This risk assessment, commissioned by the Toy Industries of Europe, considers the effects of pigmentary TiO_2 on the principal target organ for non-neoplastic effects following

inhalation, the lung, with pulmonary effects including lung epithelial cell injury, cholesterol granulomas and fibrosis being reported in experimental animals following sub-chronic and chronic exposures. The appearance of neoplastic lesions in the lung was argued to be based on lung overload due to exposure concentrations being above the maximum tolerated dose, leading to impairment of alveolar macrophage-mediated clearance and a known particular sensitivity of rat lung to inhaled particles, including TiO_2. The MoA was described as a cascade of events from particle uptake by alveolar macrophages, neutrophil activation, production of ROS and induction of anti- and pro-inflammatory changes and inflammation. The applicability of these findings to humans was still considered to be debatable. Pigment grade TiO_2 was argued to be non-genotoxic and non-mutagenic and robust, but with occupational epidemiology studies showing no link between increased risk of lung cancer following occupational exposure to pigment grade TiO_2. The risk assessment followed a threshold approach and established a DNEL based on an inflammatory endpoint as an initial event in lesion formation which would be protective for all later pathological changes. The PoD was selected from the study of Muhle *et al.* (1991) with a NOAEC of 5 mg/m^3. This NOAEC was converted to a 24 h exposure value of 888 $\mu g/m^3$ (5x6/24x5/7) and then to a DNEL of 35.5 $\mu g/m^3$ applying assessment factors for interspecies differences (2.5) and intraspecies variation (10). Estimates of exposure were calculated according to guidelines from ECHA and RIVM (Van Engelen *et al.*, 2008) for a number of toy products containing pigment grade TiO_2 at a range of concentrations, the use of which had been assessed as generating the potential for inhalation exposure. Risk characterisation was carried out using the Risk Characterisation Ratio (RCR) based on the estimated exposure and the exposure time adjusted DNEL (e.g. 35.5 x24/0.045) for the use of a toy 0.27x per day for 10 minutes = 0.045 h per day).

Using this approach, the RCR was found to be <1 for the majority of toy products and usage scenarios considered, indicating that the risk posed by potential inhalation of TiO_2 from the use of toys is minimal. The exception to this was the use of powder paints under worst-case conditions, which had a RCR of 9.1 and TIE concluded that there may be a risk associated with such use. The typical use scenario for powder paints had an RCR of 0.44.

EFSA, 2021 (oral exposure)

EFSA re-evaluated the safety of the food additive titanium dioxide (E171). EFSA considered that studies with TiO_2 NPs < 30 nm were of limited relevance to the safety assessment of E171. EFSA concluded that, although gastrointestinal absorption of TiO_2 particles is low, they may accumulate in the body. Studies on general and organ toxicity did not indicate adverse effects with either E171 up to a dose of 1,000 mg/kg body weight per day or with TiO_2 NPs (> 30 nm) up to the highest dose tested of 100 mg/kg bw per day. No effects on reproductive and developmental toxicity were observed up to a dose of 1,000 mg E171/kg bw per day, the highest dose tested in the EOGRT study available. It was noted that some observations (potential immunotoxicity, potential inflammation and potential induction of aberrant crypt foci with E171 and potential neurotoxicity with ultrafine TiO_2 particles) may indicate adverse effects. With respect to genotoxicity, it was concluded that TiO_2 particles have the potential to induce DNA strand breaks and chromosomal damage, but not gene mutations. No clear correlation was observed between the physico-chemical properties of TiO_2 particles and the outcome of either *in vitro* or *in vivo* genotoxicity assays. Concerns for the genotoxicity of TiO_2 particles that may be present in E171 could therefore not be ruled out. Several modes of action for the genotoxicity may operate in parallel. There was uncertainty as to whether a threshold mode of action could be assumed. A cut-off value for TiO_2 particle size with respect to genotoxicity could not be identified. The final conclusion was that E171 can no longer be considered as safe when used as a food additive.

Food Standards Agency UK. *(COT position paper January 2022) (oral exposure)*

Early 2022 the Committee on Toxicity (COT) and the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COM) of the UK Food Standards Agency (FSA UK, 2022) presented an interim position paper on titanium dioxide that included an overview of the various evaluations of TiO_2 authorized as food additive E171 in the EU. The TiO_2 review was initiated by the 2021 evaluation of TiO_2 as E171 food additive by the EFSA Panel on Food Additives and Flavourings (FAF) that additionally was endorsed by EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). Both UK FSA committees summarised and evaluated the 2021 EFSA Opinion on E171, with a particular focus on the endpoints related to genotoxicity. The COM questioned the quality of the dataset and robustness of some of the studies used by the EFSA panel to draw its conclusions and noted that the overall data considered by EFSA were heterogenous. Regarding the mode of action for genotoxicity, the COM agreed that the evidence indicated an indirect interaction with DNA with a threshold for genotoxicity. The COT were in agreement with the COM's view and further noted the large discrepancy between the underlying dataset and the conclusions drawn by EFSA. Both COM and COT considered the EFSA conclusions not justifiable based on the available evidence. Based on the outcome of the COM and COT evaluations, the UK FSA has now initiated a review on the safety of TiO_2 as food additive that might be available early 2023.

Health Canada (2022) (oral exposure)

Health Canada summarised the state of the science concerning the carcinogenicity of titanium dioxide (TiO_2) used as a food additive. The adverse effects associated with oral exposure to TiO_2 are noted to be largely derived from non-standard studies that administered stable, homogenised suspensions of ultrasonically dispersed particles. The initial concerns with human exposure to TiO_2 particles arose in part from a non-guideline rat study funded by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) in which animals were exposed to food-grade TiO_2 dispersed in drinking water at a human relevant dose (~10 mg/kg bw/d) for 100 days (Bettini *et al.,* 2017). TiO_2 particles were reported to have accumulated in Peyer's patches and exposed animals developed large aberrant crypt foci (ACF) at higher rates than unexposed controls. However, the findings of ACF in the colon by Bettini *et al.* have not been replicated in subsequent studies, even at doses orders of magnitude higher. While the intensive sample preparation steps are necessary and appropriate for particle characterisation and hazard identification for nanoscale materials in general, in the opinion of Health Canada's Food Directorate they do not fully represent exposure to TiO_2 as a constituent of food. Several negative studies in which food-grade TiO_2 was administered via the diet were accorded the highest weight in this review. In addition, the available evidence indicates food-grade TiO_2 is not genotoxic *in vivo*, although the number of studies available is limited and more research is recommended to confirm these findings. Overall, Health Canada's Food Directorate did not identify any compelling health concerns for the use of TiO_2 as a food additive. While some uncertainties in the database were identified that would benefit from further research, Health Canada concluded that the weight of available evidence suggests these data gaps are not significant enough to warrant a more precautionary approach at this time.

Food Standards Australia – New Zealand (FSANZ, 2022) (oral exposure)

Food Standards Australia – New Zealand (FSANZ) also evaluated the carcinogenicity of TiO_2 used as food additive. FSANZ commissioned a review a number of years ago to consider the potential health risks associated with TiO_2 and other food additives containing particles in the nanoscale. The review, published in 2016, concluded there was insufficient evidence at that time to support a risk assessment. The recent evaluation largely corroborates the above findings of Health Canada. In addition, it was observed that the recently conducted extended one-generation reproductive toxicity study in rats with food-

grade TiO_2 administered via the diet at doses up to 1,000 mg/kg bw.day found no evidence of systemic toxicity, developmental or reproductive toxicity, developmental neurotoxicity or developmental immunotoxicity. Based on the data currently available, FSANZ concludes there is no evidence to suggest that dietary exposures to food-grade TiO_2 are of concern for human health.

6.7.3 Exposure assessment

Application of TiO_2 in toys

Titanium dioxide can be used in toys in a number of ways, including as a pigment in craft materials (e.g. chalks, pencils, etc.), as a pigment in paints applied to toys, and in polymeric toy materials (e.g. acrylonitril-butadiene-styrene, polyethylene, polypropylene, polyvinyl chloride, etc.). According to information from Toy Industries of Europe (TIE), the TiO_2 content in polymeric toy materials varies between 1 to 10% (of the total material). In other toys or toy materials, concentrations generally range up to 30% with the highest levels in white pencils containing up to 51% TiO_2. The TiO_2 used in all toy products is pigment grade, with no deliberate use of ultrafine TiO_2. Based on the information provided by the toy pigment industry using titanium dioxide on two different white pigments, a size below 10 µm, the size indicated in EU 2020/217 for a carcinogenic hazard, may be present in the white pigments. In addition, the presence of an ultrafine fraction in the pigments cannot be excluded. However, the provided data (TIE, 2020a) suggest that such an ultrafine fraction would not be present to a significant degree.

When TiO_2 is used as a colouring agent in polymers used to manufacture toys, the TiO_2 is fixed within the polymer matrix. Release of TiO_2 from this matrix is considered unlikely. Potential exposure is only possible when pieces of the toy break off due to mouthing (see oral exposure below).

Inhalation exposure

Table 6.12 summarises the results of the inhalation exposure assessment of Section 6.4.2 that will be used for risk assessment for children playing with toys containing TiO_2.

Table 6.12: Inhalation exposure results for the four selected scenarios

Scenario	Duration (min)	Air conc. PM_{10}-TiO_2 "realistic high" (µg/m³)	Air conc. PM_{10}-TiO_2 "upper bound" (µg/m³)
1. Casting kit	10	347	695
2. Chalk	45	-	42.5
3. White colour pencil	45	-	10
4. Powder paint	10	23.2	579

Oral exposure

The following data will be used for the risk assessment after oral exposure for children playing with toys containing TiO_2:

Table 6.13: Oral exposure results for the three selected scenarios

Scenario	Frequency	Exposure (mg TiO_2/day)
1 Finger paint	1x (single event)	120
	18x per year	5.9
2. White colouring pencil	2x per day	8.2

| 3. Lipstick | 1x per day | 2.0 |
| Aggregated oral exposure | repeated exposure | 16.1 |

Aggregated TiO₂ exposure from toys (for definition: see Glossary)

Exposures to different toys on the same day may occur and may be aggregated per route of exposure (see Glossary for definition). Aggregated oral exposure was considered for the three oral exposure scenarios. This aggregated oral exposure is: 16.1 mg TiO₂/day. Concomitant oral and inhalation exposure is likely. However, the oral exposure resulting from the lung clearance and transport by the mucociliary escalator is rather low, and even orders of magnitude lower in view of the high oral exposures in the evaluated scenarios. Therefore, an aggregated oral exposure including the mucocilliary route was considered not relevant. In addition, the inhalatory and oral routes may result in exposure of different target organs. For inhalation, aggregation of different exposure sources is not relevant, since the exposures will not occur simultaneously, and the endpoint is a concentration-related effect (inflammation).

Although the evaluated exposure scenarios have the highest possibility for TiO₂ exposure, it should be noted that the use of some toys containing TiO₂ that were not evaluated in this Opinion may possibly result in exposure for children. Other sources of TiO₂ exposure, e.g., via food, cosmetics etc., were not considered.

6.7.4 Toxicological Point of Departure

6.7.4.1 Introduction

For both inhalation and oral exposure, adverse effects of exposure to TiO₂ particles could be identified, including direct effects such as lung and GIT oxidative stress and inflammation and indirect effects such as altering the immune system responses.

A genotoxic effect of TiO₂ in both ultrafine and non-ultrafine forms was demonstrated in several *in vitro* or *in vivo* studies, indicating a potential for chromosomal damage.

Therefore, there may be a risk for genotoxicity due to TiO₂ exposure (both ultrafine and non-ultrafine forms) both after inhalation and oral uptake. The WoE for genotoxicity is, however, weak to moderate.

TiO₂ released from toys will probably have unknown surface modifications and may also contain a fraction of particles in the ultrafine scale. Specifically, the ultrafine form could undergo internalisation and potentially induce a genotoxic effect (a genotoxic effect was also noted for the non-ultrafine form that had not undergone internationalisation).

6.7.4.2 PoD for inhalation

Although there is limited evidence in epidemiological studies for the induction of lung cancer in occupational settings, recent re-evaluation of epidemiological studies applying more sophisticated statistics revealed a HWSE for occulational exposure (Canu *et al.,* 2022). These results in combination with various animal studies clearly indicate a possible carcinogenic effect of TiO₂ in the lung after inhalation exposure. From the available rodent studies and the AOP suggested, the mechanisms by which TiO₂ can induce lung tumours in rats after inhalation can operate via impaired clearance and persistent inflammation. Therefore, also considering the moderate WoE for genotoxic potential of a possible nanofraction after inhalation exposure, the SCHEER concludes that the PoD for inhalation carcinogenicity can be based on a threshold for these effects as proposed by the SCCS (2020).

The short-term PoD was selected based on Bermudez *et al.* (2002, 2004): the NOAECs in these studies were determined to be 0.5 mg/m^3 for ultrafine TiO$_2$ and 10 mg/m^3 for fine TiO$_2$.

6.7.4.3 PoD for oral exposure

The available studies after oral exposure are not sufficient to draw firm conclusions on the potential carcinogenicity of TiO$_2$ particles. The induction of oxidative stress and inflammation in the GIT indicates a possible indirect or promoting effect of TiO$_2$ on tumour development. Therefore, also considering the weak WoE for genotoxic potential of a possible nanofraction after oral exposure, the SCHEER concludes that the PoD for oral carcinogenicity can be based on the threshold for these effects. However, a cut-off value for TiO$_2$ particle size with respect to the genotoxicity as reported could not be identified.

The SCHEER agrees with the EFSA that there are no studies appropriately designed and conducted to investigate the potential oral carcinogenicity of TiO$_2$ (ultrafine) particles. Repeated dose toxicity studies on general and organ toxicity (Warheit *et al.*, 2015) and reproductive and developmental toxicity with E171 (EFSA, 2021) did not indicate adverse effects up to a dose of 1,000 mg/kg bw per day. For possible effects of TiO$_2$ as E171 at lower doses, such as indications for immunotoxicity, inflammation as well as neurotoxicity, uncertainties were noted (EFSA 2021). This was found in oral studies with both pigmentary and ultrafine TiO$_2$. The oral LD50 for the TiO$_2$ was found to be > 5,000 mg/kg bw. Therefore, the SCHEER concluded that for the risk assessment, the PoD of 1,000 mg/kg bw per day would be used for repeated exposure. For a single event the POD used is 5,000 mg/kg bw, being the lowest possible value for the LD50 in rats.

6.7.5 Human Equivalent Concentrations (HEC)

For deriving the human equivalent concentration (HEC) at inhalation, a dosimetric adjustment factor (DAF) was used to convert the rat 6-h NOAEC of 0.5 mg/m^3 (from Bermudez *et al.,* 2004) to a 24-h HEC based on species-specific information on deposition, pulmonary surface area, and breathing volume (See Table 6.14 and details in Annex V). The DAF was calculated as the ratio of the steady state load/lung surface area of the rat and the steady state load/lung surface area of humans. Deposition per pulmonary surface area is the key dose metric for inflammatory effects. The DAF was calculated using the Multi Pathway Particle Deposition (MPPD) v3.04. to estimate the pulmonary deposition fraction to bothhuman and rat lungs.

The HEC is an exposure calculation based on the deposition of particles in humans in which the toxicokinetic part is limited to the deposition process in the lung. It is assumed that the effects in the lung (*i.e.*, accumulation of particles as initiating effect, leading to overwhelming of the clearing capacity, persistent inflammation and subsequently systemic effects, see Section 6.2.2) are the only adverse effects and that TiO$_2$ that is absorbed and systemically available does not cause any other adverse effects.

The 24h-HEC was calculated based on the NOAEC without adjustment for the duration of the study exposure period of 90 days – semi-chronic), since children are considered to be exposed for a short period of time only. HECs were calculated for children at specific ages (23 months, 3 and 6 years, as indicated in the MPPD model v3.04) by applying a DAF to the NOAEC used as the POD. Table 6.14 shows time-adjusted HECs for 10 and 60 minutes based on the NOAEC of 0.5 mg/m^3 for ultrafine TiO$_2$. This time adjustment is based on the assumption that concentration x time is constant. Results are shown with and without taking the difference in elimination from the lung (alveolar clearance) between rats and humans into account. If this difference is taken into account, this is based on a half-time for particles of 60 days in rat, and 400 days in human. Elimination constants are calculated as follows:

Formula 4:

Elimination constant = − ln(0.5)/ half-life (MAK 2020, ANSES 2019)

Formula 5:

in rat; *elimination constant = -(ln0.5)/60 = 0.0116/day*

Formula 6:

In human; *elimination constant= -(ln0.5)/400 = 0.00173/day*

Also, a factor of 5/7 was applied to compensate for 5 days per week exposure in the animal studies, and 7 days per week exposure for children.

Table 6.14: HECs at ages of 23 months, 3 and 6 years derived from the NOAEC for ultrafine TiO$_2$ of 0.5 mg/m^3 (See Annex III)

	23 months		3 years		6 years	
	-	+	-	+	-	+
10 minutes adjusted HEC (mg/m^3.day)	10.9	1.6	11.6	1.7	11.4	1.7
60 minutes adjusted HEC (mg/m^3.day)	1.9	0.3	1.9	0.3	1.9	0.3

 - = not corrected for human elimination, + = corrected for human elimination

The human equivalent concentration (HEC) was calculated using the same method to convert the rat NOAEC of 10 mg/m^3 for pigmentary fine TiO$_2$ (from Bermudez *et al.,* 2002). The simulation was performed using whole body exposure and with the following particle properties: density (4.3g/cm^3), MMAD (1.44µm) and GSD (1.71). See Table 6.15.

Table 6.15: HECs at ages of 23 months, 3 and 6 years derived from the NOAEC for pigmentary fine TiO$_2$ of 10 mg/m^3 (See Annex III)

	23 months		3 years		6 years	
	-	+	-	+	-	+
10 minutes adjusted HEC (mg/m^3.day)	233.5	34.8	248.1	37.0	240.0	35.8
60 minutes adjusted HEC (mg/m^3.day)	38.9	5.8	41.4	6.2	40.0	6.0

 - = not corrected for human elimination, + = corrected for human elimination

6.7.6 Risk characterisation

6.7.6.1 TiO2 as colouring agent for polymers

The application of TiO$_2$ as a colouring agent for polymers used to produce toys is considered to pose no or negligible risk to children, as the potential release of the TiO$_2$ from the polymers is considered negligible to non-existent due to the fixation of the TiO$_2$ within the polymer. Potential exposure is only possible when pieces of the toy break off due to mouthing (see oral exposure below).

6.7.6.1 Inhalation

The MoS was derived by comparing the HEC to the exposure estimate. It was calculated for the estimated realistic high exposure and the estimated upper-bound (*i.e.*, worst-case) exposure and with and without taking into account pulmonary clearance (elimination). For chalk and white colour pencil, no realistic high scenario estimate was calculated. The calculation was performed for both Bermudez *et al.* studies: for ultrafine TiO_2 with NOAEC= 0.5 mg/m^3 (see summary in Table 6.16 for realistic high exposure) and for fine TiO_2 with NOAEC= 10mg/m^3) (see summary in Table 6.17 for realistic high exposure).

Table 6.16: MoS* calculated for the 4 inhalation scenarios at realistic high exposure to ultrafine TiO_2 (NOAEC = 0.5 mg/m^3)

Scenario	Duration (min)	23 months		3 years		6 years	
		-	+	-	+	-	+
1 Casting kit	10	32	**4.7**	33	**5.0**	33	**4.9**
2 Chalk	45	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3 White colour pencil	45	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4 Powder paint	10	472	71	501	75	493	74

* - = not corrected for human elimination, + = corrected for human elimination; a MoS >= 25 is considered safe (see explanation below), n.d. not determined.

Table 6.17: MoS* calculated for the 4 inhalation scenarios at realistic high exposure to fine TiO_2 (NOAEC = 10 mg/m^3)

Scenario	Duration (min)	23 months		3 years		6 years	
		-	+	-	+	-	+
1 Casting kit	10	673	100	715	107	692	103
2 Chalk	45	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3 White colour pencil	45	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4 Powder paint	10	10064	1501	10696	1595	10346	1543

* - = not corrected for human elimination, + = corrected for human elimination; a MoS >= 25 is considered safe (see explanation below), n.d. not determined.

For upper-bound estimates, the MoS-values are as presented below.

Table 6.18: MoS* calculated for the 4 inhalation scenarios at upper-bound exposure to ultrafine TiO_2 (NOAEC = 0.5 mg/m^3)

Scenario	Duration (min)	23 months		3 years		6 years	
		-	+	-	+	-	+
1 Casting kit	10	**16**	**2.3**	**17**	**2.5**	**16**	**2.5**
2 Chalk	45	44	**6.5**	46	**6.9**	45	**6.7**

| 3 White colour pencil | 45 | 185 | 27 | 196 | 29 | 193 | 29 |
| 4 Powder paint | 10 | **19** | **2.9** | **21** | **3.1** | **20** | **3.0** |

[*] - = not corrected for human elimination, + = corrected for human elimination; a MoS >= 25 is considered safe (see explanation below)

This upper-bound exposure for ultrafine TiO_2 in several exposure scenarios results in a MoS that is below 25 when calculated including elimination from the lung: for scenario 1 casting kits, for scenario 2 chalk, and for scenario 4 powder paint for all age groups. For white pencils for all age groups, the MoS is above 25, although barely when elimination is included.

Table 6.19: MoS[*] calculated for the 4 inhalation scenarios at upper-bound exposure to fine TiO_2 (NOAEC = 10 mg/m^3)

Scenario	Duration (min)	23 months		3 years		6 years	
		-	+	-	+	-	+
1 Casting kit	10	336	51	357	53	345	52
2 Chalk	45	921	137	979	146	947	141
3 White colour pencil	45	3951	589	4200	626	4063	606
4 Powder paint	10	409	61	434	65	420	63

[*] - = not corrected for human elimination, + = corrected for human elimination; a MoS >= 25 is considered safe (see explanation below)

The upper-bound exposure results for fine TiO_2 in lowest MoS for the casting kit to be 51, for the chalk in a lowest MoS of 137, for the white colour pencils a lowest MoS of 589, and for the powder paint in a lowest MoS of 61, all scenarios for the age at 23 months and the risk calculated including elimination.

The MoS should be evaluated with consideration of the uncertainties in its derivation:
- Toxicodynamic differences rat-humans (factor of 2.5): toxicokinetic differences have already been taken into account in the extrapolation from the rat-NOAEC to a HEC using the DAF and the elimination rate.
- Intraspecies variability in the human population (factor of 10)
- Uncertainties with regard to the exposure assessment (addressed by the realistic high – upper-bound range).

The MoS therefore should be minimally 25 in order to consider an exposure to be safe (*i.e.*, having no or negligible risk for toxic effects).

It can be concluded that, based on the realistic high exposure estimates for ultrafine TiO_2 and the time-adjusted HECs, the MoS-values as such indicate safe use for exposure scenario 4 for powder paints, but not for exposure scenario 1 for casting kit for all age groups, if elimination is considered.

Based on upper-bound exposure estimates for ultrafine TiO_2, safe use is not indicated for casting kits, chalk, and powder paint for all age groups, if elimination is considered. Safe use for ultrafine TiO_2 with no or negligible risk is only indicated for white colour pencils.

For fine TiO_2, all scenarios show safe use with no or negligible risk based on the realistic and upper-bound exposure estimates and the time-adjusted HECs.

These conclusions will be combined with the WoE-assessment in Section 6.7.6.4.

6.7.6.2 Oral exposure

The MoS was calculated for the three selected oral exposure scenarios for direct exposure, of a 15 kg child, whereas indirect exposure (mouthing) was considered to be negligible. The PoD of 1,000 mg/kg bw per day was compared to the estimated repeated exposures (Table 6.13). The results of these repeated exposures are shown in Table 6.20. The MoS for the acute exposure scenario for finger paint is 625.

Table 6.20: MoS[*] calculated for the three selected oral exposure scenarios (pigment grade TiO$_2$)

Scenario	MoS
4. Finger paint	2564
5. White colouring pencil	1818
6. Lipstick	7692

[*] A MoS >= 100 is considered to pose no or negligible risk (see explanation below)

The MoS should be evaluated with consideration of the uncertainties in its derivation:
- Toxicokinetic and dynamic differences rat-humans (factor of 10)
- Intraspecies variability in the human population (factor of 10)
- Uncertainties with regard to the exposure assessment (addressed by the worst-case approach)

The MoS therefore should be minimally 100 in order to consider an exposure to be "safe" (*i.e.,* having no or negligible risk for toxic effects).

It can be concluded that, based on the worst-case exposure estimates for a 15-kg child and the PoDs of 1,000 mg/kg bw.day for repeated exposure and 5,000 mg/kgbw for acute exposure, the MoS-values indicate safe use for the three exposure scenarios considered. This conclusion will be combined with the WoE-assessment in Section 6.7.6.4.

6.7.6.3 Aggregated exposure to TiO$_2$ in different toys

Oral exposure

The worst-case aggregated oral exposure is 16.1 mg/day or 1.1 mg TiO$_2$/kg bw.day for a 15 kg child (see Table 6.13), resulting in a MoS of 909.

This MoS should also be minimally 100 (see Section 6.7.6.2) in order to consider an exposure to be safe (*i.e.,* having negligible risk for toxic effects). It can be concluded that this MoS-values indicates safe use for worst-case aggregated oral exposure of a 15-kg child.

6.7.6.4 Weight of evidence

The WoE for all lines of evidence needed for the risk characterisation is presented here below, leading to the final conclusions in the next section:

Particle size (Section 6.2)

Pigmentary fine TiO$_2$ should not contain a nanofraction. The TiO$_2$ used in all toy products is pigment grade, with no deliberate use of ultrafine TiO$_2$. The presence of an ultrafine fraction in the pigments cannot be excluded. However, the provided data (TIE, 2020a) suggest that such an ultrafine fraction would not be present to a significant degree. Due to the limited data available on the particle size distribution of TiO$_2$ in toys, the WoE is weak for the conclusion that no ultrafine fraction is present.

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Exposure (Section 6.4)

The WoE for the lack of TiO_2 release from polymers used to manufacture toys is strong for the TiO_2 content as reported for food contact materials, but is evaluated as moderate for the lack of release from toy materials as no data from toy materials themselves are available.

The WoE for the exposure estimation for the inhalatory and oral scenarios is summarised in Table 6.21. Details can be found in Sections 6.4.2.4 and 6.4.2.7, respectively.

The weight of evidence for the various *inhalation* exposure scenarios is as follows:

As described in Table 6.9, the uncertainty related to scenario 3 (pencil use) is very small, because 5 different pencils were tested in a specific, relevant use scenario and the emissions were measured at a distance of 3 cm and 50 cm directly as $TiO_2 < 10$ μm. Based on the available and measured data, the WoE for the exposure scenario for the use of pencils is strong.

For the calculation of scenario 2 (chalk), the uncertainty on exposure is somewhat higher because the calculations were based on an experiment with chalk where only particles in the air ($PM_{2.5}$, PM_{10} and total suspended particulates) were measured. However, the assumption that the same weight fraction of TiO_2 is in the air as in the chalk itself is quite plausible. Based on the measured data on chalk, the WoE for the extrapolation for the release of TiO_2 particles from chalk can be considered moderate as the measurement did not determine the emission of TiO_2 itself.

Large uncertainties are associated with scenarios 1 and 4, because they are based on an experiment with cosmetic talcum powder (hydrous magnesium silicate), and not with the materials of the respective toys (gypsum or powder paint). In addition, no information was available on the dustiness of the cosmetic products used for the measurements. Therefore, based on considerations regarding usability, it was assumed that 1% of the amount used can be airborne and that the dustiness of TiO_2 in the toys is comparable to that of talcum. Large uncertainties are associated with this assumption, but a 1% release is considered very conservative, so it is nevertheless plausible that the upper bound cannot be exceeded in reality. In view of the uncertainties for the emission scenario 1 (casting kit) and scenario 4 (powder paint) the WoE is considered weak.

For the *oral* exposure calculation, the uncertainty related to the finger paint scenario is large, resulting is a weak WoE (low quality, low consistency). For white colouring pencils, the uncertainty related to the indirect ingestion scenario is large, resulting is a weak WoE (low quality, low consistency). For lipstick/lipgloss, there is somewhat less uncertainty, resulting in a moderate WoE for the oral exposure calculation (medium quality, low consistency).

Table 6.21: WoE for each exposure scenario

Scenario	Toy	WoE
Inhalation		
1	casting kit	weak
2	chalk	moderate
3	white colour pencil	strong
4	powder paint	weak
Oral		
1	finger paint	weak
2	white colour pencil	weak
3	lipstick/lip gloss	moderate

Toxicokinetics (Section 6.5)

Both oral and inhalation exposure can result in internal uptake of TiO_2 particles albeit at relatively low amounts. The WoE for low uptake after inhalation exposure is considered strong in view of high-quality data and high consistency.

The WoE for oral exposure is considered moderate to strong, as there is some variation in the amount of Ti that can be detected in the body after oral exposure. In addition, there is considerable difference in the quality of the published results with respect to the characterisation of the TiO_2 materials used.

Repeated dose toxicity (oral, inhalation) (Section 6.6):

Based on the absence of inflammation responses for ultrafine TiO_2 (P25) in a 90-day repeated dose toxicity study, the no-observed-adverse-effect concentration (NOAEC) was found to be 0.5 mg/m^3, whereas for fine TiO_2, a NOAEC of 10 mg/m^3 was observed (Bermudez *et al.*, 2002, 2004). The best estimates of a human exposure threshold for pulmonary inflammation (in occupational setting) are 1.0 mg/m^3 and 0.11 mg/m^3 for fine (MMAD 2.1 μm) and ultrafine (MMAD 0.8 μm) TiO_2 particles, respectively (Dankovic *et al.*, 2007).

A NOAEL was determined for general toxicity after oral exposure of 1,000 mg/kg bw per day for 90 days, after oral gavage of rutile-type alumina-coated pigment-grade TiO_2 particles (Warheit *et al.*, 2015, EFSA, 2021). However, several uncertainties remain regarding possible immunotoxic, genotoxic and carcinogenic activity after oral exposure, for which the use of TiO_2 particles as E171 food additive could not be considered safe.

The toxicological and AOP data of fine and ultrafine TiO_2 are both considered highly consistent and of medium to high quality for both inhalatory and oral routes of exposure. For oral exposure, however, uncertainties remain regarding immunotoxic, genotoxic and carcinogenic activity (see Sections 6.6.5 and 6.6.6), diminishing the reliability and consistency of the NOAEL. Regarding possible effects of TiO_2 as E171 at lower doses, such as indications for immunotoxicity, inflammation as well as neurotoxicity, uncertainties were noted (EFSA, 2021). Therefore, the overall WoE for the inhalatory NOAECs is considered strong but for oral exposure, the WoE for the NOAEL is judged to be weak.

Carcinogenicity (Section 6.6.5)

Although there is limited evidence in epidemiological studies for the induction of lung cancer in occupational settings, the WoE obtained in combination with various animal studies is strong for a possible carcinogenic effect of TiO_2 in the lung after inhalation exposure. Recently, a re-evaluation of previously published epidemiological studies showed a Health Worker Survivor Effect (HWSE) with a positive association between lagged cumulative exposure to TiO_2 and lung cancer mortality. Whether the carcinogenic effect is due to a specific effect of TiO_2 (ultrafine) particles or due to a general carcinogenic effect of particles in the lung is yet unknown. Discussions are ongoing as to whether to classify inhaled Poorly Soluble Low Toxicity (PSLT) particles as a class for carcinogenic hazard, of which TiO_2 is considered to be an example (Borm and Driscoll, 2019).

The available studies after oral exposure are not sufficient to draw firm conclusions on the potential carcinogenicity of TiO_2 particles. However, the induction of oxidative stress and inflammation in the GIT indicates a possible indirect or promoting effect of TiO_2 on tumour development. The WoE for tumour promoting activity of TiO_2 particles in the GIT is moderate, whereas the WoE for tumour induction in the GIT is uncertain to weak.

Genotoxicity (Section 6.6.6)

Overall, based on the results of *in vitro* and *in vivo* genotoxicity studies the SCHEER is of the opinion that the pigmentary TiO_2 grades can be considered to have no genotoxic potential after oral and inhalation exposure, provided the presence of a nanofraction can

be excluded. The relative contributions of the postulated modes of action to the genotoxicity elicited by TiO_2 ultrafine particles are unknown and there is uncertainty as to whether a threshold dose for any mode of action could be established.

Considering there are several uncertainties regarding the genotoxicity of TiO_2 particles (existence of both positive and negative results with different endpoints, various quality of the studies in terms of the characterisation of the TiO_2 used in studies), the WoE for genotoxicity is weak to moderate. For inhalation exposure, the WoE of genotoxic hazard of TiO_2 is moderate, based on high quality but relatively low consistency of the results. However, for oral exposure, the WoE of genotoxic hazard of TiO_2 is weak.

6.7.7 Final Conclusions

Release from toy materials/toys

The application of TiO_2 as colouring agent for polymers used to produce toys is considered to pose no or negligible risk to children, as the potential release of the TiO_2 from the polymers is considered negligible to non-existent due to the fixation of the TiO_2 within the polymer. Potential exposure is only possible when pieces of the toy break off due to mouthing (see oral exposure below).

Inhalation exposure

Based on the MoS-values, it can be concluded that toys containing TiO_2 can be used safely in the realistic high- and upper-bound exposure scenarios considered, when this TiO_2 does not contain ultrafine fractions.

When an ultrafine fraction is assumed to be present, safe use is not indicated for exposure scenario 1 (casting kit, realistic high- and upper-bound estimate), scenario 2 (chalk, upper-bound estimate) and scenario 4 (powder paint, upper-bound estimate). The WoE for the inhalation risk characterisation is strong for the hazard characterisation and weak to strong for the exposure assessment.

The main uncertainty in the hazard characterisation is connected to the relatively low consistency of the genotoxicity results. There may be a risk for both ultrafine and non-ultrafine forms of TiO_2 genotoxicity, but it remains uncertain whether this will affect the threshold approach followed. The weight of evidence for the exposure estimations varies from weak (casting kit, powder paint) to moderate (chalk) and strong (white colour pencil). However, the uncertainty in the exposure scenarios is addressed by basing the risk assessment on a conservative approach that uses the upper-bound exposure estimations.

Therefore, it can be concluded that white colour pencil (scenario 3, weak WoE for particle size distribution, but strong WoE for exposure and for the hazard characterisation) can be used safely by children of different age groups even when an ultrafine fraction is present in the TiO_2 preparation, with regard to possible inhalation exposure.

It cannot, however, be concluded that casting kits, chalk, and powder paint containing an ultrafine-fraction can be used safely by children, taking into account the low MoS-values. However, when it can be demonstrated with high certainty that no ultrafine-fraction is present in TiO_2 in toys and toy materials, safe use for all products with a TiO_2 content above 1%, highlighted in Table 6.5, is indicated based on the exposure estimations of this Opinion.

For upper-bound estimates, considered as worst-case scenarios, the MoS-values are as presented in Tables 6.22 and 6.23.

Table 6.22: MoS* calculated for the 4 inhalation scenarios at upper-bound exposure to ultrafine TiO2 (NOAEC = 0.5 mg/m³)

Scenario	Duration (min)	23 months	3 years	6 years
		+	+	+
1 Casting kit	10	**2.3**	**2.5**	**2.5**
2 Chalk	45	**6.5**	**6.9**	**6.7**
3 White colour pencil	45	27	29	29
4 Powder paint	10	**2.9**	**3.1**	**3.0**

* + = corrected for human elimination; a MoS >= 25 is considered safe

For ultrafine TiO2, this upper-bound exposure in several exposure scenarios results in a MoS that is below 25 when calculated including elimination from the lung: for scenario 1 casting kits, for scenario 2 chalk, and for scenario 4 powder paint for all age groups. So, the inhalation exposures to ultrafine TiO2 released from casting kits, chalk, and powder paint can not be considered safe.

For white pencils for all age groups, the MoS is above 25, and the use of ultrafine TiO2 can be considered to pose no or negligible risk regarding inhalation exposure based on the upper-bound exposure estimates.

For fine TiO2, the upper-bound exposure for the casting kit results in a lowest MoS of 51, for the chalk in a lowest MoS of 137, for the white colour pencils a lowest MoS of 589, and for the powder paint in a lowest MoS of 61, all scenarios were envisaged for the age of 23 months and the risk was calculated including elimination.

For the evaluated uses of fine TiO2 in casting kits, chalk, white colour pencils and powder paint, the MoS show safe use with no or negligible risk after inhalation exposure based on the upper-bound exposure estimates.

Table 6.23: MoS* calculated for the 4 inhalation scenarios at upper-bound exposure to fine TiO2 (NOAEC = 10 mg/m³)

Scenario	Duration (min)	23 months	3 years	6 years
		+	+	+
1 Casting kit	10	51	54	55
2 Chalk	45	137	146	141
3 White colour pencil	45	589	626	606
4 Powder paint	10	61	65	63

* + = corrected for human elimination; a MoS >= 25 is considered safe

Oral exposure

Based on the MoS-values only, it might be concluded that toys containing TiO2 can be used with no or negligible risk in the worst-case oral exposure scenarios considered. However, the WoE for the oral risk characterisation is uncertain for the hazard characterisation and weak to moderate for the exposure assessment. The uncertainty in the hazard characterisation is connected to uncertainties regarding immunotoxic, genotoxic and carcinogenic activity. Regarding possible effects of TiO2 as E171 at lower doses, such as indications for immunotoxicity, inflammation as well as neurotoxicity, uncertainties were

noted (EFSA, 2021). The WoE for the exposure estimations is weak for the finger paint and white colour pencil scenarios and moderate for the lipstick scenario. The uncertainty in the exposure estimates is addressed by a worst-case approach.

Although there is uncertainty on the hazard characterisation, the MoS for oral exposure for the pigmentary fine TiO$_2$ is sufficiently high to indicate safe use. When the absence of an ultrafine fraction can be demonstrated with appropriate methodology, pigmentary TiO$_2$ in toys can be considered to show safe use with no or negligible risk after oral exposure.

Table 6.24 Summary of conclusions regarding safety of toys containing TiO$_2$

	Fine particles	Ultrafine particles
Inhalation		
Casting kit	safe	safe use not determined conclusively
Chalk	safe	safe use not determined conclusively
White colour pencil	safe	safe
Powder paint	safe	safe use not determined conclusively
Oral		
Finger paint	safe	safe use not determined conclusively
White colour pencil	safe	safe use not determined conclusively
Lipstick/ lip gloss	safe	safe use not determined conclusively

7. RECOMMENDATIONS FOR FUTURE WORK

To determine the safey of using pigmentary TiO$_2$ particles in toys, it is essential to know the number size distribution of the particles, including both constituent particles and agglomerates/aggregates. In addition, it should be demonstrated that plausibly no or a negligible ultrafine fraction is present in the pigmentary TiO$_2$ preparations.

In view of the uncertainty on the potential genotoxicity of pigmentary fine TiO2, further studies to the genotoxicity of fine TiO2 are recommended, including clear demonstration of the absence of an ultrafine fraction in these preparations investigated.

In view of the lack of data on the release of TiO$_2$ from toys and/or toy materials, migration and TiO$_2$ release studies are recommended.

In view of the uncertainty of the oral hazard characterisation of pigmentary TiO$_2$, further toxicity studies after oral exposure are warranted.

8. CONSIDERATION OF THE RESPONSES RECEIVED DURING THE PUBLIC CONSULTATION

A public consultation on this Opinion was open on the website of the Scientific Committees from 3rd of June to 4th of July 2022. Information about the public consultation was broadly communicated to national authorities, international organisations and other stakeholders. Seven manufacture organisations (TiO$_2$ producers and toy manufactures), one manufacturer, three governmental organisations, one public organisation, and two individuals participated in the public consultation, providing input to different parts of the Opinion, resulting in 122 contributions collected in a table presented as Annex VI.

Several commenters report that the vast majority of the TiO$_2$ grades used in toys do not contain 1% or more particles with an aerodynamic diameter of ≤ 10 µm and need not be classified as Category 2 carcinogen and subject to a risk assessment. The SCHEER notes that it would be up to the manufacturer to demonstrate in its risk assessment that its product does not fall under the CMR rules of the Toy Directive 2009/48/EC. In this Opinion, the SCHEER provided the risk assessment for the cases that toys contain 1% or more of particles below ≤ 10 µm. The SCHEER is not aware, or has not been informed about TiO$_2$ pigmentary product composition for specific applications (e.g. paints) that would not be included in the requirements as indicated in the Toy Directive.

Several comments indicated that nanomaterials are not included in the pigmentary TiO$_2$ products used in toys. However, the information on the size and size distribution of the pigmentary TiO$_2$ products is limited. Therefore, SCHEER included in its Opinion the risks associated with TiO$_2$ products containing both an ultrafine and/or fine particle fraction.

In addition, several recently published Reports/Opinions of regulatory advisory committeees and/or agencies were discussed and included in the evaluation by SCHEER.

Based on the comments received, the SCHEER Opinion on the risks associated with the use of TiO$_2$ pigment in childrens toys was modified in several locations. A more detailed evaluation was performed for genotoxicity effects, and the oral risk characterisation was adapted.

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10. GLOSSARY OF TERMS

Aggregated exposure	Exposure to one agent from different exposure pathways and/or exposure routes (Heinemeyer *et al.*, 2021).
Fine particles	Microscale particles with an aerodynamic diameter above 0.1 µm
Inhalable fraction	The fraction comprising droplets/particles with MMAD of ≤100 µm
Mucociliary escalator	Removal of particles from the conducting airways and the bronchial region together with mucus into the oral cavity, from which they can be swallowed
Respirable fraction	The fraction of the inhalable particles that enter the deepest part of the lung, the non-ciliated alveoli: conventionally these are the particles with a MMAD ≤4-5 µm.
Thoracic fraction	The fraction of the inhalable particles that pass the larynx and penetrate into the conducting airways and the bronchial region of the lung: conventionally these are the particles with a MMAD ≤10 µm
Ultrafine particles	Nanoscale particles with an aerodynamic diameter of 100 nm (0.1 µm) or less

11. LIST OF ABBREVIATIONS

ADME	absorption, distribution, metabolism, excretion (toxicokinetics)
bw	body weight
CMR	Carcinogenic, Mutagenic, toxic for Reproduction
DAF	Dosimetric Adjustment Factor
DNEL	Derived No-effect Level
ECHA	European CHemical Agency
EFSA	European Food Safety Agency
GIT	Gastrointestinal Tract
HEC	Human Equivalent Concentration
HWSE	Health Worker Survivor Effect
LOAEL	Lowest-Observed-Adverse-Effect Level
MMAD	Mass Median Aerodynamic Diameter
MoS	Margin of Safety
NOAEC	No-Observed-Adverse-Effect Concentration
NOAEL	No-Observed-Adverse-Effect Level
PBPK	Physiologically Based PharmacoKinetic (model)
PoD	Point of Departure
PSLT	Poorly Soluble Low-Toxicity

RCR   Risk Characterisation Ratio

SCCS   Scientific Committee on Cosmetic Safety

SCHEER   Scientific Committee on Health, Environmental and Emerging Risks

TIE   Toys Industries of Europe

WoE   Weight of Evidence

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ANNEXES

Annex I: Toys Industry data on release and content

Table A-I.1. Data provided by TIE on TiO₂ release from various brands of pencils

Sample number of pencils used	Type of pencil	Distance cm	Filter	Mass TiO$_2$ <10 μm mg/m^3	Mass >10 μm mg/m^3	TiO$_2$ content/colour	Test report No	Reference
6	Faber-Castell, White Colour GRIP notebook & board	3	3A 3B 4A 4B	0.010 0.012 0.002 0.005	0.024 0.409 0.038 0.037	>30% Could be up to max 51%	60376879-003 Ann 2-3	Ann 2-2
		50	3C 4C	0.006 0.002	0.004 0.052			
6	Staedtler Mars GmbH & Co. KG, White, ergo soft 157	3	1A 1B 2A 2B 3A 3B	0.002 0.007 0.002 0.004 0.003 0.006	0.006 0.01 0.008 0.01 0.003 0.013	White Could be up to max 51%	60412480-003 Ann 2-5	Ann 2-4
		30	1C 2C 3C	0.003 0.001 0.005	- - 0.007			
6	Brand not provided, White, Colour 16	3	1A 1B 2A 2B 3A 3B	0.019 0.015 0.014 0.020 0.016 0.015	0.027 0.020 0.025 0.085 0.064 0.039	White Could be up to max 51%	60419230-002002002 Ann 2-7	Ann 2-6
		50	1C 2C 3C	0.005 0.016 0.009	0.016 0.040 0.030			
6	Stabilo International GmbH, White Type 0520	3	1A 1B 2A 2B 3A 3B	0.003 0.007 0.005 0.008 0.005 0.006	0.018 0.047 0.003 0.025 0.049 0.100	White Could be up to max 51%	60428931-001 Ann 2-9	Ann 2-8
		50	1C 2C 3C	0.003 0.003 0.013	0.009 0.003 0.012			

Distance = distance of use of coloured (=white) pencils to measuring point. Measuring time 100 min.
Threshold alveolar fraction (< 5 μm) 1.25 mg/m^3, respirable fraction (< 10 μm) 10 mg/m^3.

1

Table A-I.2. Data provided by TIE on TiO$_2$ release from various types of wax crayons

Summary data were provided from tests performed with wax crayons (white) containing various amounts of TiO$_2$

Sample number of crayons used	Distance cm	Filter	Mass TiO$_2$ <10 µm mg/m^3	Mass >10 µm mg/m^3	Presence of TiO$_2$	Reference
6	3	1A	0.0002	-	4%	Ann 3-1
		1B	0.003	0.002	white	60410916-
		2A	0.0001	-		001
		2B	-	-		
		3A	0.0001	0.002		Ann 3-2
		3B	-	0.004		60410916-
	50	1C	0.001	-		002
		2C	0.001	-		
		3C	0.0001	-		
6	3	1A	0.001	0.003	20%	Ann 3-3
		1B	0.0004	-	white	60414436-
		2A	0.002	-		002
		2B	0.001	-		
		3A	0.001	-		
		3B	0.0003	-		
	50	1C	-	-		
		2C	0.00007	0.005		
		3C	0.001	-		
	3	1A	0.0006	0.003	White	Ann 3-4444
		1B	0.0004	-	P460	60414436-
		2A	0.002002002002	-		004
		2B	0.0007	-		
		3A	0.0006	-		

		3B	0.0003	-		
	50	1C	-	-		
		2C	0.00007	0.005		
		3C	0.001	-		
6	3	1A	0.0006	-	white	Ann 3-6
		1B	0.003	-		60418994-
		2A	0.0006	-		001
		2B	0.0004	-		
		3A	0.0001	-		
		3B	-	-		
	50	1C	-	-		
		2C	-	-		
		3C	-	-		
6	3	1A	0.0006	-	White	Ann 3-7
		1B	0.003	-	Colour 16	60418994-2
		2A	0.0006	-		
		2B	0.0004	-		
		3A	0.0001	-		
		3B	-	-		
	50	1C	-	-		
		2C	-	-		
		3C	-	-		

Distance = distance of use of coloured pencils to measuring point. Measuring time 100 min.
Threshold alveolar fraction (< 5 μm) 1.25 mg/m^3, respirable fraction (< 10 μm) 10 mg/m^3.

Table A-I.3. Data provided by TIE on TiO$_2$ content of various wax crayons

Sample number wax crayons	Colour	Presence of TiO$_2$ mg/kg	Presence of TiO$_2$ %	Reference
1	White	122000	20.4	Ann 3-5
2	Red	130	0.022	
3	Black	170	0.028	
4	Purple	16000	2.67	
5	Orange	250	0.042	
6	Brown	270	0.045	
7	Yellow	220	0.037	
8	Green	600	0.100	
9	Blue	180	0.030	

Determination of the theoretical content of titanium dioxide after decomposition and quantification of titanium by ICP-OES acc. to DIN EN ISO 11885 resp. ICP-MS acc. to DIN EN ISO 17294-2

Table A-I.4. Data provided by TIE on TiO$_2$ release from abrasion of white finger paint (steel pencils scratching on applied dry finger paint)

Application quantity mg/cm^2	Distance cm	Filter	Mass TiO$_2$ <10 µm mg/m^3	Mass TiO$_2$ >10µm mg/m^3	TiO$_2$ content /colour	Report No	Reference
8.264	3	1A 1B 2A 2B 3A 3B	>0.012 >0.045 >0.038 >0.030 0.014 0.003	>0.048 >0.314 >0.144 >0.026 - -	white	60428792-001 60428792-002	Ann 4-1 Ann 4-2
	30	1C 2C 3C	>0.032 >0.033 >0.021	>0.0 >0.048 -			

Distance = distance of use of coloured (=white) finger paint paper surface to measuring point. Measuring time 100 min.

Table A-I.5. Data provided by TIE on TiO$_2$ release from use of Modelling Compound (use simulation: rubbing on sandpaper)

Sample number of fit formed modelling compound used	Distance cm	Filter	Mass TiO$_2$ <10 µm mg/m^3	Mass >10 µm mg/m^3	TiO$_2$ content/colour	Reference
6 rods	3	1A 1B 2A 2B 3A 3B	0.004 0.006 0.005 0.006 0.002 0.003	0.005 0.014 0.002 0.08 0.036 0.009	rosa	Ann 5-1 60429249-001 Ann 5-2 60429249-002
	30	1C 2C 3C	0.004 0.005 0.004	- 0.057 -		

Distance = distance of use of white modelling compound on paper surface to measuring point.

Figure A-1.1 Summary of the TiO₂-dust release studies provided by EWIMA and its members (Annex 6 as provided by TIE Figures on overall measurements as performed)
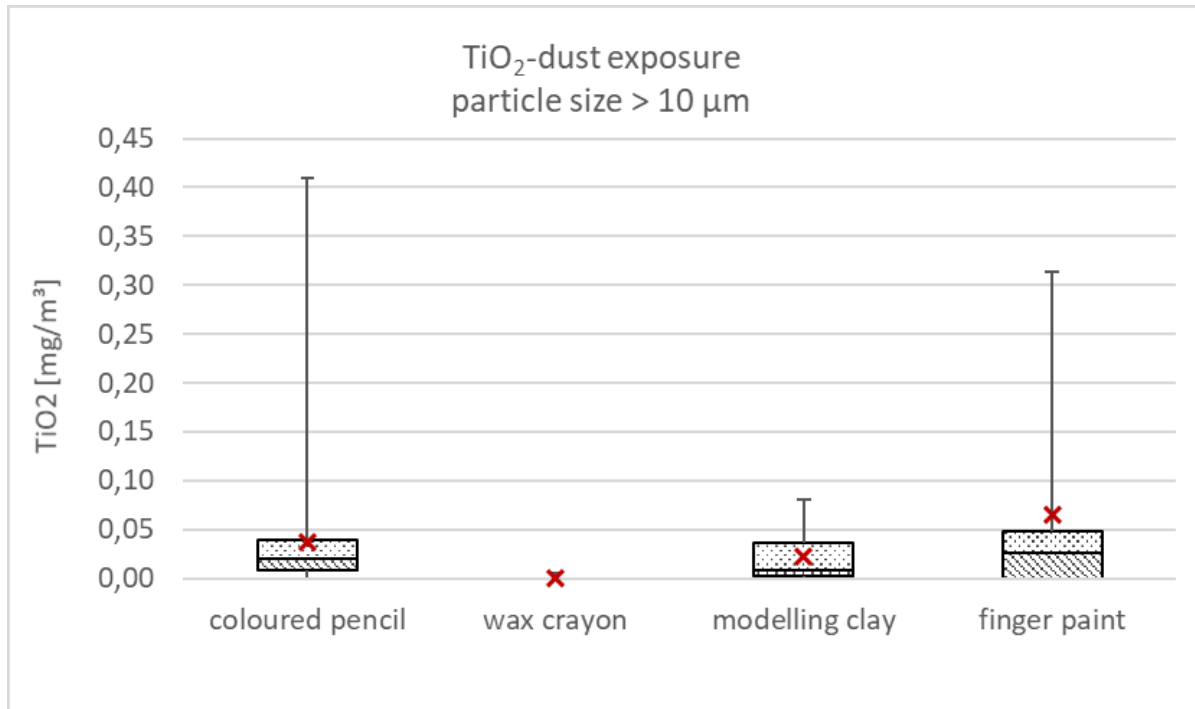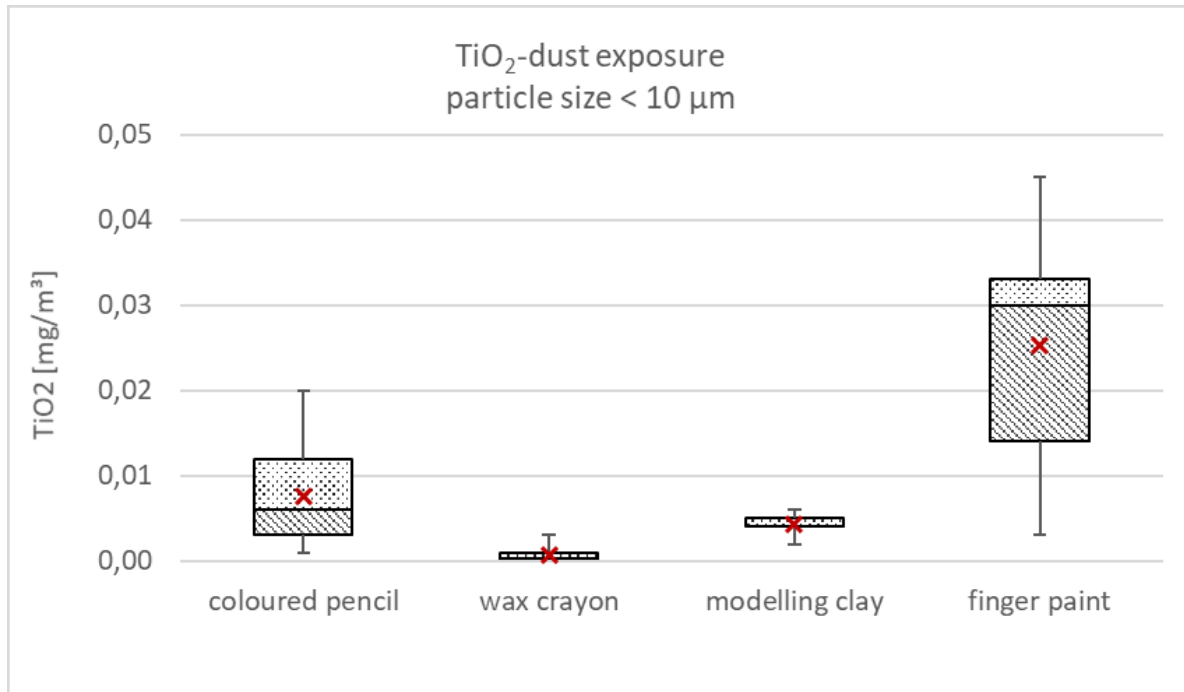
Table A-I.6: Results of TiO$_2$-dust release studies provided by EWIMA and its members

Product category	Coloured pencil	Wax cryon	Modelling clay	Finger paint
Number of tests (different brand/products)	11 (4)	9 (4)	3 (1)	3 (1)
Concentration TiO$_2$ in the product (% w/w)	10 – 33	4 - 20	1	2
Measurements per test	3	3	3	3
Number of samples per measurement	6	6	6	6
Sampling time (min)	100	100	100	100
TiO$_2$ < 10µm (mg/m^3)	0.001-0.020 (0.006)	0.000-0.003 (0.000)	0.002-0.006 (0.004)	0.003-0.045 (0.030)
Range (median) Mean (±SD	0.008 (±0.005)	0.001 (±0.001)	0.004 (±0.001)	0.025 (±0.013)
TiO$_2$ >10µm (mg/m^3)	0.000-0.409 (0.020)	0.000-0.005 (0.000)	0.000-0.080 (0.009)	0.000-0.314 (0.026)
Range (median) Mean (±SD)	0.035 (±0.70) *0.037(±0.07)*	0.001 (±0.001)	0.023 (±0.027)	0.064 (0.098) *0.064 (±0.104)*

In *italics* recalculated by SCHEER.

Annex II: Calculation of scenario air concentrations

Table A-III.1: Air concentrations calculated by applying adjustment factors to measured air concentrations (upper bound)

Parameter	Scenario 1 casting kit	Scenario 2 chalk	Scenario 3 pencil	Scenario 4 powder paint
Cair_meas (mg/m3)	8.42	0.17	0.02	8.42
Vmeas/Vscen	0.385	5	0.5	0.385
wfscen/wfmeas	0.015	0.05	1	0.25
ascen/ameas	14.3	1	1	0.714
Cair_scen (mg/m3)	**0.695**	**0.0425**	**0.01**	**0.579**

Annex III: Calculation of the air concentrations of TiO₂ for four selected use scenarios

Table A-III.1: Air concentrations

Scenarios	Weight Fraction TiO2	Air conc. PM$_{10}$-TiO2 TIE report (µg/m3)	Air conc PM$_{10}$-TiO2 SCHEER "realistic"* (µg/m3)	Air conc PM$_{10}$-TiO2 SCHEER "upper bound" (µg/m3)	Remarks	Uncertainties/WoE	Time per event (min)	Events per day
Scenario 1 (casting)	0.015	75.3	347	695	"Realistic": 1% of 500g airborne "Upper bound": 1% of 1,000g	Measurement with talcum powder, airborne unknown	10	1; 2
Scenario 2 (chalk)	0.05	104	-	42.5		Measurements with chalk, 4 different chalks tested	45	1; 2
Scenario 3 (pencil)	0.51	13	-	10		Measurements of TiO₂ for one pencil type	45	1; 2
Scenario 4 (powder paint)	0.25	5020	23.2	579	"Realistic": 1% of 2g airborne "Upper bound": 10% of 50g	Measurement with talcum powder, airborne unknown	10	1; 2

*Realistic scenario only calculated for scenarios with high uncertainty, to illustrate the uncertainty around the calculations

Annex IV: Genotoxicity tables

Table A-IV.1: Summary of selected genotoxicity papers (found on potential differences in genotoxicity of ultrafineTiO₂ depending on coating)

	Cell type	TiO₂-NP characteristics	Was there any effect of coating on genotoxicity observed?	Reference
1	Hamster lung fibroblasts V79	1. Nano-TiO₂ MTI5 (anatase) from MTI Corporation (Richmond, CA). 2. P25 (anatase/rutile) from Evonik Industries (Düsseldorf, Germany). 3. Nanofilament rutile from Sigma–Aldrich. 4. Vive Nano Titania(−) (rutile) from Vive Nano Inc. (Toronto, ON) – it is a negatively charged water-dispersible rutile nanoparticle powder stabilised by sodium polyacrylate. According to the manufacturer, less than 22% of its weight is TiO₂. 5. Hombitan LW-S (H. Bulk anatase) from Sachtleben Chemie (Duisberg, Germany).	**Comet assay** Yes, the coating (Vive Nano Titania) **decreased** cytotoxicity and genotoxicity in comet assay comparing to other TiO₂ forms.	Hamzeh M. *et al.*, 2013. In vitro cytotoxicity and genotoxicity studies of Titanium dioxide (TiO₂) nanoparticles in Chinese hamster lung fibroblast cells. Toxicology in Vitro 27 (2): 864–873. doi:10.1016/j.tiv.2012.12.018.
2	C3A rat hepatocytes	1. NM 101 (anatase; 9 nm) 2. NRCWE 001, rutile 10 nm from NanoAmor (Houston, USA) and used for production of: 3. NRCWE 002, rutile 10 nm with positive charge and 4. NRCWE 003, rutile 10 nm with negative charge 5. NRCWE 004, rutile 94 nm from NaBond.	**Comet assay** Yes, negative charge coating (NRCWE03) **decreased** DNA damage in comet, BUT on the other hand, positive coating (NRCWE02) slightly **increased** the DNA-damaging effect of NRCWE01. Genotoxicity was most evident following exposure to NM 101 (TiO₂ 7 nm) and NRCWE 002 (positively charged TiO₂ 10 nm).	Kermanizadeh A *et al.*, 2012. An in vitro liver model - assessing oxidative stress and genotoxicity following exposure of hepatocytes to a panel of engineered nanomaterials. Particle and Fibre Toxicology 9 (1): 28. doi: 10.1186/1743-8977-9-28.
3	Human renal proximal tubule	The same NMs as above	**Comet assay** Yes, positive charge coating (NRCWE02) was associated with **increased** DNA	Kermanizadeh A *et al.* 2013. An in vitro assessment of panel of

	epithelial cells HK-2		damage in comet assay comparing to NRCWE01 (no coating).	engineered nanomaterials using a human renal cell line: cytotoxicity, pro-inflammatory response, oxidative stress and genotoxicity. BMC Nephrology 14 (1): 96. doi: 10.1186/1471-2369-14-96.
4	BEAS-2B human lung epithelium	1. titanium(IV) oxide nanopowder (rutile phase from Sigma-Aldrich product no. 637262; originally labelled as 99.5% pure, but particle characterisation showed that the particles contained <5% SiO$_2$ as coating material; particle size 10x40 nm), 2. titanium (IV) oxide nanopowder (anatase-phase from Sigma-Aldrich product no. 637254; 99.7%, particle size <25 nm) 3. titanium (IV) oxide powder (rutile-phase from Sigma-Aldrich product no. 224227; 99.9%, particle size <5 μm)	**Comet assay** Yes, nanosized SiO$_2$-coated rutile was a less effective inducer of cell toxicity and DNA damage in BEAS-2B cells than nanosized anatase or fine rutile. The reason for this difference is unclear, but it may be related to the SiO$_2$-coating that increases the hydrophilicity of the nanosized rutile. The coating may reduce the ability of rutile to catalyze reactive radical generation.	Falck G *et al.*, 2009. Genotoxic effects of nanosized and fine TiO$_2$. Hum Exp Toxicol 28(6–7): 339–352. doi: 10.1177/0960327109 105163
5	Human PBL	Four nanosized TiO$_2$: - NM-102 - NM-103 (surface modified with dimethicone) - NM-104 (surface modified with glycerine) - NM-105	**Micronucleus test** Yes, coating increased genotoxicity, as both rutiles coated NM-103 (dimethicone) and NM-104 (glycerine) induced more pronounced effects, comparing to NM-102 anatase and not active NM-105 (rutile-anatase).	Tavares AM *et al.*, 2014. Genotoxicity evaluation of nanosized titanium dioxide, synthetic amorphous silica and multi-walled carbon nanotubes in human lymphocytes. Toxicol in Vitro 28(1): 60–69. doi:

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| 6 | A549 human bronchoalveolar lung cancer cells | 1. commercial TiO_2 NP (84% anatase, 16% brookite crystal phase composition) from Colorobbia Italia SpA as colloidal nanosuspension (nanosol) used to prepare:

2. citrate coated TiO_2

3. silica coated TiO_2

4. TiO_2 Aeroxide® P25 used as benchmark material | **Micronucleus and comet assay**

Both coated and uncoated TiO_2 were positive, though in some cases the effect after exposure to coated NPs was slightly less or more pronounced. | Stoccoro A *et al.*, 2017. Multiple endpoints to evaluate pristine and remediated titanium dioxide nanoparticles genotoxicity in lung epithelial A549 cells. Toxicology Letters, 276, 48–61. |
| 7 | BEAS-2B | 1. NM-100 (anatase, 50–150 nm)

2. NM-101 (anatase, 5–8 nm, coated)

3. NM-103 (rutile, 20–28 nm, coated) | **Micronucleus and comet assay with Fpg**

A weak genotoxic effect of the tested TiO_2 materials was observed with an induction of oxidised bases for all three materials of which NM-100 was the most potent. When the comet slides were briefly exposed to lab light, a clear induction of DNA strand breaks was observed for the anatase materials, but not for the rutile. | Di Bucchianico S *et al.*, 2017. Genotoxicity of TiO_2 nanoparticles assessed by mini-gel comet assay and micronucleus scoring with flow cytometry. Mutagenesis 32: 127–137. |

Note: the top partial row above row 6 contains the reference "10.1016/j.tiv.2013.06.009".

Table A-IV.2: Uptake of TiO₂ by cells (NP=nanoparticles / ultrafine fraction; NM=nanomaterials)

Test system/ Test object	Exposure conditions (concentration/duration/metabolic activation)	Information on the characteristics of the test substance	Result	Reliability/ Comments	Relevance of the result	Ref ID_ authors_year
In vivo rat TEM, TEM-EDX	Oral administration, 7 days	TiO2 E171, NM2105	Both E171 and TiO2 NPs were localised in the area of nuclei in colon and liver cells	1	High	Bettini *et al.*, 2017. Food-grade TiO2 impairs intestinal and systemic immune homeostasis, initiates preneoplastic lesions and promotes aberrant crypt development in the rat colon. Scientific Reports, 7, 40373.
In vivo mice, TEM	Daily doses of 10 and 15 mg/kg bw via i.v. injection to mice on two consecutive days, animals were sacrificed 28 days after the last i.v. injection	TiO2 NPs (anatase 22 nm, NM-102)	TiO2 NPs were localised in the area of nuclei of hepatocytes in all mice exposed to either dose of NM, albeit without a clear dose-related effect. The NMs inside nuclei were always surrounded by the basophilic heterochromatin	1	High	Louro H, Tavares A, Vital N, Costa PM, Alverca E, Zwart E, de Jong W, Fessard V, Lavinha J and Silva MJ, 2014. Integrated approach to the in vivo genotoxic effects of a titanium dioxide nanomaterial using LacZ plasmid based transgenic mice. Environmental and Molecular Mutagenesis, 55, 500–509. https://doi.org/10.1002/em.21864

In vitro Lung Epithelial A549 cells, TEM, Rahman	10 µg/ ml TiO2 nanoparticles	TiO2NPs P25 Degussa	Uptake of TiO2 NPs in the Nucleus by both Raman Imaging and TEM	1	High	Ahlinder et al. Large Uptake of Titania and Iron Oxide Nanoparticles in the Nucleus of Lung Epithelial Cells as Measured by Raman Imaging and Multivariate Classification. Biophysical Journal, 105 July 2013, 310–319
In vitro A549 human lung carcinoma cells TEM	4h exposure, 50 µg/ml	TiO2-A12, TiO2-A25 and TiO2-R20 TiO2-A25 (AEROXIDE P25) were from Degussa	Small NPs 12 nm TiO2-A12 accumulating inside the nucleus	2	Limited	Jugan M-L, Barillet S, Simon-Deckers A, Herlin-Boime N, Sauvaigo S, Douki T and Carriere M, 2012. Titanium dioxide nanoparticles exhibit genotoxicity and impair DNA repair activity in A549 cells. Nanotoxicology, 6, 501–513.
In vitro HepG2 cells TEM	1 µg/ml	TiO2 30-70 nm	Subcellular localisation of TiO2 NPs inside cytoplasm and nucleus was confirmed using TEM	2 Positive Fpg comet assay and micronucleus even in low concentration of 1 µg/ml	Limited	Shukla RK, Kumar A, Gurbani D, Pandey AK, Singh S and Dhawan A, 2013. TiO2 nanoparticles induce oxidative DNA damage and apoptosis in human liver cells. Nanotoxicology, 7, 48–60.
In vitro V79 cell, TEM	3, 15 and 75 µg/cm2 24 h treatment	NM 105 anatase/rutile, 15-24 nm	NP found in cytoplasma and nucleus by TEM	1	High	Kazimirova et al. Effects of Titanium Dioxide Nanoparticles on the Hprt Gene Mutations in V79 Hamster Cells. NANOMATERIALS ,10 Issue: 3, No 465, 2020
In vitro V79 cells, TEM	25 µg/mL 6 h and 24 h.	TiO2 NPs Nanopowder	Accumulation and cellular localisation of TiO2 NPs in	1	High	Jain AK, Senapati VA, Singh D, Dubey K, Maurya R and Pandey AK, 2017. Impact of anatase titanium dioxide nanoparticles on mutagenic and genotoxic

		Sigma, anatase <25 nm	V79 cells sand nuclei			response in Chinese hamster lung fibroblast cells (V-79): the role of cellular uptake. Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association, 105, 127–139.
In vitro HBE cells, TEM	50 µg/mL of TiO$_2$ NPs for 24 h: 17nm-SA, 17nm-LA, 117nm-SA and 117nm-LA	TiO2 NPs primary sizes 17 and 117 nm	Intracellular uptake of TiO$_2$ agglomerates by HBE cells, TEM Some TiO$_2$ agglomerates close to the nucleus, small NPs in nucleus in agglomerate	1	High	Murugadoss S, Brassinne F, Sebaihi N, Petry J, Cokic SM, Van Landuyt KL, Godderis L, Mast J, Lison D, Hoet PH and van den Brule S, 2020. Agglomeration of titanium dioxide nanoparticles increases toxicological responses in vitro and in vivo. Part Fibre Toxicol, 17, 10.
In vitro human peripheral lymphocyte, TEM	20, 50, 100, 200 µg/ml, 4h	TiO2-NPs in the anatase crystal phase (<25 nm, Sigma-Aldrich	Uptake in nucleus only in one cell. Data from this study did not show any cyto- or genotoxic potential of TiO2-NPs despite NP uptake into the nucleus.	3 Uptake in nucleus only in one cell. Pictures unclear.	Low	Hackenberg S, Friehs G, Kessler M, Froelich K, Ginzkey C, Koehler C, Scherzed A, Burghartz M and Kleinsasser N, 2011. Nanosized titanium dioxide particles do not induce DNA damage in human peripheral blood lymphocytes. Environmental and Molecular Mutagenesis, 52, 264–268.

Table A-IV.3: DNA binding of TiO$_2$ (NP=nanoparticles=ultrafine fraction)

Test system/ Test object	Exposure conditions (concentration/duration/metabolic activation/methods of detection)	Information on the characteristics of the test substance	Result	Reliability/ Comments	Relevance of the result	Ref ID_ authors_year
DNA binding in vivo, mice liver	Livers of TiO2 NPs-treated ICR mice by i.p. 5, 10, 50, 100 and 150 mg/kg bw per day for 14 days UV–Vis absorption spectroscopy, circular dichroism (CD), extended X-ray absorption fine structure (EXAFS) spectroscopy and gel electrophoresis.	TiO2 NPs (anatase, 5nm)	A dose-dependent increase in the content of TiO2 NPs in liver DNA identified by ICP-MS spectroscopy indicated changes in the DNA conformation. EXAFS spectroscopy indicated that anatase TiO2 NPs could be bound with the oxygen or phosphorus atoms of the nucleotide and nitrogen atoms of base pairs in DNA. Significant hypochromicities were observed.	2	Limited	Li N, Ma L and Wang J (2010). Interaction Between Nano-Anatase TiO2 and Liver DNA from Mice In Vivo. Nanoscale Res Lett. 5(1): 108–115.

| Sprague–Dawley rat | After intranasal administration (300 µg/rat per day for 45 days), the interaction between TiO2 particles and liver tissue

UV–Vis absorption spectrometry, atomic force microscopy (AFM), TEM, micro-synchrotron radiation X-ray fluorescence (m-SRXRF) and gel electrophoresis | a) nanoanatase (d < 25 nm); b) micro-rutile (d < 5 µm); c) a mixture of 5–10% rutile and 90–95% anatase (d < 100 nm). | DNA binding (hypochromicity) was observed with the TiO2 NPs anatase and TiO2 NPs rutile/anatase mixture but not with micro rutile. According to the authors, TiO2 NPs anatase can insert itself between DNA base pairs covalently but whether this binding is covalent via P–O–Ti–O bond is questionable. | 2 | Limited | Jin C, Tang Y, Fan XY, Ye XT, Li XL, Tang K, Zhang YF, Li AG and Yang YJ (2013). In vivo evaluation of the interaction between titanium dioxide nanoparticle and rat liver DNA. Toxicology and Industrial Health, 29. |
| DNA binding to human genomic DNA (in vitro) | *In vitro* DNA binding capacity of TiO2 NPs (< 100 nm)

UV–Vis spectroscopy | TiO2 NPs | Hyperchromic effect, probably due to strong stacking interactions between human genomic DNA and TiO2 NPs. | 2 | Limited | Patel S, Patel P, Sachin B, Undre SR, Pandya MS and Sonal B (2016). DNA binding and dispersion activities of titanium dioxide nanoparticles with UV/vis spectrophotometry, fluorescence spectroscopy and physicochemical analysis at physiological temperature. Journal of |

						Molecular Liquids 213: 304-311. Patel S, Patel P and Bakshi SR (2017). Titanium dioxide nanoparticles: an in vitro study of DNA binding, chromosome aberration assay, and comet assay. Cytotechnology, 69: 245–263.
DNA binding to calf thymus DNA	Methods: 1) UV-visible spectroscopy; 2) fluorescence quenching; 3) circular dichroism (CD); 4) docking analysis	TiO2 NPs, rutile, 14 nm (XRD)	A strong binding affinity of TiO2 NPs with DNA. The hyperchromic behaviour confirms unwinding of double-stranded DNA. Molecular docking analysis revealed a selective binding of TiO2 NPs with A-T bases in minor groove of DNA.	2	Limited	Ali K, Abul QF, Dwivedi S, Abdel-Salam EM, Ansari SM, Saquib Q, Faisal M, Al-Khedhairy AA, Al-Shaeri M and Musarrat J. (2018). Titanium dioxide nanoparticles preferentially bind in subdomains IB, IIA of HSA and minor groove of DNA. Journal of Biomolecular Structure and Dynamics, 36, 2530–2542.

DNA Binding of TiO2NPs alone and in combination with Doxorubicin (DOX) to calf thymus DNA in vitro	Methods: UV–Vis absorption Spectroscopy and circular dichroism (CD); DNA thermal denaturation studies; flow cytometry and fluorescence microscopy for in vitro experiments	TiO2 NPs	Interaction of TiO2 NPs with DNA leading to changes in the secondary structure of the DNA helix.	2	Limited	Hekmat A, Saboury AA, Divsalar A and Seyedarabi A (2013). Structural effects of TiO2 nanoparticles and doxorubicin on DNA and their antiproliferative roles in T47D and MCF7 cells. Anti-Cancer Agents in Medicinal Chemistry, 13.
Structural changes in calf thymus DNA	Calf thymus DNA, combined treatment of TiO2 NPs anatase (< 10 nm) + paclitaxel (PTX) in comparison to single exposures to either compound. UV–Vis and CD spectrometry, thermal denaturation and fluorescence emission spectra	TiO2 NPs anatase (< 10 nm) + paclitaxel (PTX) in	Formation of a complex between DNA and TiO2 NPs	1	High	Hekmat A, Afrough M, Tackallou SH and Ahmad F (2020). Synergistic effects of Titanium dioxide nanoparticles and Paclitaxel combination on the DNA structure and their antiproliferative role on MDA-MB-231 cells. Journal of Nanoanalysis 7: 152–165.
Interaction with DNA (from salmon sperm)	TiO2 NPs (21 nm) interaction with salmon DNA. Analytical techniques (capillary electrophoresis coupled with UV and Fourier transform infrared spectroscopy).	TiO2 NPs (21 nm)	The ability of TiO2 NPs (21 nm) to interact with DNA confirmed. Electrostatic interactions of	1	High	Alsudir S, Lai EPC. (2017) Electrosteric stabilization of colloidal TiO2 nanoparticles with DNA and

| | | | TiO2 NPs via the sugar-phosphate backbone were demonstrated with double-stranded and single-stranded DNA. | | | polyethylene glycol for selective enhancement of UV detection sensitivity in capillary electrophoresis analysis. Anal Bioanal Chem. 409,1857-1868. doi: 10.1007/s00216-016-0130-8 |

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Annex V: Calculation of the Human Equivalent Concentration (HEC)

Calculations for ultrafine TiO₂ with NOAEC = 0.5 mg/m3 (Bermudez *et al.*, 2004)

For deriving the human equivalent concentration (HEC), a dosimetric adjustment factor (DAF) was used to convert the rat 6-h NOAEC of 0.5 mg/m^3 (from Bermudez *et al.*, 2004) to a 24-h HEC based on species-specific information on deposition, pulmonary surface area, and breathing volume. Deposition per pulmonary surface area is the key dose metric for inflammatory effects. The DAF was calculated using the Multi Pathway Particle Deposition (MPPD) v3.04. to estimate the pulmonary deposition fraction to human and rat lungs.

As previously described by the SCCS (see TIO₂ Opinion, SCCS/1617/20), the SCHEER considers the deposition in pulmonary region as the most relevant dose metric for the current assessment and for HEC calculation.

The HEC was calculated as follow:

HEC = NOAEC x (deposition rate/lung surface area) rat/ (deposition rate /lung surface area) human.

Bermudez *et al.* 2004 used Fisher rat (this strain is not available in the MPPD model). For the deposition fraction calculation, the long Evans symmetric was used according to MPPD2 since the Long Evans rat serves as an approximate model for other rat strains using the same morphometry. The simulation was performed using whole-body exposure and with the following particle properties: density (4.3g/cm^3), MMAD (1.44µm) and GSD (2.6).

Calculation and results are presented below (Table A-VI.1 and Figure A-VI.A):

Table A-V.1: Calculations of deposition values calculated by the SCHEER using Bermudez *et al.* 2004

	MPPD Parameter
Rat	NOAEC = 0.5
Tidal Vol (mL)	2.1
Breaths/min	102
VE (mL/min)	214.2
Fractional deposition (PU) (see figure A)	0.056
Alveolar surface area (m2)	0.297
Clearance	Not used
deposition rate [1]	0.003084

(1) Deposition rate rat = 0.056 x (2.1/1,000,000) 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
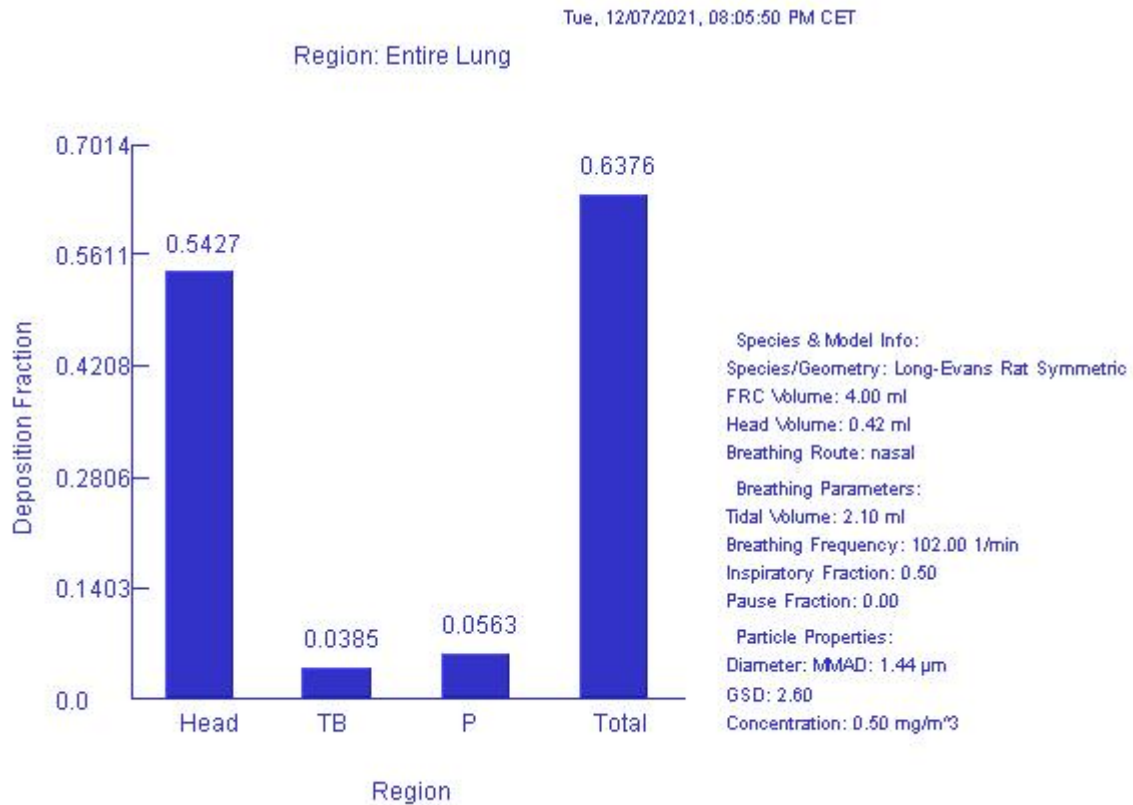
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Figure A-V.A: Calculation of fractional deposition in rat, by using the default parameter of the software:

In humans, the deposition fraction of the pulmonary regions was calculated with the default setting airway morphometry for humans by applying age-specific symmetric for children aged 23-months, three years, and six years. Respiratory frequencies, bodyweights and other input parameters are listed in the table (A-VI.2) below.

Children aged 2 to <3 years, and 3 to <6 years were considered to be the age groups that are most susceptible to experiencing high exposures on the basis of their relatively higher respiratory rates compared to other age groups (EPA US, 2008).

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Table A-V.2: Input parameters and its values used in MPPD model

Age	Upper respiratory tract volume(ml)	Functional residual capacity (ml)	Tidal volume (ml)	Breathing frequency (per minute) [1]	Body weight (Kg) [1]	Alveolar surface area (m²)[2]
23 months	6.96	40.34	86.79	27	12	12
3 years	9.47	57.46	121.3	24	16	16
6 years	21.03 [3]	740 [3]	209 [4]	19.2 [4]	27	27
8 years	21.03	740	278.2	17	34	34

(1) Data published by Anses (Leconte S, Rousselle C, Bodin L, Clinard F, Carne G. Refinement of health-based guidance values for cadmium in the French population based on modelling. Toxicol Lett. 2021 Apr 1; 340:43-51, and used by EFSA Opinion on PFAAS (Risk to human health related to the presence of perfluoroalkyl substances in food, Nov 2020)).
(2) Not specified in MPPD model, data are estimated by Lenfant 2000 (cited by Fröhlich *et al.*, 2016) where the estimation of alveolar surface is equal to1 m^2/kg bw.
(3) Not specified in MPPD model, data are from 8 years old children in the MPPD model
(4) Not specified in MPPD model, data are from from Poorbahrami *et al.*, 2021. Poorbahrami K, Vignon-Clementel IE, Shadden SC, Oakes JM. (2021) A whole lung in silico model to estimate age dependent particle dosimetry. Sci Rep 11:11180. doi: 10.1038/s41598-021-90509-8.

The exposure scenario was fixed to oronasal-mouth breathing and the exposure conditions were calculated based on a constant exposure and deposition only (*i.e.*, no clearance included in these calculations).

Calculation and results are presented below (Table A-VI.3 and Figures A-VI.B, A-VI.C and A-VI.D) for different age groups.

Table A-V.3: calculations of HEC and estimated deposition values calculated by the SCHEER using Bermudez *et al.* 2004

Human	23 month	3 years	6 years
Tidal Vol (mL)	86.79	121	209
Breaths/min	27	24	19
VE (mL/min)	2343	2904	3971
Fractional deposition (PU)	0.2426	0.2461	0.3087
Alveolar surface area (m2)	12	16	27
Clearance Human	Not used	Not used	Not used
deposition rate [1]	0.82	1.03	1.77
DAF	**0.15**	**0.16**	**0.16**
24-hour Adjusted HEC (mg/m3.day)	**0.076**	**0.081**	**0.079**
1-hour Adjusted HEC (mg/m3.day)	**1.827**	**1.938**	**1.906**
10 min Adjusted HEC (mg/m3.day)	**10.961**	**11.625**	**11.431**

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[1] Deposition rate human = fractional deposition x (tidal volume/1000000) x respiratory rate x hours x 24 = XX m^3/day

> These **HECs DO NOT take into account** the elimination constant in rats and humans, expressed in days:
>
> Elimination constant = −ln(0.5)/elimination half-time (MAK 2020, ANSES 2019)
>
> In rats, the elimination constant = -(ln0.5)/60 = 0.0116/day.
>
> In humans, elimination constant= -(ln0.5)/400 = 0.00173/day
>
> **If clearance is taken into account, the HEC should be divided by a factor of 6.7, given the difference in elimination rates between human and rats (humans have a lower elimination rate).**
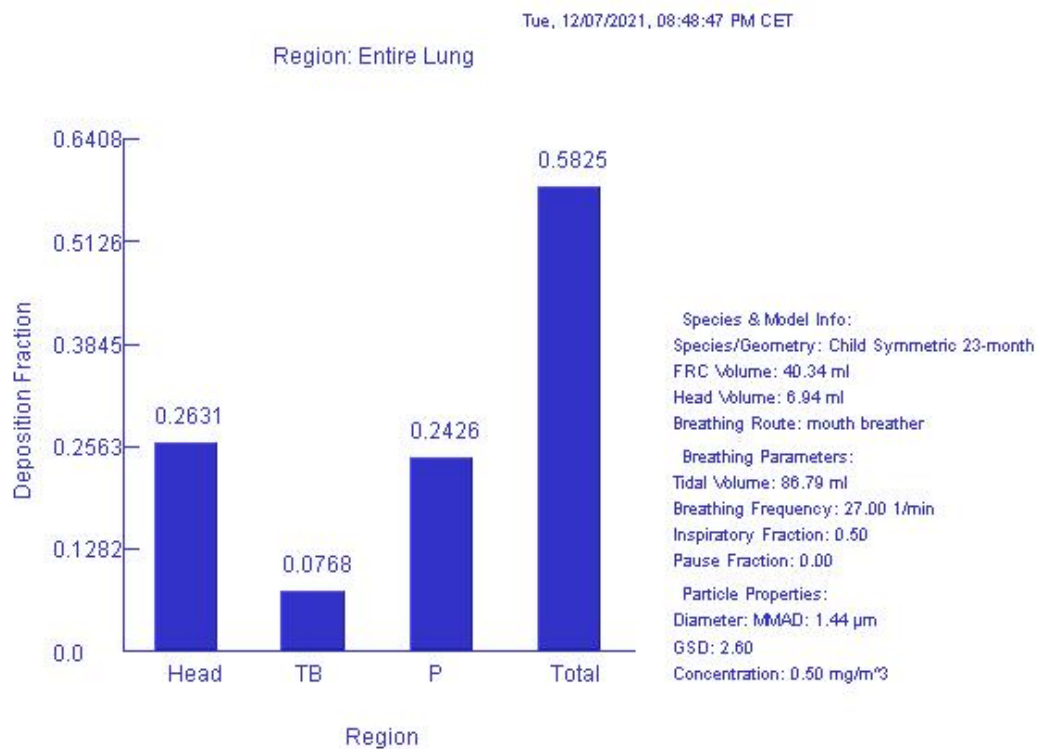

Figure A-V.B: Calculation of fractional deposition in 23-month-old children, by using the default parameter of the software
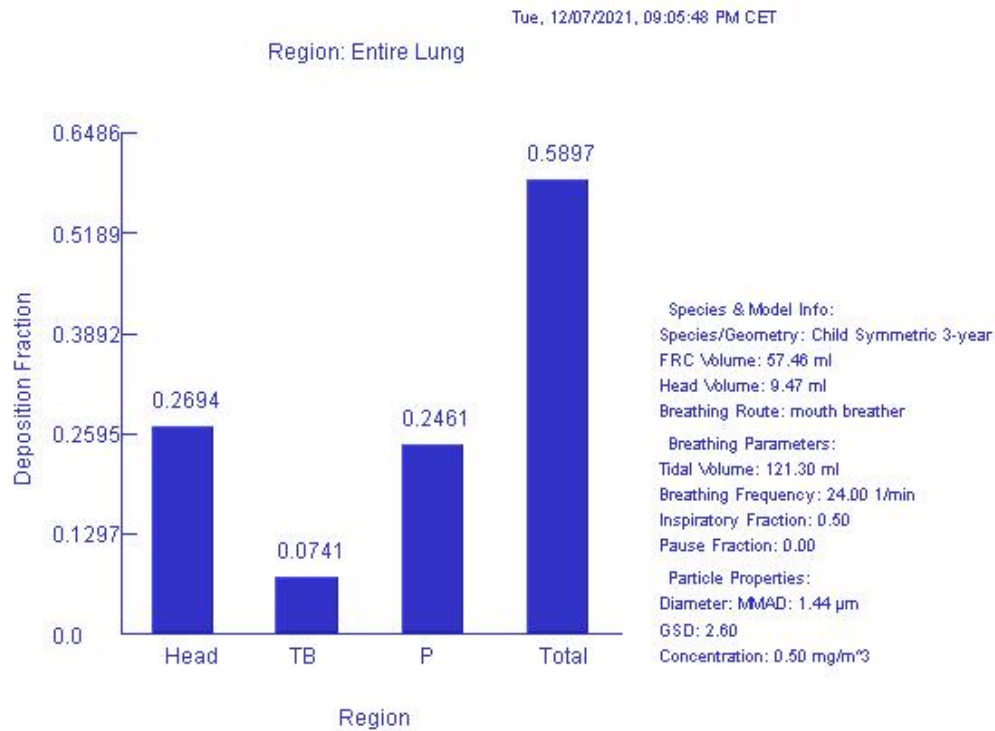
Figure A-V.C: Calculation of fractional deposition in 3-year-old children, by using
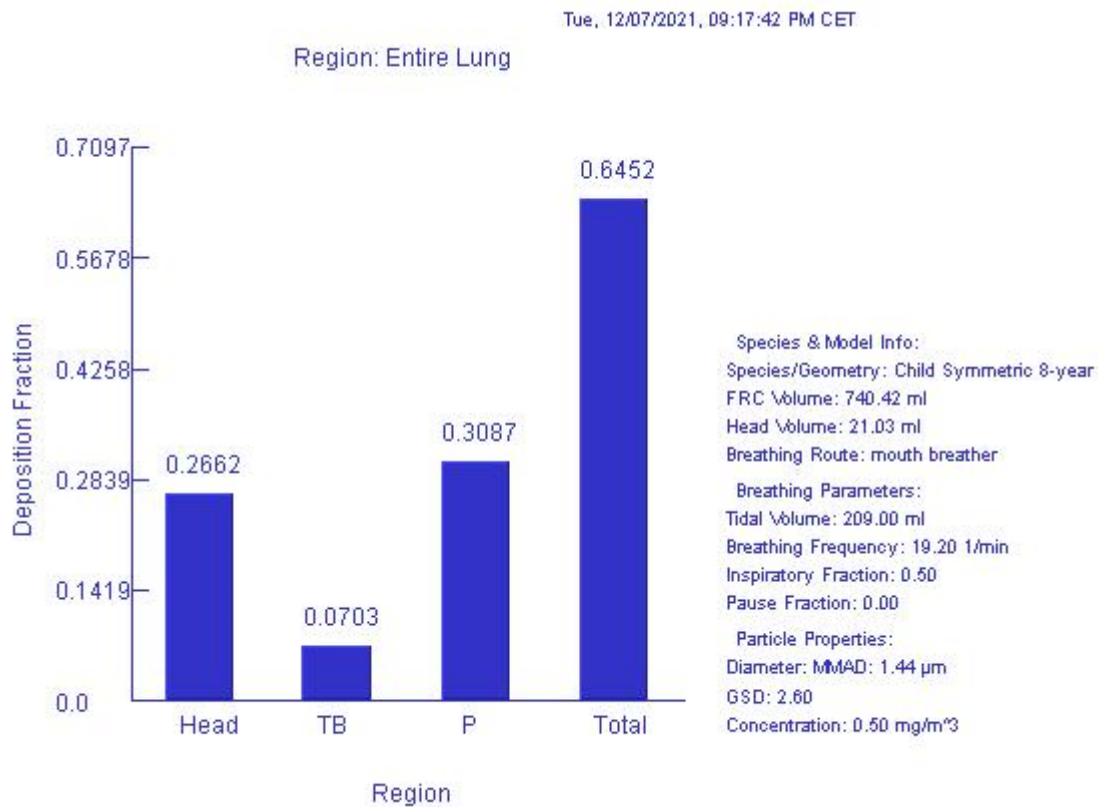the default parameter of the software

Figure A-V.D: Calculation of fractional deposition in 6-year-old children, by using the default parameter of the software

Calculations for fine TiO₂ with NOAEC =10 mg/m3 (Bermudez *et al.*, 2002)

Bermudez *et al.* (2002) used the Fisher rat (this strain is not available in the MPPD model). For the deposition fraction calculation, the Long Evans symmetric was used according MPPD2 since the Long Evans rat can serve as an approximate model for other rat strains using the same morphometry. The simulation was performed using whole-body exposure and with the following particle properties: density 4.3 g/cm^3, MMAD 1.44 μm, GSD 1.71. Calculation and results are presented below (Tables A-V.4 and A-V.5 and Figures A-V.E, A-V.F, A-V.G and A-V.H) for different age groups.

Table A-V.4: Calculations of deposition values calculated by the SCHEER using Bermudez *et al.* 2002

	MPPD Parameter
Rat	NOAEC = 10
Tidal Vol (mL)	2.1
Breaths/min	102
VE (mL/min)	214.2
Fractional deposition (PU) (see figure A)	0.056
Alveolar surface area (m2)	0.297
Clearance	Not used
deposition rate [1]	0.00317812

[1] 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

Table A-V.5: calculations of HEC and estimated deposition values calculated by the SCHEER using Bermudez *et al.* 2002

Human	23 months	3 years	6 years
Tidal Vol (mL)	86.79	121	209
Breaths/min	27	24	19
VE (mL/min)	2343	2904	3971
Fractional deposition (PU)	0.2426	0.2461	0.3087
Alveolar surface area (m2)	12	16	27
Clearance Human	Not used	Not used	Not used
deposition rate [1]	0.79197055	0.993586176	1.733198544
DAF	0.16	0.17	0.17
24-hour Adjusted HEC (mg/m3.day)	1.621	1.723	1.667
1-hour Adjusted HEC (mg/m3.day)	38.913	41.356	40.007
10 min Adjusted HEC (mg/m3.day)	233.479	248.136	240.044

[1] Deposition rate human = fractional deposition x (tidal volume/1000000) x respiratory rate x hours x 24 = XX m^3/day
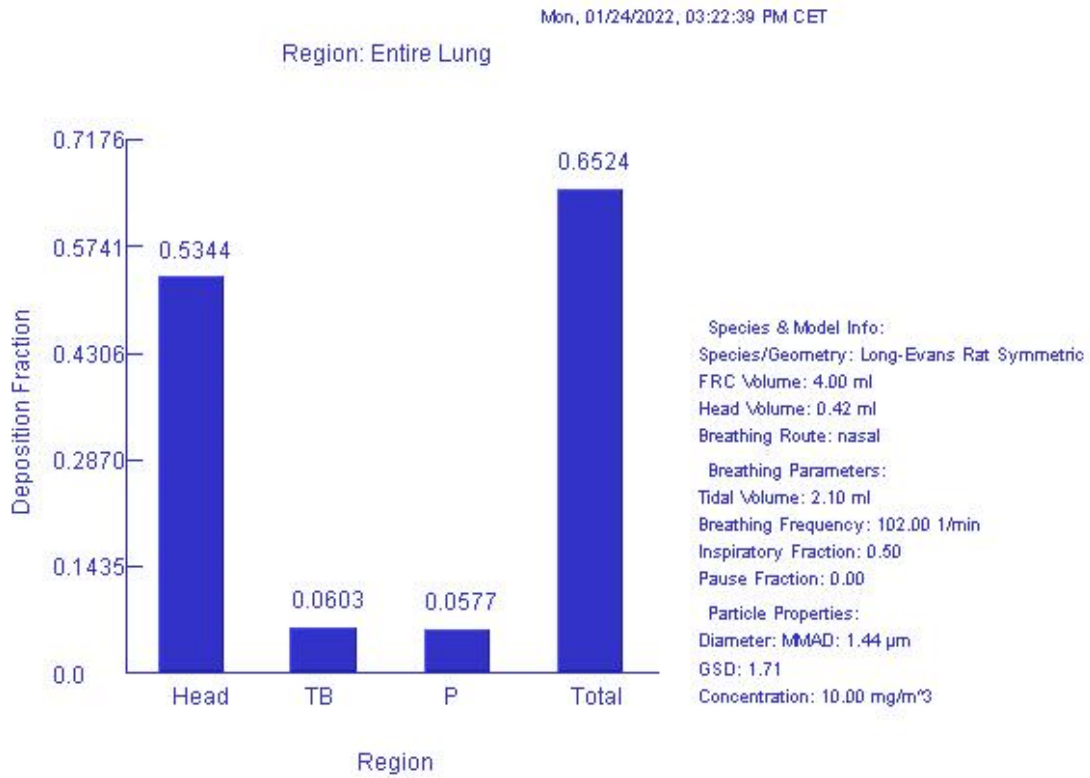
Figure A-V.E : Calculation of fractional deposition in rat, by using the default parameters of the software
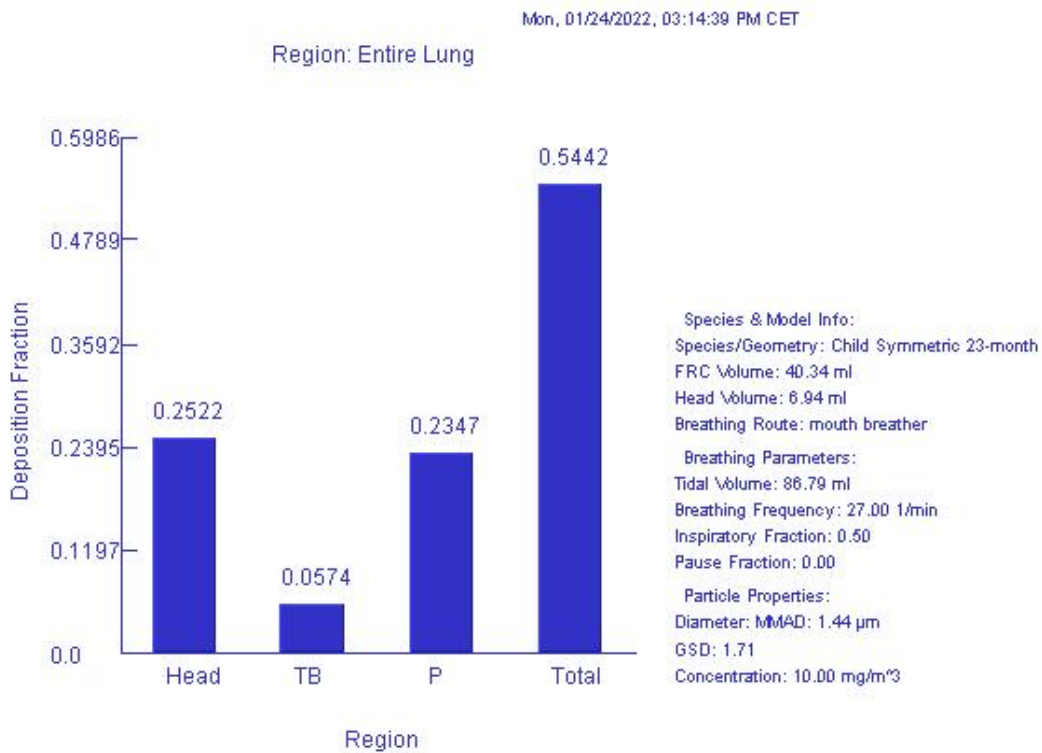
Figure A-V.F: Calculation of fractional deposition in 23-month-old children, by using the default parameters of the software
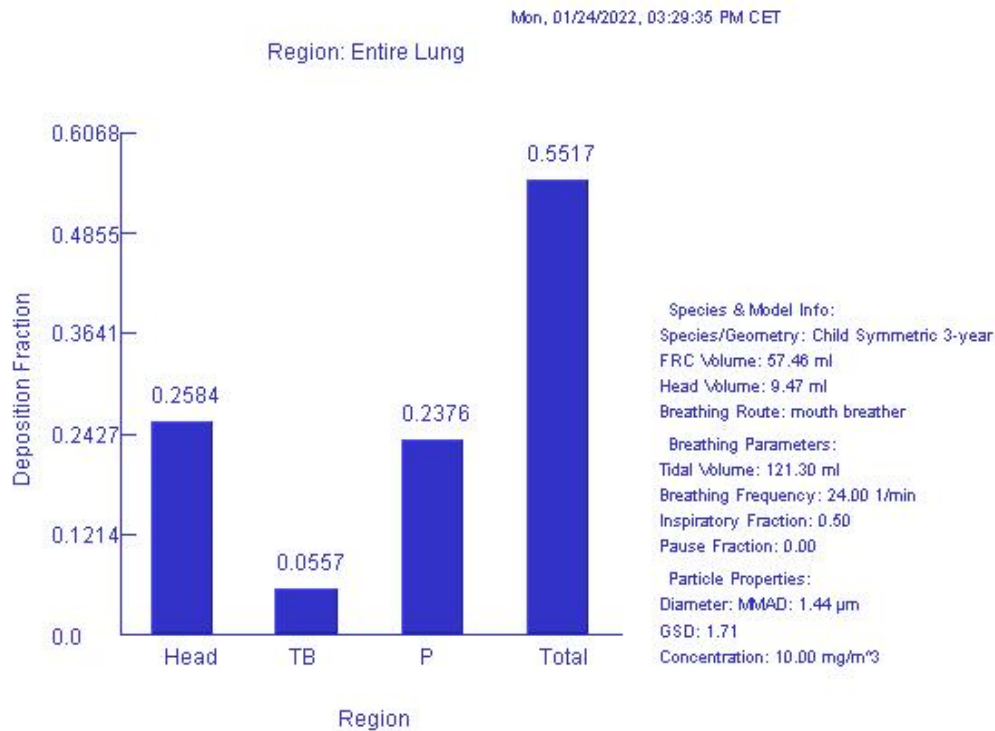

Figure A-V.G: Calculation of fractional deposition in 3-year-old children, by using the default parameter of the software

Mon, 01/24/2022, 03:41:31 PM CET

Region: Entire Lung

Species & Model Info:
Species/Geometry: Child Symmetric 8-year
FRC Volume: 740.42 ml
Head Volume: 21.03 ml
Breathing Route: mouth breather

Breathing Parameters:
Tidal Volume: 209.00 ml
Breathing Frequency: 19.20 1/min
Inspiratory Fraction: 0.50
Pause Fraction: 0.00

Particle Properties:
Diameter: MMAD: 1.44 µm
GSD: 1.71
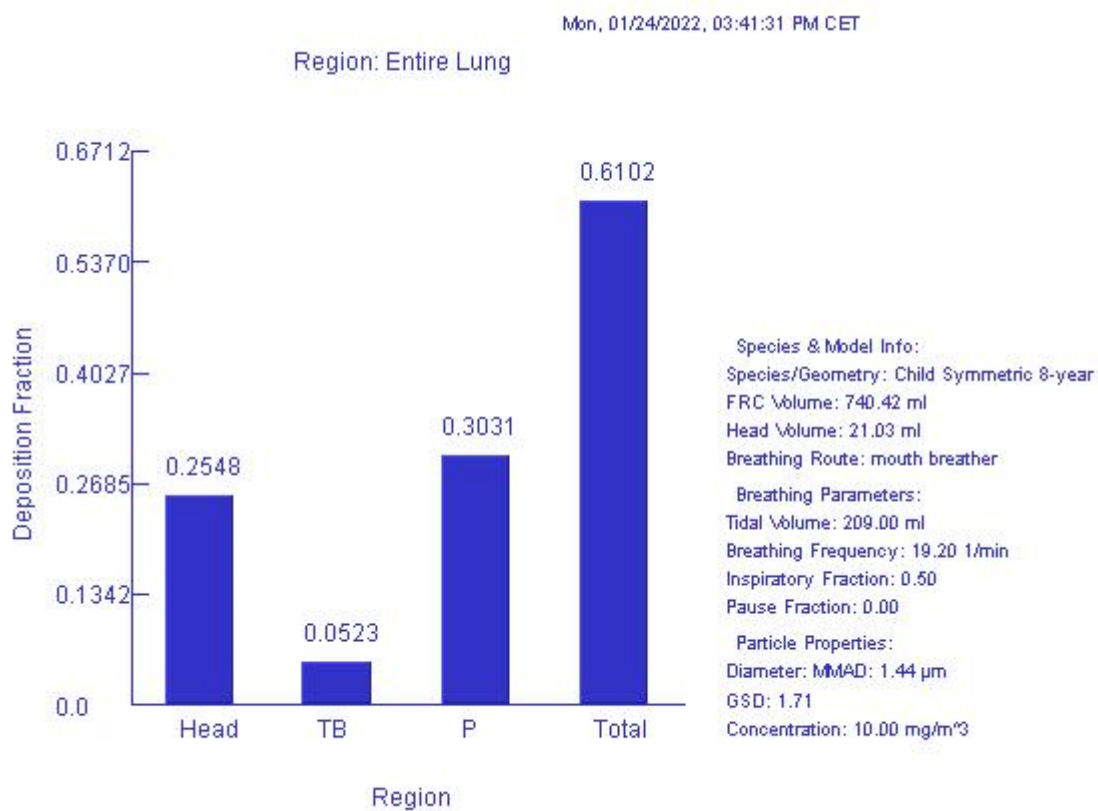Concentration: 10.00 mg/m^3

Figure A-V.H: Calculation of fractional deposition in 6-year-old children, by using the default parameter of the software