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Risk assessment of the use of **titanium dioxide** as a marker in feed

RIVM letter report 2024-0171
J.A. de Heer et al.

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Colophon

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J.A. de Heer (author), RIVM
R.C. Sprong (author), RIVM
S.E.C.G. ten Voorde (author), RIVM
S.M.F. Jeurissen (author), RIVM

Contact:
Suzanne Jeurissen
Preventie, Leefstijl en Gezondheid / Chemische Voedselveiligheid (PLG-CVV)
suzanne.jeurissen@rivm.nl

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Synopsis

Risk assessment of the use of titanium dioxide as a marker in feed

The white food colourant titanium dioxide (E 171) was used as a food and feed additive until 2022. Thereafter, the use of this additive in these products was banned in the European Union. According to the European Food Safety Authority (EFSA), it is uncertain whether the use of E 171 in food in safe.

In spite of this ban, the Netherlands Food and Consumer Product Safety Authority (NVWA) is frequently asked to make an exemption for the use of titanium dioxide as a marker in research on feed. This research includes 'digestibility studies' to investigate whether animals are able to digest the nutrients in feed well. Titanium dioxide makes this research easier. The NVWA has asked the Dutch National Institute for Public Health and the Environment (RIVM) to perform a risk assessment for this use.

RIVM has assessed the possible health effects of titanium dioxide in feed for three farm animals: dairy cows, chickens (laying hens and broilers) and pigs (sows, piglets and fattening pigs). It performed a similar assessment for humans that eat these animal products (consumers). Finally, RIVM assessed the health effects of titanium dioxide in feed for dogs and cats (companion animals).

The conclusions of ESFA formed the basis for the risk assessment, especially the conclusion on the possibility that titanium dioxide can damage DNA. According to EFSA, this is uncertain, but cannot be ruled out. Therefore, it is not clear if the use of this substance as a marker in feed is safe for companion animals, dairy cows, laying hens and sows. This is also true for consumers.

For short-living farm animals (broilers, piglets and fattening pigs), this is different. This is because possible damage to DNA will only cause adverse effects after a long time. For these animals, RIVM investigated whether the marker was harmful for their general health and their organs. Harmful effects cannot be excluded for these animals either, but it is expected that this risk is low. However, it is uncertain whether it is safe to consume animal products made from animals that have had feed with titanium dioxide.

Keywords: titanium dioxide, feed additive, food additive, E 171

Publiekssamenvatting

Risicobeoordeling over het gebruik van titaniumdioxide als een markerstof in diervoeder

De witte kleurstof titaniumdioxide (E 171) is tot 2022 als additief in voedsel en diervoeder gebruikt. Daarna is het gebruik van dit additief in deze producten in de Europese Unie verboden. Volgens de Europese voedselveiligheidsautoriteit (EFSA) is het namelijk niet zeker dat het gebruik van E 171 in voedsel veilig is.

Toch krijgt de Nederlandse Voedsel- en Warenautoriteit (NVWA) regelmatig de vraag om een uitzondering te maken voor het gebruik van titaniumdioxide als markerstof in onderzoek naar diervoeder. In zogeheten verteringsstudies wordt gemeten of dieren de voedingsstoffen in het diervoeder goed kunnen verteren. Titaniumdioxide maakt dit onderzoek eenvoudiger. De NVWA heeft daarom het RIVM gevraagd een risicobeoordeling voor dit gebruik uit te voeren.

Het RIVM heeft gekeken naar mogelijke gezondheidseffecten van titaniumdioxide in diervoeder voor drie soorten landbouwhuisdieren: melkkoe, kip (leghen en vleeskuiken) en varken (zeug, big en vleesvarken). Dit is ook gedaan voor de mensen die producten van deze dieren eten (consument). Ten slotte is gekeken naar gezondheidseffecten van het gebruik in diervoeder voor honden en katten (gezelschapsdieren).

De basis voor de risicobeoordeling zijn de conclusies van de EFSA. En dan vooral de conclusies over de mogelijkheid dat titaniumdioxide DNA kan beschadigen. Volgens de EFSA is dat onzeker, maar kan het niet worden uitgesloten. Daarom is niet duidelijk of het gebruik van deze stof als markerstof in diervoeder veilig is voor gezelschapsdieren, melkkoe, leghen en zeug. Dat geldt ook voor de consument.

Voor landbouwhuisdieren die kort leven (vleeskuiken, big en vleesvarken), ligt dit anders. Dat komt omdat eventuele beschadigingen aan het DNA pas na een langere tijd schadelijke effecten veroorzaken. Voor deze dieren is onderzocht of de markerstof schadelijk is voor hun gezondheid in het algemeen en voor hun organen. Ook voor hen kunnen schadelijke effecten niet worden uitgesloten, maar is de kans daarop naar verwachting klein. Het is wel onzeker of het veilig is om producten te eten van dieren die diervoeder met titaniumdioxide hebben gehad.

Kernwoorden: titaniumdioxide, diervoederadditief, voedseladditief, E 171

Contents

Summary

Titanium dioxide is a white pigment that was used as an additive (E 171) in food and feed in the European Union (EU). In 2022, the EU banned the use of titanium dioxide as a food and feed additive[1,2] following the conclusions of the re-evaluation of titanium dioxide (E 171) by the European Food Safety Authority (EFSA). Although titanium dioxide is no longer permitted as an additive in animal feed, the Netherlands Food and Consumer Product Safety Authority (NVWA) can grant permission for its use as a marker substance in digestibility studies, to aid the estimation of nutrient uptake. Since such requests are frequently received by the NVWA, the NVWA requested RIVM to perform a risk assessment for the use of titanium dioxide as a marker in feed for dairy cows, chickens, pigs, cats and dogs.

To this aim, the potential risks of the use of titanium dioxide as a marker in test feed for farm animals (dairy cow, laying hen, broiler, piglet, fattening pig and sow) are assessed, addressing the health risks for the animals and for the consumer of animal products. In addition, the potential animal health risks for its use in test feed for cats and dogs are assessed. Related to this, the maximum amount of titanium dioxide that can be used as a marker in feed without having adverse health effects for the concerned species and the consumers, was calculated.

In addition to the risk assessment, an overview of several existing risk assessments on titanium dioxide used as a food or feed additive and the current European legislation on the use of titanium dioxide in food, feed and (veterinary) medicinal products is included in the report.

To calculate the exposure, the NVWA provided exposure scenarios for the requested species, including concentrations of titanium dioxide in feed and exposure durations based on their received requests. This was combined with data on default body weights and feed intake (EFSA, 2012; NRC, 2006). The estimated exposure to titanium dioxide used as a marker in feed ranged between 14 mg/kg bw/day for dogs and 300 mg/kg bw/day for laying hens and broilers.

The current risk assessment was based on the conclusions made by EFSA (EFSA, 2021a,b). The EFSA Panel on Food Additives and Flavourings (FAF; EFSA, 2021a) could not rule out a concern for genotoxicity, and given the many uncertainties, they concluded that E 171 can no longer be considered as safe when used as a food additive. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP; EFSA, 2021b) endorsed this conclusion and considered that it also applies to titanium dioxide as a feed additive for all animal species. Because genotoxicity of titanium dioxide particles cannot be

[1] Commission Regulation (EU) 2022/63 of 14 January 2022 amending Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council as regards the food additive titanium dioxide (E 171). Available via: http://data.europa.eu/eli/reg/2022/63/oj.
[2] Commission Implementing Regulation (EU) 2021/2090 of 25 November 2021 concerning the denial of authorisation of titanium dioxide as a feed additive for all animal species. Available via: http://data.europa.eu/eli/reg_impl/2021/2090/oj.

ruled out, potential concerns on the safety of the additive for the target species (especially for long-living animals and reproductive animals), consumers and users cannot be excluded (EFSA, 2021b). Several other (inter)national bodies have reached a different conclusion that there is insufficient evidence for a concern with respect to genotoxicity. On the one hand, this discrepancy reflects uncertainties in the toxicological database and differences in the test materials considered relevant for the food and feed additive. On the other hand, it reflects a different approach for handling these uncertainties, with EFSA following a more conservative approach.

In the current risk assessment, a distinction is made between short-living animals, i.e. animals for meat production (broilers, piglets and fattening pigs) and long-living animals (companion animals, i.e. cats and dogs), and reproductive animals (dairy cows, laying hens and sows).

For long-living animals and reproductive animals, a potential health risk cannot be excluded because genotoxicity of titanium dioxide particles cannot be ruled out. Due to this potential risk, no safe maximum amount of titanium dioxide as a marker in feed can be established for these animals.

In contrast to these animals, animals for meat production have a relative short life, i.e. their productive lifespan is usually much lower than their natural life expectancy. If any genotoxic and carcinogenic effects would occur, they reveal after longer periods. Therefore, these risks are considered negligible for short-living animals. For these animals the risk of titanium dioxide as a marker in feed was assessed taking into account general and organ toxicity, not genotoxicity and carcinogenicity. In the EFSA opinion on titanium dioxide (E 171) no adverse effects of E 171 were observed related to general and organ toxicity in short term studies up to a daily dose of 1000 mg/kg bw (EFSA, 2021a). In addition, no adverse effects related to general and organ toxicity and developmental and reproductive toxicity were observed in an Extended One-Generation Reproductive Toxicity (EOGRT) study at the highest dose tested of 1000 mg/kg bw/day. Therefore, in the present report a safe daily dose of 10 mg/kg bw is calculated for short-living animals based on a No Observed Adverse Effect Level (NOAEL) of 1000 mg/kg bw/day and an uncertainty factor of 100. The estimated exposure to titanium dioxide as a marker in feed, of all short-living animals taken into account in this assessment is higher than the safe daily dose for general and organ toxicity. Therefore, a risk of adverse health effects due to titanium dioxide cannot be fully excluded. However, it is expected that the risk is low because this safe dose was based on a NOAEL in rats which was the highest dose tested in the study. In general, no adverse general or organ toxicity adverse effects were seen in the studies available.

When considering only animal health for short-living animals, the calculated maximum amounts of titanium dioxide as a marker in feed range from 167 mg/kg feed for broilers to 250 mg/kg feed for fattening pig based on the safe daily dose of 10 mg/kg bw. This is well below the amount of titanium dioxide in feed used in the exposure scenarios for short-living animals as provided by NVWA (5000 mg/kg feed). It should

be noted that the calculated maximum amounts are conservative values, since they are based on a NOAEL in a rat study which was the highest dose tested. It might be considered if lower doses of titanium dioxide are also effective as a marker in feed. Alternatively, tolerance studies in the target species could be performed and be used as a basis for a safe level instead of the data from rats in combination with an uncertainty factors for interspecies differences. Please note that when consumer health is taken into account, no safe maximum amount of titanium dioxide as a marker in feed can be established, since potential health risks for the consumers of the animal products cannot be excluded because genotoxicity of titanium dioxide particles cannot be ruled out. Therefore, it could be investigated if it is feasible to use other alternatives as a marker compound in feed.

Concluding, for long-living animals (companion animals, i.e. cats and dogs), reproductive animals (i.e. dairy cows, laying hens and sows) and consumers of animal products a potential health risk cannot be excluded for the use of titanium dioxide as marker in feed, since a risk of genotoxicity cannot be ruled out for titanium dioxide. Therefore, no safe maximum amount in feed can be determined for these animals.

For short-living animals (animals for meat production, i.e. broilers, piglets and fattening pigs), adverse effects on animal health due to titanium dioxide for general and organ toxicity cannot be fully excluded at the provided use levels, although it is expected that the risk is low. The safe maximum amount of titanium dioxide in feed was estimated to be 167 mg/kg for broilers, 200 mg/kg for piglets and 250 mg/kg for fattening pigs. Please note that when consumer health is taken into account, no safe maximum amount of titanium dioxide as a marker in feed can be established, since potential health risk for the consumers of animal products cannot be excluded because genotoxicity of titanium dioxide cannot be ruled out.

1 Introduction

Titanium dioxide is a white pigment that was used as an additive (E 171) in food and feed in the European Union (EU). Titanium dioxide is also presently used in a wide variety of other products such as (veterinary) medicinal products, personal care products, paints and plastics. In 2022, the EU banned the use of titanium dioxide as a food and feed additive[3,4] following the conclusions of the re-evaluation of titanium dioxide by the European Food Safety Authority (EFSA). The EFSA Panel on Food Additives and Flavourings (FAF) concluded that based on all the evidence available, a concern for genotoxicity could not be ruled out, and E 171 can no longer be considered as safe when used as a food additive (EFSA, 2021a). The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) endorsed this conclusion and considered that it also applies to titanium dioxide as a feed additive for all animal species (EFSA, 2021b).

Titanium dioxide is also regularly used as an inert marker in feed in digestibility studies to aid the estimation of nutrient uptake. Although titanium dioxide is no longer permitted as an additive in animal feed, the NVWA can grant permission for its use as a marker substance[5]. Since such requests ('ontheffingsaanvragen') are frequently received by the NVWA, the NVWA requested RIVM to perform a risk assessment for the use of titanium dioxide as a marker in feed for dairy cattle, pigs, chickens, cats and dogs. The NVWA would like to use this risk assessment to evaluate such requests and – if possible - determine general conditions required for the safe use of titanium dioxide as a marker in feed.

To this aim, the potential risks of the use of titanium dioxide as a marker in test feed for farm animals (dairy cattle, pigs and chickens) are assessed, addressing the health risks for the animals and for the consumer of animal products. In addition, the potential animal health risks for its use in test feed for cats and dogs are assessed. Related to this, the maximum amount of titanium dioxide that can be used as a marker in feed without having adverse health effects for the concerned species and the consumers, was calculated.

Chapter 2 of this report provides an overview of the current European legislation on the use of titanium dioxide in food, feed and (veterinary) medicinal products. Although titanium dioxide is also used in other regulatory frameworks such as cosmetics, these are outside the scope of the current report. Chapter 3 summarizes the conclusions of several existing risk assessments on titanium dioxide used as a food or feed additive. Besides EFSA, the FAO/WHO Joint Expert Committee on Food

[3] Commission Regulation (EU) 2022/63 of 14 January 2022 amending Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council as regards the food additive titanium dioxide (E 171). Available via: http://data.europa.eu/eli/reg/2022/63/oj.
[4] Commission Implementing Regulation (EU) 2021/2090 of 25 November 2021 concerning the denial of authorisation of titanium dioxide as a feed additive for all animal species. Available via: http://data.europa.eu/eli/reg_impl/2021/2090/oj.
[5] Besluit Diergeneesmiddelen 2022, article 2.1. Available via: https://wetten.overheid.nl/BWBR0046416/2022-03-11

Additives (JECFA), as well as several other national and international bodies, have recently performed risk assessments of titanium dioxide. The recent EFSA opinions on titanium dioxide (EFSA, 2021a,b) will be used as a basis for the current risk assessment. Chapter 4 contains the risk assessment and Chapter 5 the discussion and conclusion.

2 Overview of European legislation on titanium dioxide

This report provides an overview of the current European legislation on the use of titanium dioxide in food, feed and (veterinary) medicinal products. Although titanium dioxide is also used in other regulatory frameworks such as cosmetics, these are outside the scope of the current report.

2.1 Food additive

Up to 2022, titanium dioxide used to be authorised as a food additive (E 171) according to Annex II of the Regulation 1333/2008[6]. However, as follow up of the EFSA opinion of 2021 on the re-evaluation of titanium dioxide for use as a food additive (EFSA, 2021a), the use of E 171 as food additive was prohibited[3]. As a result, from 7 August 2022 onwards, it is not allowed to place foods containing E 171 on the market in the EU. Only foods produced before this data may remain on the market until their date of minimum durability or 'use by' date.

Currently, E 171 is listed in Part B1 of Annex II to Regulation 1333/2008[6] because it is still allowed in (veterinary) medicinal products (see section 2.3).

Titanium dioxide used as the food additive E 171 needs to comply with the specifications laid down in Regulation (EC) 231/2012[7].

2.2 Feed additive

Titanium dioxide was authorised as a feed additive (colourant) for all animal species by Directive 70/524/EEC and was included in the register of feed additives in accordance with Regulation (EC) 1831/2003[8]. After re-evaluation by the EFSA FEEDAP Panel (EFSA, 2021b), the use of titanium dioxide as a feed additive was prohibited (Regulation 2021/2090[9]). By 20 March 2022, stocks of titanium dioxide and premixtures containing titanium dioxide had to be withdrawn from the marked. Feed materials and compound feed, containing titanium dioxide or premixtures with titanium dioxide, had to be withdrawn by 20 June 2022.

Titanium dioxide used as feed additive needed to comply with the specifications laid down for the food additive E 171 (EFSA, 2021b).

[6] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. Consolidated version, 28/10/2024. Available via https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02008R1333-20241028.
[7] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. Available via: http://data.europa.eu/eli/reg/2012/231/oj.
[8] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. Available via http://data.europa.eu/eli/reg/2003/1831/oj.
[9] Commission Implementing Regulation (EU) 2021/2090 of 25 November 2021 concerning the denial of authorisation of titanium dioxide as a feed additive for all animal species. Available via http://data.europa.eu/eli/reg_impl/2021/2090/oj.

2.3 (Veterinary) medicinal products

In accordance with Directive 2009/35/EC[10], the use of colours in human and veterinary medicinal products is restricted to those authorized for use in foods according to Regulation (EC) No 1333/2008[6]. Although E 171 is banned since 2022, there is an exception for use as a colour in (veterinary) medicinal products pending the development of adequate alternatives to replace it while ensuring the quality, safety and efficacy of the medicinal products concerned. It was laid down that after three years this has to be evaluated[3].

This exception is based on a scientific analysis provided by EMA in 2021. According to EMA, E 171 is used on a great scale in human and veterinary medicinal products. Approximately 91,000 human medicinal products and 800 veterinary medicinal products contained E 171 in the EU according to EU Trade Associations (EMA, 2021). It has multiple functions in those products, with the main function being a colourant and opacifier, particularly providing protection from UV-light and uniform appearance. Compared to other excipients, titanium dioxide also allows for a thinner, less fragile outer layer of a tablet. The latter helps to ensure that the active ingredient of the medicine becomes available quickly after ingestion (EMA, 2021). Sudden prohibition of E 171 could lead to shortages on the European market, according to EMA. EMA also pointed out that it should be possible, from a technical point of view, to find alternatives, both as colour and for other uses. However, the use of alternatives to replace E 171 needs to be carefully assessed and could take several years depending on the complexity of reformulation and studies required (EMA, 2021).

E 171 may be used in (veterinary) medicines until February 2025. The EC is expected to present new information on the use of titanium dioxide in medicines, reviewing the necessity to maintain E 171 for exclusive use as colour in (veterinary) medicinal products or to delete it from the Union list of food additives. The review of the EC will be based on an updated assessment of the European Medicines Agency (EMA), which includes information on the progress made to develop alternatives, the possible impacts on quality, safety and efficacy, and on the availability of (veterinary) medicinal products[3].

[10] Directive 2009/35/EC of the European Parliament and of the Council of 23 April 2009 on the colouring matters which may be added to medicinal products. Available via: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32009L0035.

3 Overview of existing risk assessments on titanium dioxide used as a food or feed additive

3.1 EFSA

3.1.1 Food additive

Titanium dioxide (E 171) consists of small particles. When EFSA re-evaluated titanium dioxide previously in 2016 they considered that the food additive mainly consisted of microparticles, with a nano-sized fraction (<100 nm) less than 3.2% by mass (EFSA, 2016). However, it was noted that no limits for the particle size were set in the EU specifications. Based on the available information, EFSA concluded that E 171 did not raise concern with respect to genotoxicity and was not carcinogenic via the oral route. A NOAEL of 2250 mg/kg bw/day from a carcinogenicity study in rats (NCI, 1979) was used to calculate a margin of safety. It was concluded that that the use of E 171 was not of concern. However, no health-based guidance value was established due to some uncertainties and data gaps. EFSA recommended that an extended one-generation reproduction toxicity (EOGRT) study should be performed, and that EU specifications for E 171 should include a better description of the particle characteristics.

Based on new information, EFSA recommended in 2019 that the EU specifications for E 171 should include additional parameters related to its particle size distribution including a number-based distribution median value of the minimum external dimension (>100 nm) measured by electron microscopy, which is equivalent to less than 50% of constituent particles by number with a minimum external dimension <100 nm (EFSA, 2019).
E 171 usually contains a fraction of nano-sized particles <100 nm, but based on the presence of the fraction of nanoparticles in E 171, it falls under the scope of the EFSA Guidance on nanotechnology, which was broadened in its 2018 revision to also cover materials containing a fraction of particles, less than 50% in the number–size distribution (EFSA, 2018).
Therefore, the re-evaluation of titanium dioxide in 2021 was performed in line with the data requirements specified in this EFSA Guidance on nanotechnology (Note that the EFSA guidance on nanotechnology was updated in 2021 (EFSA, 2021c,d).

EFSA (2021a) reviewed studies with E 171, which contains both nanoparticles and larger particles, and studies with titanium dioxide nanoparticles (>30 nm). However, the Panel considered that studies with titanium dioxide nanoparticles <30 nm were of limited relevance to the safety assessment of E 171, since their occurrence in the food additive is low (<1% by number).

EFSA concluded that E 171 has a low oral systemic availability, probably less than 0.5%. They also concluded that E 171 may pass the placenta. In studies in rats with titanium dioxide nanoparticles with sizes between 7 and 90 nm, half-lives of around 200 to 450 days were observed. EFSA concluded that although the gastrointestinal absorption of titanium

dioxide nanoparticles is low, they may accumulate in the body due to their long half-life.

No adverse effects of E 171 were observed related to general and organ toxicity in short term studies up to a daily dose of 1000 mg/kg bw (no higher doses were tested). For titanium dioxide nanoparticles (>30 nm) tested at daily doses of up to 100 mg/kg bw (no higher doses tested) for up to 90 days, also no adverse effects were observed in general and organ toxicity studies.

In addition, no adverse effects related to developmental and reproductive toxicity were observed in an Extended One-Generation Reproductive Toxicity (EOGRT) study at the highest dose tested of 1000 mg/kg bw/day. However, EFSA noted that some findings related to immunotoxicity and inflammation with E 171 and neurotoxicity with titanium dioxide nanoparticles, together with the potential induction of aberrant crypt foci with E 171, may indicate adverse effects.

In the previous evaluation (EFSA, 2016), EFSA evaluated a carcinogenicity study in mice and rats with E 171 mixed through the diet (NCI, 1979) and concluded that E 171 was not carcinogenic to rats and mice. However, in its 2021 opinion, EFSA concluded that this study was not appropriate to conclude on the chronic toxicity and carcinogenicity of titanium dioxide nanoparticles.

EFSA reviewed over 300 studies on genotoxicity and concluded that titanium dioxide nanoparticles have the potential to induce DNA strand breaks and chromosomal damage, but not gene mutations. Results appeared to be independent of the particle size of titanium dioxide particles, and no clear cut-off value for titanium dioxide particle size could be defined. Based on all the evidence available, EFSA could not rule out a concern for genotoxicity of nanoparticles that may be present in E 171, and given all uncertainties, they concluded that E 171 can no longer be considered as safe when used as a food additive.

3.1.2 Feed additive

In 2021, EFSA evaluated the safety of titanium dioxide as feed additive for all animal species (EFSA, 2021b). Besides the safety for the target species and the consumer, EFSA also evaluated the safety for the user and for the environment, as well as the efficacy of the feed additive. These latter aspects were outside the scope of the current risk assessment and not summarized here. Titanium dioxide used as feed additive needed to comply with the specifications laid down for the food additive E 171 (EFSA, 2021b), indicating that it concerns the same substance. Like for food additives, EFSA concluded that titanium dioxide used as feed additive can no longer be considered as safe for all animal species. Although the oral bioavailability of titanium dioxide is low, particles of titanium dioxide can accumulate in the body due to their long half-life. Because genotoxicity of titanium dioxide particles cannot be ruled out, this raises potential concerns on the safety of the additive for the target species (especially for long-living animals and reproductive animals) and consumers. It was noted by EFSA that specific data in target species were absent. In addition, no data on the absorption, distribution, metabolism and excretion of titanium dioxide in

target species were available. Also, no residue studies in relevant food-producing target species were available. Therefore, EFSA was not able to conclude on the safety of titanium dioxide for target species and consumers.

To explore if more recent toxicological studies in target species have become available, a literature search was performed to collect all toxicological studies in cattle, pigs, chickens, cats and dogs published after the EFSA opinion, however, no new studies were identified (Annex 1).

3.2 Assessments by other institutes

3.2.1 JECFA

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) re-evaluated the safety of titanium dioxide (International Numbering System for Food additives (INS) No. 171) in 2023 (FAO/WHO, 2024a,b). Previously, in 1969, the Committee evaluated titanium dioxide and established an Acceptable Daily Intake (ADI) "not specified" based on an absence of significant absorption and a lack of toxicological effects in the available laboratory animal and human studies at that time (FAO/WHO, 1970).

INS171 is equivalent to E 171, except that the titanium dioxide coating of pearlescent pigments is included in the specifications of specific types of E 171, but not for INS171 (FAO/WHO, 2024a,b). The titanium dioxide coating of pearlescent pigments (INS176) was therefore not considered in the re-evaluation by JECFA.

JECFA considered studies using E 171, as well as with two other specified test substances, relevant for the their evaluation. JECFA evaluated studies with titanium dioxide nanoparticles in the re-evaluation, but it was concluded that these were not representative of INS171 because these have significantly lower particle size distribution ranges.
JECFA concluded that gastrointestinal absorption of INS171 is very low. They did not specifically address accumulation of INS171 (nanoparticles) in the body. No adverse effects of INS171 were observed in short term toxicity studies with daily doses tested up to 15 g/kg bw and 5 g/kg bw in mice and rats, respectively. In addition, JECFA concluded that there was no evidence for carcinogenicity, and reproductive or developmental toxicity. JECFA noted that the available data did not provide convincing evidence of genotoxicity for INS171, but recognized the limitations in current methodologies particular with respect to the testing of poorly soluble particulate materials. Although there were uncertainties in the genotoxicity data, JECFA noted that INS171 was not carcinogenic in an 2-year study in rats and mice considered adequate (NCI, 1979). Epidemiological studies investigating the relation between titanium dioxide exposure and health effects were deemed insufficient to draw any conclusions. Altogether, JECFA concluded that considering the very low oral absorption of INS 171, and in the absence of any identifiable hazard associated with INS 171 in the diet, the ADI "not specified" could be reaffirmed.

3.2.2 National bodies outside the EU

Recent national assessments from the United Kingdom (COT, 2024), Canada (Health Canada, 2022) and Australia and New Zealand (FSANZ, 2022) are available and will be discussed in more detail below.

3.2.2.1 Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment

The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) of the UK evaluated titanium dioxide used as food additive in 2022 and updated their statement in 2024 (COT, 2024). They considered studies on both E 171 and titanium dioxide nanoparticles but deemed the latter less relevant for E 171. The COT concluded that there was little evidence in the literature to suggest that there was a health concern related to genotoxicity induction by titanium dioxide, especially for exposure to the micro sized titanium dioxide fraction via the oral route. COT noted that most studies in the literature showing adverse effects in genotoxicity used nano-sized material. Therefore, they considered the genotoxicity risk from E 171 to be low. In addition, the COT considered that the data indicated that titanium dioxide did not induce aberrant crypt foci, inflammation and immunotoxicity, reproductive and developmental toxicity, and neurotoxicity. The COT derived a health based guidance value of 10 mg/kg bw/day, based on a NOAEL of 1000 mg/kg bw/day obtained from an extended one generation test and using an uncertainty factor of 100 (a factor 10 for interspecies variability and a factor 10 for individual variability) (COT, 2024).

3.2.2.2 Health Canada

Health Canada evaluated the safety of titanium dioxide in 2022 (Health Canada, 2022). They concluded that titanium dioxide is poorly absorbed in the gastrointestinal tract. According to Health Canada, titanium dioxide containing nanoparticles may produce toxic effects in various test systems when dispersed and stabilized in simple matrices such as water but not when mixed with food. They therefore considered studies with titanium dioxide dispersed in water as less relevant than titanium dioxide mixed with food, since titanium dioxide used as food additive in food is present in the form of larger agglomerates that are not dispersed to the same degree. In addition, they noted that proteins and other macromolecules present in food adsorb to titanium dioxide particles, forming a “corona” that alters particle size and the surface characteristics that govern their interactions with the gut environment. Also, according to Health Canada, the physical-chemical characteristics of titanium dioxide may result in reactive radicals, potentially introducing artifacts in toxicity testing. Overall, Health Canada’s Food Directorate did not identify any compelling health concerns for the use of titanium dioxide as a food additive. They identified uncertainties in the database but judged that these data gaps were not significant enough to warrant a more precautionary approach at the time of evaluation (Health Canada, 2022).

3.2.2.3 Food Safety Australia and New Zealand

In 2022, Food Safety Australia and New Zealand (FSANZ) performed a risk assessment on titanium dioxide used as a food additive (FSANZ 2022). Like the COT and Health Canada, FSANZ concluded that there is

no evidence to suggest that dietary exposures to food-grade titanium dioxide are of concern for human health. According to FSANZ, the concerns of titanium dioxide as a food additive are primarily based on studies with titanium dioxide nanoparticles or on studies in which food-grade titanium dioxide has been dispersed in water by sonication and administered by oral gavage or via drinking water. They questioned the relevance of such studies for assessing the human health risk from dietary exposure to food-grade titanium dioxide for similar reasons as Health Canada (see section 3.1.3.2). Titanium dioxide in food is not sonicated. In addition, there is no evidence that titanium dioxide in foodstuffs is broken down into smaller particles in the gastrointestinal tract. Moreover, exposure to titanium dioxide via gavage or drinking water does not take into the proactive effect (‘corona’ formation) of the food matrix (FSANZ, 2022).

3.3 Differences and uncertainties in the assessments

Compared to the data available for the EFSA evaluation, no new key studies were identified that were used in the more recent evaluations by other institutes. An important difference between the EFSA assessment and the other recent assessments is that EFSA also considered and took into account studies with titanium dioxide nanoparticles (particle size >30 nm) in their assessment, whereas in the other assessments, these studies were considered not, or less relevant. Also, EFSA included studies in which titanium dioxide was dispersed in drinking water, whereas these were excluded by others. In addition, other authorities consider *in vivo* studies in which animals are not exposed through the diet (e.g. oral gavage studies) as less representative for human oral exposure in case of the food additive. EFSA, on the other hand, considers studies in which the titanium dioxide is well dispersed using high-energy dispersion methods, best known in oral gavage studies, as able to cover the risks.

Apart from the differences between the risk assessments mentioned above, all risk assessments acknowledged that there are uncertainties in the toxicological database, especially related to the possible effects of the nanoparticles in titanium dioxide. These uncertainties are handled in different ways. EFSA concluded that given the uncertainties in genotoxicity, possible genotoxic effects of titanium dioxide cannot be excluded, and that therefore, also in combination with other uncertainties in the data, it is uncertain whether the use of titanium dioxide as a food additive is safe. In contrast, the other institutes concluded that there is insufficient evidence for a concern with respect to genotoxicity (RIVM, 2024).

4 Risk assessment

4.1 Exposure assessment

To assess the risk of the use of titanium dioxide as a marker in feed, first the exposure of the animals to titanium dioxide has to be estimated. The NVWA has provided data on the requested species, including concentrations in feed and exposure durations based on the requests ('ontheffingsaanvragen') that were received for the use of titanium dioxide as a marker in feed from applicants. These are listed in Table 1. This was combined with default body weights and feed intake values reported by EFSA (2012) and by the United States National Research Council (NRC, 2006). The exposure to titanium dioxide was estimated for all target species (Table 1).

Table 1 Exposure scenarios for different animal species considered for the risk assessment of titanium dioxide.

Species	Body weight (kg)	Feed intake (kg/day)	Concentration titanium dioxide (g/kg)[c]	Exposure duration (days)[c]	Calculated exposure (mg/kg bw/day)
Broiler	2[a]	0.12[a]	5	36	300
Piglet	20[a]	1[a]	5	42	250
Fattening pig	50[a]	2[a]	5	42	200
Sow (lactating)	200[a]	6[a]	5	28	150
Laying hen	2[a]	0.12[a]	5	36	300
Dairy cow	650[a]	20[a]	1	42	31
Cat	4[b]	0.06[b]	1	28	15
Dog	25[b]	0.36[b]	1	28	14

[a] EFSA FEEDAP (2012); [b] NRC (2006); [c] As provided by NVWA

4.2 Risk assessment

In the current risk assessment, the potential risks of titanium dioxide as a marker in test feed on animals health is assessed. For the risk assessment, we assumed that titanium dioxide used as an inert marker in animal feed complies with the specifications of E 171. The recent EFSA opinions on titanium dioxide (EFSA, 2021a,b) will be used as a basis for the current risk assessment. A distinction is made between short-living animals, i.e. animals for meat production (broilers, piglets and fattening pigs) and long-living animals (companion animals, i.e. cats and dogs), and reproductive animals (dairy cows, laying hens and sows). Also, the health risk for the consumer of animal products is assessed.

This risk assessment is conducted in accordance with the EFSA guidance on the assessment of the safety of feed additives for the target species (EFSA, 2017a) and the EFSA guidance on the assessment of the safety of feed additives for consumers (EFSA, 2017b). With respect to genotoxicity, these guidances only describe how to determine if a

substance is genotoxic. In the EFSA guidance on the submission of data for food additives (EFSA, 2021e), EFSA recommended to use the threshold of toxicological concern (TTC) for genotoxic compounds of 0.15 µg/person/day for unavoidable impurities in food additives that are carcinogenic and genotoxic and for which no carcinogenicity data are available. Such approach is not included in the guidances for feed additives.

EMA developed a guideline for setting acceptable limits, for genotoxic impurities that arise during synthesis and/or storage of a drug substance in the (veterinary) medicinal product, that are expected to pose negligible carcinogenic risk in target species and the human consumer (EMA, 2018), using the TTC for substances for which no carcinogenicity data are available.

The guideline is not intended for intentionally added substances, however, the approach outlined in the EMA guideline is used in the current risk assessment to give an indication of the (potential) risk of genotoxicity for the target species by comparing the estimated exposure for the target species and the consumer to a level that is acceptable for genotoxic impurities in veterinary medicines. As this approach covers both animals and humans, this method was used.

4.2.1 Short-living animals

For short-living animals (animals for meat production, i.e. broilers, piglets, and fattening pigs) the productive lifespan is usually much lower than their natural life expectancy. If any genotoxic and carcinogenic effects would occur, they reveal after longer periods. Therefore, these risks are considered negligible for short-living animals. For these animals, the risk of titanium dioxide as a marker in feed was assessed for general and organ toxicity, not for genotoxicity and carcinogenicity.

No toxicity studies in the target species are available. According to the EFSA guidance on the assessment of the safety of feed additives for the target species (EFSA, 2017a), an appropriate NOAEL or benchmark dose lower confidence level (BMDL) can also be derived from toxicity studies in laboratory animals (EFSA, 2017a). To derive a safe daily dose in the target species, this point of the departure should then be divided by an uncertainty factor of 100 accounting for intra- an interspecies differences.

In the available toxicity data of titanium dioxide in laboratory animals, no adverse effects related to general and organ toxicity were observed up to a daily dose of 1000 mg/kg bw. It has to be noted that there were some uncertainties regarding immunotoxicity, neurotoxicity and inflammation. In an EOGRT Study, no adverse effects related to general and organ toxicity or developmental and reproductive toxicity were observed in rats exposed to up to 1000 mg/kg bw/day titanium dioxide (E 171) up to 18 weeks. According to EFSA, immunotoxicity was not sufficiently assessed in this study, hence no conclusion could be drawn on this aspect based on this study. In the current risk assessment, a NOAEL of 1000 mg/kg bw/day for general and organ toxicity was identified in this study.

It is noted that in an earlier risk assessment EFSA identified a NOAEL of 2250 mg/kg bw/day (the highest dose tested) in a carcinogenicity study in rats and mice (EFSA, 2016). However in the most recent risk assessment, EFSA considered that this study was not appropriate to ascertain the absence of a potential to elicit chronic toxicity and carcinogenicity by titanium dioxide nanoparticles, presumably because of the test substance used. This NOAEL is therefore not used in the current risk assessment.

In the current risk assessment the NOAEL from the EOGRT study was used. In accordance with the EFSA guidance (EFSA, 2017a), the NOAEL of 1000 mg/kg bw/day is divided by an uncertainty factor of 100 (10 for interspecies, 10 for intraspecies differences) to determine a safe daily dose in the target species of 10 mg/kg bw for animal health. This safe daily dose can be compared to the estimated exposure as shown in Table 1. It can be concluded that the estimated exposure of food-producing animals to titanium dioxide, which ranges between 200 mg/kg bw/day for fattening pigs and 300 mg/kg bw/day for broilers, is a factor 20 to 30 higher than the safe daily dose. Therefore, a risk for general and organ toxicity cannot be fully excluded for these exposure scenarios. However, it is expected that the risk is low, because the safe dose was based on a NOAEL in rats which was the highest dose tested in the study. In general, no adverse general or organ toxicity adverse effects were seen in the studies available, whereas on the other hand, there are remaining uncertainties for titanium dioxide regarding immunotoxicity, neurotoxicity and inflammation upon oral exposure. Please note that this assessment only considers animal health, a potential health risk cannot be excluded for consumers of animal products since genotoxicity cannot be ruled out.

4.2.2 Long-living animals and reproductive animals

EFSA could not rule out genotoxicity of titanium dioxide based on all the evidence available raising potential concern for target species especially long-living animals and reproductive animals (EFSA, 2021b). Therefore, potential health risks cannot be excluded for dogs, cats, dairy cows, laying hens and sows.

To give an indication of the (potential) risk of genotoxicity for the target species, the estimated exposure was compared to a level that is acceptable for genotoxic impurities in veterinary medicines, using the following approach as described in the EMA guideline for genotoxic impurities (EMA, 2018):

- From a target species safety perspective, acceptable limits for mutagenic impurities in veterinary medicinal products (VMPs) corresponding to an intake with a theoretical 1 in 100,000 excess lifetime risk of cancer can be justified. This represents a small theoretical increase in risk when compared to overall lifetime incidence of developing any type of cancer, but is acceptable as the animal is expected to receive a health benefit from the VMP.
- Acceptable intakes can be derived using substance specific carcinogenicity data for the concerned impurity, or be based on the threshold of toxicological concern for genotoxic carcinogens, resulting in an acceptable intake of 0.025 µg/kg bw/day. Established cancer risk assessments are based on daily lifetime

exposures. Estimation of risk based on less-than-lifetime exposures can result in higher acceptable intakes of impurities for a short time that correspond to a similar risk level. For this approach the acceptable cumulative lifetime exposure is uniformly distributed over the actual exposure days of the animal.

The available substance specific carcinogenicity studies were not appropriate to conclude on the chronic toxicity and carcinogenicity of titanium dioxide (nanoparticles). Therefore, the estimated exposure is compared to the threshold of toxicological concern of 0.025 µg/kg bw/day. The estimated exposure of long-living animals and reproductive animals (14,000-300,000 µg/kg bw/day; see Table 1) is much higher that the threshold of toxicological concern. As a refinement the less-than-lifetime approach is used, because the animals will only be exposed to titanium dioxide for a short time. The threshold of toxicological concern corrected for less-than-lifetime exposure is calculated for cats and dogs separately, using the following formula (EMA, 2018):

$$= \frac{0.025\ (\mu g/kg\ bw/day) * 365\ (days) * expected\ lifespan\ (years)}{study\ duration\ (days)}$$

The threshold of toxicological concern corrected for less-than-lifetime exposure ranges from 1.8 µg/kg bw/day for laying hens to 4.9 µg/kg bw/day for sows (Table 2). Again it can be concluded that the estimated exposure of dogs, cats, dairy cows, laying hens and sows is over 3000 times higher than the threshold of toxicological concern (after correcting for less-than-lifetime exposure). This indicates that the estimated exposure in the used scenarios highly exceeds the level that is acceptable for genotoxic impurities in veterinary medicines. It is noted that EMA justifies a theoretical 1 in 100,000 excess lifetime risk of cancer, as the animals have a benefit from the VMPs, while in general, when there is no benefit, a 1 in 1,000,000 excess lifetime risk of cancer is considered acceptable. In the current case titanium dioxide is added as a colouring agent in feed, therefore there is no health benefit and it could be argued that the threshold of toxicological concern used should be lower. This will however not affect the conclusion, as the estimated exposure would still exceed a lower threshold of toxicological concern.

Table 2 Estimated exposure in long-living animals and reproductive animals and the threshold of toxicological concern corrected for less-than-lifetime (LTL) exposure.

Animal	Exposure (µg/kg bw/day)	TTC (µg/kg bw/day)	Expected lifespan (years)	Exposure duration (days)	TTC corrected for LTL exposure (µg/kg bw/day)
Sow (lactating)	150,000	0.025	15[a]	28	4.9
Laying hen	300,000	0.025	7[b]	36	1.8
Dairy cow	31,000	0.025	17[c]	42	3.7
Cat	15,000	0.025	14[d]	28	4.6
Dog (beagle)	14,000	0.025	12[e]	28	3.9

[a] https://vetmed.iastate.edu/sites/default/files/vcs/Pot%20Bellied%20Pig%20Care.pdf
[b] https://livestock.extension.wisc.edu/articles/life-cycle-of-a-laying-hen/
[c] https://sentientmedia.org/how-long-do-cows-live/
[d] https://mcvoordieren.nl/kennisbank/hoe-oud-wordt-een-kat/
[e] https://www.beaglewereld.nl/hoe-oud-wordt-een-beagle/

It addition to the potential risks related to genotoxicity, it is noted that the exposure of long-living animals and reproductive animals in the exposure scenarios considered is higher than the safe daily dose that was estimated for short-living animals for general and organ toxicity. Therefore, a risk for general and organ toxicity cannot be fully excluded in these scenarios, although is it expected that this risk is low. This is because this safe dose was based on a NOAEL that was set at the highest dose tested, and because no adverse effect related to general and organs toxicity were found in studies with laboratory animals.

4.2.3 *Consumer safety*

For all food producing animals the (potential) risk for the consumer of animal products needs to be assessed. This includes short-living animals, even though genotoxicity may not be considered of concern for animal health because of their relatively short life time.
EFSA could not rule out genotoxicity of titanium dioxide based on all the evidence available raising potential concern for the consumer of animals that were given titanium dioxide in feed (EFSA, 2021b). The current risk assessment was based on the conclusion of EFSA. A potential health risk for consumers can therefore no be excluded when titanium dioxide is used as marker in feed, since genotoxicity cannot be ruled out.
To give an indication of the (potential) risk of genotoxicity for the consumer, the estimated exposure was compared to a level that is acceptable for genotoxic impurities in veterinary medicines using the pragmatic approach as described in the EMA guideline for genotoxic impurities (2018) is used.

In the context of this guideline the 'virtually safe dose' to assess consumer safety is a level of human exposure to a genotoxic carcinogen associated with a tumour incidence of ≤ 1 in 10^6. This means that the exposure to this dose during the whole lifetime is associated with an

additional cancer risk of 1 in 1,000,000. The virtually safe dose can be calculated based on substance-specific carcinogenicity data or – in the absence of substance-specific data – the threshold of toxicological concern approach considers a dose of 0.0025 µg/kg bw/day or 0.15 µg/person/day.

According to the pragmatic approach of EMA, it is assumed that the amount ingested by the consumer will be lower than the 'virtually safe dose', associated with a tumour incidence of ≤ 1 in 10^6, if the amount ingested by the target species will be below the threshold of toxicological concern for the target species (see section above) of 0.025 µg/kg bw/day. In other words, if the exposure is safe for the target species it will also be safe for the consumer of this species, taking into account that the substance will be distributed, metabolized and (partly) excreted in the target species before consumption.

Estimation of risk based on the less-than-lifetime approach is not applicable in this situation, because the potential consumer exposure to residues could be chronic even if target species exposure is for only a short duration.

It can be concluded that the estimated exposure to titanium dioxide for the target species (ranging from 31,000 – 300,000 µg/kg bw/day; Table 1) highly exceeds the threshold of toxicological concern (0.025 µg/kg bw/day). This indicates that in the used scenarios the 'virtual safe dose' that is acceptable for genotoxic impurities in veterinary medicines is exceeded.

For fine tuning of the consumer risk, data need to be available on the distribution of titanium dioxide in the body of the target species (or actual residue data) combined with the consumption data of the edible commodities. This should then be compared with the threshold of toxicological concern of 0.0025 µg/kg bw/day for human exposure, associated with a tumour incidence of ≤ 1 in 10^6.

4.2.4 Safe maximum amount in feed

For long-living animals and reproductive animals and consumers a safe maximum amount for titanium dioxide as a marker in feed cannot be established, as the potential risk for genotoxicity could not be excluded.

For short-living animals a safe maximum amount in feed can be calculated by multiplying the estimated safe daily dose in the target species (10 mg/kg bw; see section 4.2.1) by the default body weight of the animal and dividing by the default feed intake listed in Table 1. The calculated safe maximum amounts in feed are 167 mg/kg feed for broilers, 200 mg/kg feed for piglets and 250 mg/kg feed for fattening pigs. This is below the amount of titanium dioxide in feed used in the exposure scenarios for short-living animals as provided by NVWA (5000 mg/kg feed; see Table 1). It should be noted that the estimated safe daily dose in the target species may be conservative as it is derived from a the highest dose administered in a rat study. Tolerance studies in target species, or toxicity studies in laboratory animals using higher dose levels, would be required to investigate if higher values are safe. Please note that when consumer health is taken into account, no safe

maximum amount of titanium dioxide as a marker in feed can be established, since potential health risk for the consumers of animal products cannot be excluded because genotoxicity of titanium dioxide cannot be ruled out.

5 Discussion and conclusion

5.1 Discussion

In the current risk assessment, the recent EFSA opinions on titanium dioxide (2021a,b) were used as basis. For reproductive animals (i.e. dairy cows, laying hens and sows) and long-living animals (companion animals, i.e. cats and dogs) and for consumers of animal products, it cannot be concluded that the use of titanium dioxide as a marker in test feed is safe, because potential genotoxicity of titanium dioxide cannot be ruled out. However, as described in Chapter 3, several other (inter)national bodies have reached the different conclusion that there is insufficient evidence for a concern with respect to genotoxicity. On the one hand, this discrepancy reflects uncertainties in the toxicological database and uncertainties in the relevance of the composition of the test materials used. On the other hand, it reflects a different approach for handling these uncertainties, with EFSA following a more conservative approach. A quick search for recent literature showed that >30 publications studying or reviewing the genotoxicity of titanium dioxide have been published since the publication of the EFSA opinion (data not shown), indicating that research into the genotoxicity of titanium dioxide is ongoing. Well-conducted and suitable genotoxicity studies with representative test materials would be required to decrease the uncertainty with respect to the genotoxicity of titanium dioxide.

For short-living animals (animals for meat production, i.e. broilers, piglets and fattening pigs) the health risk was assessed for general and organ toxicity, since the risks for genotoxicity and carcinogenicity were considered negligible given their short lifespan. When considering only animal health aspects, safe maximum amounts in feed were calculated for these animals based on a NOAEL obtained from a study in rats. These values might be conservative as they were based on a NOAEL which was the highest dose tested in the study and, in general, no general or organ toxicity adverse effects were seen in the studies available.
The calculated safe maximum amounts are lower than the use levels provided by the NVWA (a factor 20-30, depending on the species). It might be considered if lower doses of titanium dioxide would be also effective as a marker in feed. Alternatively, tolerance studies in the target species could be performed and be used as a basis for a safe level instead of the data from rats in combination with an uncertainty factor for interspecies differences. In addition, since potential health risks for the consumer cannot be excluded, it could be investigated if it is feasible to use other alternatives as a marker compound in feed.

Although the use of titanium dioxide as an additive in food and feed is banned in the EU, titanium dioxide can still be used as an excipient in human and veterinary medicines. This status is temporary, pending the development of adequate alternatives to replace it, while ensuring the quality, safety and efficacy of the medicinal products concerned, or regulatory alterations ensuring that it can be used as an excipient in future. For medicines, both the risks and the benefits are taken into

account, while compounds deliberately added to food and feed should be safe. Developments of alternatives for titanium dioxide in medicinal products could also be relevant for the application in food and feed.

5.2 Conclusion

For long-living animals (companion animals, i.e. cats and dogs), reproductive animals (i.e. dairy cows, laying hens and sows) and consumers of animal products a potential health risk cannot be excluded for the use of titanium dioxide as marker in feed, since a risk of genotoxicity cannot be ruled out for titanium dioxide. Therefore, no safe maximum amount in feed can be determined for these animals.

For short-living animals (animals for meat production, i.e. broilers, piglets and fattening pigs), the estimated exposure based on the exposure scenarios provided for the use of titanium dioxide as a marker in feed exceed the calculated safe daily dose. Therefore, adverse effects on animal health due to titanium dioxide for general and organ toxicity cannot be excluded at the provided use levels. However, it is expected that the risk is low, because the safe dose was based on a NOAEL in a rat study which was the highest dose tested. In general, no general or organ toxicity adverse effects were seen in the studies available. Based on the safe daily dose, the safe maximum amount of titanium dioxide in feed was estimated to be 167 mg/kg for broilers, 200 mg/kg for piglets and 250 mg/kg for fattening pigs. Please note that when consumer health is taken into account, no safe maximum amount of titanium dioxide as a marker in feed can be established, since potential health risk for the consumers of animal products cannot be excluded because genotoxicity of titanium dioxide cannot be ruled out.

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Annex 1 Literature search toxicity studies in farm animals, cats and dogs

In October 2024 a literature search was conducted to collect information about adverse effects of titanium dioxide in farm animals and cats and dogs. Both EMBASE and PubMed were search. See Table 3 for the specific search strings and number of results.

Table 3 Overview of the search strings used to collect articles studying the toxicity of titanium dioxide in farm animals and cats and dogs and the number of results.

PubMed	Embase
(("titanium dioxide"[Title/Abstract]) OR (e171[Title/Abstract]) OR (tio2[Title/Abstract])) AND ((ruminant[Title/Abstract]) OR (cow[Title/Abstract]) OR (poultry[Title/Abstract]) OR (broiler[Title/Abstract]) OR ("laying hen"[Title/Abstract]) OR (pig[Title/Abstract]) OR (sow[Title/Abstract]) OR (piglet[Title/Abstract]) OR (cat[Title/Abstract]) OR (dog[Title/Abstract])) AND (("acute toxicity"[Title/Abstract]) OR ("subacute toxicity"[Title/Abstract]) OR ("sub acute toxicity"[Title/Abstract]) OR ("chronic toxicity"[Title/Abstract]) OR ("subchronic toxicity"[Title/Abstract]) OR ("sub chronic toxicity"[Title/Abstract]) OR ("long term toxicity"[Title/Abstract]) OR (genotoxicity[Title/Abstract]) OR (carcinogenicity[Title/Abstract]) OR ("reproductive toxicity"[Title/Abstract]) OR ("developmental toxicity"[Title/Abstract]))	('titanium dioxide':ab,ti OR e171:ab,ti OR tio2:ab,ti) AND (ruminant:ab,ti OR cow:ab,ti OR poultry:ab,ti OR broiler:ab,ti OR 'laying hen':ab,ti OR pig:ab,ti OR sow:ab,ti OR piglet:ab,ti OR cat:ab,ti OR dog:ab,ti) AND ('acute toxicity':ab,ti OR 'subacute toxicity':ab,ti OR 'sub acute toxicity':ab,ti OR 'chronic toxicity':ab,ti OR 'subchronic toxicity':ab,ti OR 'sub chronic toxicity':ab,ti OR 'long term toxicity':ab,ti OR genotoxicity:ab,ti OR carcinogenicity:ab,ti OR 'reproductive toxicity':ab,ti OR 'developmental toxicity':ab,ti)
23 results	20 results (4 unique)

Title and abstract of the studies were screened to find relevant studies. None of studies were found relevant.

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National Institute for Public Health and the Enviroment, RIVM
P.O. Box 1 | 3720 BA Bilthoven
The Netherlands
www.rivm.nl/en

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