Color Additives Commonly Used in Medical Device Polymeric Materials: Derivation of Provisional Tolerable Intake (pTI) values

Table of Contents

Purpose

The purpose of this work is to document the approach used to derive generally applicable provisional tolerable intake (pTI) values for color additives commonly used in medical devices. The approach used applied uncertainty factors to extrapolate an experimentally derived no-observable-adverse-effect-levels (NOAELs) to the general human population. This uncertainty approach is described in ISO 10993-17:2002(2012). If the toxicological data was inadequate to establish a pTI, then the applicable threshold of toxicological concern was applied in accordance with ISO TS 21726:2019.

Note: Tolerable intake (TI) is an estimate of the daily exposure of a chemical substance over a specified time period that is considered to be without appreciable harm to health (ISO 10993-17:2002(2012)). TIs are calculated on the basis of body mass, and are specific to systemic toxicity (i.e., acute, subacute, subchronic, and chronic systemic toxicity, genotoxicity, carcinogenicity, and reproductive/developmental toxicity). In this document, pTIs are expressed as milligram per kilogram body weight per day (mg/kg/d).

Note: The term "provisional" is used to distinguish these values from TI values that apply to a specific medical device and its intended use, and accommodates the discovery of new toxicological evidence that will require refinement of the pTI value.

Note: The derivation of a pTI value is not an estimation of the probability that harm will occur.

Note: "Generally applicable" means the pTI values are protective for organ-specific harms or other harms that occur in tissue other than at the local site of contact (i.e., systemic toxicity). The pTI values are also protective for non-genotoxic mediated cancer and reproductive/developmental toxicities based on the available color additive specific toxicological data. In addition to the nature of the harm, "generally applicable" means the derived pTI values are protective for exposure that occurs by oral, dermal or intravenous routes, as well as for adult and pediatric populations. Lastly, the pTI values apply to impurities that are naturally present as a result of the source of the color additive (e.g., material mined from the earth) and standard processes used in the manufacture of the particular color additive.

Note: The pTI values do not apply to individuals with unique physiology not represented by the general human population, nor do they apply to unique impurities that result from supplier-specific processes (e.g., color addtive supplier or medical device manufacturer that introduces a contaminant as a result of a novel manufacturing process. Approaches for addressing these impurities are addressed in the CDRH CHRIS documentation.

The toxicological information in this report is intended for, but not limited to, supporting the development of the Center for Devices and Radiological Health (CDRH) Color Additive Hazard Risk calculator (CHRIS), available at https://dsaylor.github.io/CHRIS/.

Background

Definitions of color additives, pigments, and dyes

By definition, a color additive is any dye, pigment, or other substance not exempted by regulation and applied to a food, drug, cosmetic, or to the human body that is capable (alone or through reaction with another substance) of imparting a color to the food, drug, or cosmetic or to the human body (FDA 2020 21CFR70.3)[1]. Although this regulatory definition of a color additive does not mention medical devices, some medical devices are subject to the color additive regulatory requirements per FD&C Act of 1938[2].

[1] FDA (2020) 21CFR70.3(f) color additive. Definitions. General Provisions. Food and Drugs. Food and Drug Administration (FDA). Department of Health and Human Services (HHS). [WWW Document] URL https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm

[2] FDA (2018) Part IV: Regulating Cosmetics, Devices, and Veterinary Medicine After 1938 [WWW Document] URL https://www.fda.gov/about-fda/changes-science-law-and-regulatory-authorities/part-iv-regulating-cosmetics-devices-and-veterinary-medicine-after-1938

Note: Per regulation 21CFR70.3(f), the term color additive is defined as "any material, not exempted under section 201(t) of the act, that is a dye, pigment or other substance made by a process of synthesis or similar artifice, or extracted, isolated, or otherwise derived, with or without intermediate or final change of identity, from a vegetable, animal, mineral, or other source and that, when added or applied to a food, drug, or cosmetic or to the human body or any part thereof, is capable (alone or through reaction with another substance) of imparting a color thereto.. Substances capable of imparting a color to a container for foods, drugs, or cosmetics are not color additives unless the customary or reasonably foreseeable handling or use of the container may reasonably be expected to result in the transmittal of the color to the contents of the package or any part thereof."[1,3]

Note: FDA republished the regulatory history of color additive in the United States by Barows et al. (2003), which states: "The Color Additive Amendments of 1960 defined "color additive" and required that only color additives (except coal-tar hair dyes) listed as "suitable and safe" for a given use could be used in foods, drugs, cosmetics, and medical devices. The 1960 Amendments prescribed the factors that FDA must consider in determining whether a proposed use of a color additive is safe, as well as the specific conditions for safe use that must be included in the listing regulation. FDA updated the procedural regulations for the petition process in response to these amendments. Under these amendments, the approximately 200 color additives that were in commercial use at the time were provisionally listed and could be used on an interim basis until they were either permanently listed or terminated due to safety concerns or lack of commercial interest. Permanently listing a color additive for a proposed use was prohibited unless scientific data established its safety."[4,5]

The color additives mentioned in this report are either a pigment or a dye. Although a regulatory definitions of a dye and a pigment is not established, the Society of Dyers and Colourists recognize the following definitions of a pigment and a dye.[6]

> "*Pigments are colored, black, white or fluorescent particulate organic or inorganic solids which usually are insoluble in, and essentially physically and chemically unaffected by, the vehicle or substrate in which they are incorporated. They alter appearance by selective absorption and/or by scattering of light. Pigments are usually dispersed in vehicles or substrates for application, as for instance in the manufacture or inks, paints, plastics or other polymeric materials. Pigments retain a crystal or particulate structure throughout the coloration process. Kindly reproduced by permission of CPMA – Color Pigment Manufacturers Association, Inc.* "[6]
>
> "*Dyes are intensely colored or fluorescent organic substances only, which impart color to a substrate by selective absorption of light. They are soluble and / or go through an application process which, at least temporarily, destroys any crystal structure by absorption, solution, and mechanical retention, or by ionic or covalent chemical bonds. Kindly reproduced by permission of ETAD – The Ecological and Toxicological Association of Dyes and Organic Pigment Manufacturers*"[6]

Characteristics of pigments and dyes that impact performance and safety

As indicated in the definitions of a pigment and dye, these two types of color additives differ in how these color additives impart color, and also includes differences in chemical/physical properties; the latter impacts the range of color, transparency, resistance to fading, cost, and ease-of-use. In addition to these performance properties, toxicity of a color additive, or any other chemical compound, is an inherent property of its molecular structure and associated chemical/physical properties. Thus, differences in chemical/physical properties between pigments and dyes that impact toxicity are the following:

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[3] FDA (2017) Color Additives For Medical Devices URL https://www.fda.gov/medical-devices/premarket-approval-pma/color-additives-medical-devices

[4] FDA (2017) Color Additives History [WWW Document] URL https://www.fda.gov/industry/color-additives/color-additives-history

[5] Barrows, J.N., Lipman, A.L., Bailey, C.J. (2003) Color Additives History. Reprinted by FDA from Food Safety Magazine October / November 2003 issue. [WWW Document] URL https://www.fda.gov/industry/color-additives/color-additives-history

[6] SDC Definitions of a dye and a pigment. Society of Dyers and Colourists. [WWW Document] URL https://colour-index.com/definitions-of-a-dye-and-a-pigment

Table 1. General chemical/physical differences between dyes and pigments[8]

Property	Dye	Pigment
Molecular size	Molecule	Particle (100 to 1000 nm)
Solvent Solubility	Soluble	Insoluble
Surface Area	Higher	Lower
Toxicity	Higher	Lower

While not relevant for dyes, particle size plays a significant role in opacity and other properties of inorganic pigments (e.g., titanium dioxide). For all inorganic pigments, optimal opacity, tinctorial strength[7], dispersibility, and fastness (e.g., resistance to fading in the presence of light) occur at particle sizes between 0.1 and 1 μm. Smaller size particles impart a tranparent color, reduced tinctorial strength, less dispersibility in the polymer (i.e., reduced homogenous distribution), and reduced fastness (i.e., higher susceptibility to fading when exposed to light, weather, etc.). Similarly, larger size particles have the opposite effect on color (e.g., darker colors) and the same adverse impacts on tinctorial strength, dispersibility, and fastness.

Note: Except for carbon black, pigment particles below 100 nm are excluded from this document because particles below this size are not used for coloring because of poor optical quality (i.e., they are transparent).

Uses of pigments and dyes

Differences in how a pigment versus a dye impart color, as well as differences in chemical/physical properties, mean the production and use of a dye versus a pigment also differs. The organic and monomolecular nature of dyes has lead to many more commercially available dyes (thousands) compared to pigments (hundreds).[8] Dye molecules interact with and bind to a bind to a naturally colored substrate to impart a different color.[9] Unlike dyes, pigments are unsuitable for textile applications because they are solid particles incapable of chemical interactions. However, pigments are used to impart color to paints, inks, ceramics, and plastics because coloring of these materials does not involve chemical interactions, and instead, require modification (e.g., encapsulation with a dispersant and binder) to achieve the desired coloring effect and performance characteristics.[9,10] Compared to pigments, fewer dyes are used to color plastics, see Table 2.

Table 2. Number of commonly used dyes and pigments in polymer resins[11]

Polymer Resin	Dyes	Pigments
ABS	None reported	40
Cellusolic	None reported	53

[7] Tinctorial "\tiŋ(k)-ˈtōr-ē-əl-\ (ca. 1864) n. The relative ability of a pigment or dye to impart color value to a printing ink."(2007) Tinctorial strength. In: Gooch J.W. (eds) Encyclopedic Dictionary of Polymers. Springer, New York, NY. https://doi.org/10.1007/978-0-387-30160-0_11660
https://link.springer.com/referenceworkentry/10.1007%2F978-0-387-30160-0_11660

[8] Gregory, P. (1993) Dyes Versus Pigments: The Truth, in: Proc. of IS&T's Ninth International Congress on Advances in Non-Impact Printing Technologies. Recent Progress in Ink-Jet Technologies, Yokohama, Japan, pp. 276–278.

[9] Kiron, M.I. (2021) Requirements of Dyes and Pigments and Their Differences. [WWW Document]. URL https://textilelearner.net/dyes-and-pigments/

[10] Sirikittikul, D. (2004) I. Introduction, in: Modification of Pigment Surfaces with Polymer Monolayers. Cullivier Verlag, Gottingen, Germany, pp. 1–25.

11 Webber, T.G. (1979). Coloring of Plastics. John Wiley & Sons, New York, NY.

Table 2. Number of commonly used dyes and pigments in polymer resins.[11]

Polymer Resin	Dyes	Pigments
Ionomers	None reported	9
Polyethylene	None reported	44
Polypropylene	None reported	53
Polystyrene	14	43
Polyvinyl butyral	11	8
Thermoplastic	None reported	11
Vinyls (flexible)	None reported	43
Vinyls (rigid)	7	37

Because the majority of medical devices are comprised of plastic and other polymer resins, the majority of medical devices are colored with pigments. The color additives in this report represent the most commonly used pigments and dyes in medical devices as reported to FDA/CDRH by select medical device manufacturers.

Methodology

Obtaining toxicological data

An iterative process was used to obtain toxicological data for each color additive, see Figure 1. The process began with creating a search strategy of key terms combined with booleans (i.e., AND, OR, NOT) to obtain the necessary specifity of the search. In addition to the search strategy, searchable databases of literature known to contain chemical toxicological data were identified.

The search strategy was applied and resulted in a list of toxicological records from the identified databases that matched the search terms and included, but was not limited to, scientific articles, review articles, books, book chapters, etc. Each record in the list typically included author, title, year, abstract, etc. (i.e., metadata). Selection criteria were applied to the metadata to select specific literature records for further review. Initial search strategy, literature databases, and selection criteria parameters are presented in Table 3.

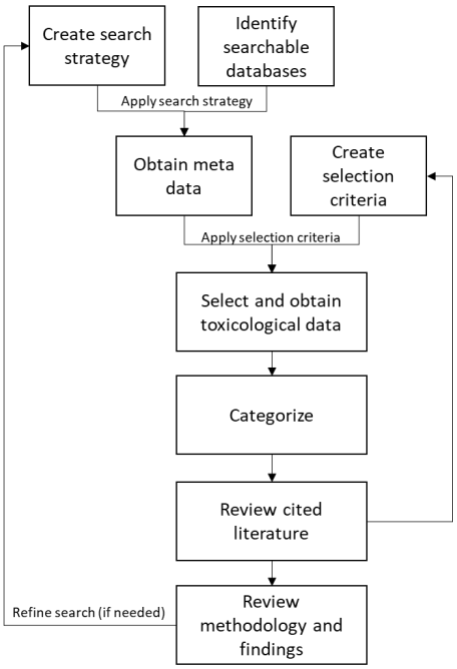


Figure 1. Obtaining toxicological data

Table 3. Literature search parameters

Parameter Type	Parameter Details
Initial Search Strategy	Includes: (color additive name OR CAS #) AND (toxicity OR toxicological OR harm OR "adverse effect" OR "toxic effect")
Literature Databases	Google Scholar, PubMed, Web of Science*
Selection criteria	Includes: (human OR adult OR child OR pediatric) OR (mice OR mouse OR rat OR rabbit OR guinea pig) OR (absorption OR tissue distribution OR metabolism OR excretion) OR (mechanism OR mechanistic OR "mode of action") OR (dose-response OR NOAEL OR LOAEL OR NEL OR LEL) OR ("in vitro" OR "in silico" OR "in chemico" OR "structure activity" OR QSAR) Excludes: environment OR river OR stream OR lake OR ocean OR atmosphere OR deer OR horse OR wildlife

Once obtained, each literature article, book, book chapter, document, report, or other type was categorized as either a health effect study, supporting study, or secondary source. A health effect study is an original report of direct *in vitro* or *in vivo* evidence of toxic effect(s), that a color additive does or does not elicit at a specified dose(s) and route/duration of treatment. A supporting study is indirect *in vitro*, *in silico*, *in chemico*, *in vivo*, or clinical evidence that a color additive does or does not elicit harm to health at a specified dose(s) and route/duration of treatment. A secondary source is information other than the original report that includes a description of chemical constituent specific toxicological data (i.e., primary health effect or supporting health effect).

Note: Examples of primary health effect data include original reports of systemic toxicity (acute, subacute, and subchronic, and chronic), genotoxicity, carcinogenicity or reproductive/developmental toxicity.

Note: Examples of supporting evidence include, but are not limited to, chemical/physical properties, toxicological structural alerts, toxicokinetics (i.e., absorption, distribution, metabolism, and elimination), mechanism/mode of toxic action, animal studies, or human epidemiological studies/clinical trials/case-reports.

Note: An example of a secondary source is a review article published in a scientific journal or a database that summarizes the toxicological findings of one or more original reports.

Other sources of color additive toxicological information were bibliography entries of health effect studies, supporting studies, and secondary source reports.

In addition to toxicological data, the following additional information for each color additive was obtained (if available): identity (e.g., name, synonyms, Chemical Abstract Service number or CAS #), chemical/physical characteristics (e.g., pigment particle size, molecular weight, solubility in water, lipophilicity), manufacture/synthesis information, impurities, etc.

Note: Molecular weight (typically in grams per mole), solubility in water, and lipophilicity are determinants of chemical toxicity. In general, determinants of particle toxicity include size (typically in nanometers), shape, and surface characteristics, see Summary of Color Additive Reported Harms (page 9) for additional information.

Toxicological data review and documentation

For each color additive, identity information, category (i.e., health effect study, supporting evidence or secondary source), *in vitro* or *in vivo* experimental study methodology, reported harms (i.e., nature of the harm and the dose at which harm does not or does occur), and reported supporting evidence were documented in each toxicological profile. Nature of harms include acute, subacute, subchronic, and chronic systemic adverse effects, genotoxicity, cancer, and reproductive/developmental toxicity. The lowest dose that resulted in an observable adverse effect (i.e., lowest-observable-adverse-effect-level or LOAEL) or no observable adverse effect (i.e., no-observable-adverse-effect-level or NOAEL) were also documented.

Study methods that were reported to be in accordance with a test guideline (e.g., Organisation for Economic Co-operation and Development or OECD) was also documented. If the study was obtained from a

secondary source that evaluated and reported data quality (e.g., European Chemicals Agency (ECHA) of the European Union), the quality of the data was documented. Observations regarding limitations of the study method and reported harms were noted for each study on a case-by-case basis. Study method and harm information not included in the toxicological profile indicates the information was not reported.

Note: LOAEL and NOAEL apply to in vivo experimental studies and are expressed as milligram per kilogram body weight per day or milligram per meter cubed for oral and inhalation toxicity studies, respectively..

Note: The term "harm" or "adverse effect" mean a biological change, such as altered morphology, physiology, growth, development, reproduction or lifespan that (a) impairs function of an organ/system, organism, or (sub)population, (b) reduces capacity to tolerate impaired function, or (c) increases susceptibility to other influences that impair function.

Note: Critical review of the reported toxicological data to establish cause-and-effect is beyond the scope of this work. No medical device specific guidances or standards are available that include the minimum objective evidence needed to determine that a chemical substance causes toxicity. The color additive toxicological data in this document may be useful for estimating the probability that harm could occur (e.g., when exposure to a color additive exceeds the pTI value).

Derivation of provisional tolerable intake (pTI) values

The method used to derive pTI values, in µg/kg/d (or mg/kg/d), was in accordance with ISO 10993-17:2002(R2012). The approach included selecting a point of departure (POD in µg/kg/d), application of uncertainty factors (UFs), calculating a modifying factor (MF), and finally, calculating the pTI value, see Figure 2. The pTI values for each color additive are intended to be generally applicable to medical devices and associated intended uses, with the exception that unanticipated impurities are dealt with separately from the pTI in the CHRIS calculator itself.

Note: Types of PODs include no-effect-level (NEL), no-observable-adverse-effect-level (NOAEL), lowest-effect-level (LEL), and lowest-observable-adverse-effect-level (LOAEL).

Note: A POD is established in relation to a biological response.

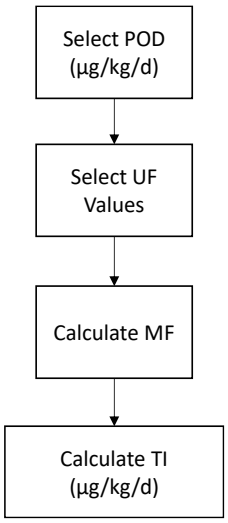


Figure 2. Derivation of TI value

When multiple types of PODs are reported, the lowest POD was selected unless the study was determined to be unreliable. If the selected POD was from an unreliable toxicological study, then the uncertainty associated with the POD was addressed in the selection and application of uncertainty factors.

Note: The adverse biological response associated with the lowest POD used in the derivation of a TI is the critical adverse health effect. A TI based on the lowest reported POD is considered protective for the critical adverse health effect and other applicable chemical specific harms known to occur at higher exposure doses.

Three UFs were applied that addressed (UF1) variability in biological response among individuals, including pediatric, (UF2) variability in biological response between animal model and humans, and (UF3) variability resulting from differences in exposure route and data quality. In general, a default value of 10 was applied for UF1 and UF2. A default value of 10 was also applied for UF3 to address variability resulting from data quality or extrapolating a POD obtained from an oral toxicity study. When a value other than the default is used, Table III.3 for the color additive includes a summary of the rationale for the value used. For example, a UF3 of 100 was used for carbon black to account for differences in exposure route and reports of polycyclic aromatic hydrocarbon impurities in some, but not all, carbon blacks (e.g., lamp black)

The modifying factor (MF) was calculated by obtaining the product of the three UFs, see Equation 1.

Equation 1 MF = UF1 x UF2 x UF3

Note: In general, application of a large modifying factor (MF) (e.g., > 10,000) could over-estimate toxicological risk for chemicals of low toxicity potential or indicate toxicological data is inadequate to derive a chemical specific pTI value.

Tolerable intake (TI) value was calculated by obtaining the quotient of the POD and MF, see Equation 2.

Equation 2 TI (µg/kg/d) = POD (µg/kg/d) ÷ MF

Summary of Color Additive pTI Values

Derived pTI values, including the selected POD and MF value used in the derivation of the pTI, for the eleven (11) color additives are presented in Table 4. For additional information on how each pTI value was derived, see the applicable page number in Table 4. For the pigments, the range of pTI values is between 0.05 (i.e., zinc oxide) and 3.3 mg/kg/d (C.I. Pigment Blue 29). The lowest pTI value of 0.05 mg/kg/d resulted from a moderately low POD (160 mg/kg/d) and large MF value (3000). The pTI value (0.15 mg/kg/d) for the three metallic phthalocyanines, which are organic pigments, is ~5x to ~10x lower compared to the pTI values of the remaining pigments, which are inorganic pigments. The lower pTI value for the metallic phthalocyanines is principally the result of a low POD value (i.e., 40 mg/kg/d). For Solvent Violet 13, an organic dye, the lowest mutagenic threshold of toxicological concern (TTC) value is applied due to insufficient toxicological data for deriving a pTI value.

Table 4. Color additive pTI values

Color Additive	Type	POD (mg/kg/d)*	MF	pTI (mg/kg/d)	Page Number
Carbon Black	Pigment (inorganic)	20,000	10,000	2.0	43
C.I. Pigment Blue 29	Pigment (inorganic)	300	90	3.3	48
C.I. Pigment Brown 24	Pigment (inorganic)	500	1,000	0.5	56
C.I. Pigment Red 101	Pigment (inorganic)	1000	1,000	1	66
C.I. Pigment Yellow 138	Pigment (inorganic)	1,000	1,000	1	72
Copper(II) Phthalocyanine	Pigment (organic)	40	270	0.15	84
Manganese(II) Phthalocyanine	Pigment (organic)	40	270	0.15	84
Phthalocyanine Green	Pigment (organic)	40	270	0.15	84
Titanium Dioxide	Pigment (inorganic)	1,000	1,000	1	109
Zinc Oxide	Pigment (inorganic)	160	3,000	0.53	113
Solvent Violet 13	Dye (organic)	NA	NA	0.0015**	122

NA = not applicable. POD = point of departure. MF = modifying factor. *All PODs are oral NOAEL or NOEL values. **Lowest (i.e., lifetime) mutagenic threshold of toxicological concern (TTC) value in accordance with ISO TS 21726:2019.

Note: "Pigment (inorganic)" indicates a pigment where the atomic structure does not include a carbon-hydrogen bond. "Pigment (organic)" and "dye (organic)" indicate color additives where the atomic structure does include a carbon-hydrogen bond.

Note: Modifying factors are based on three uncertainty factors (UFs) as described in ISO 10993-17.

ATTACHMENT A

Anatomy of a Color Additive Toxicological Profile

A toxicological profile documents reported harms and other information necessary to make informed judgements regarding the lowest reported dose below which systemic, genotoxic, carcinogenic or reproductive/developmental toxic effects are not observed. Three types of color additive information is documented in each toxicological profile, which are (1) identity (including physical-chemical characteristics), (2) reported harms and point-of-departures (e.g., a no-observable-adverse-effect-level or NOAEL), and (3) derivation of the tolerable intake (TI) value. Table 1 includes examples of information typically available for each type, as well as examples of additional information that is often (but not always) available for color additives.

Table 5. Types of color additive information with examples

Information Type	Typical Information	Examples of Additional Information
Identity	Chemical name CAS Number	Synonyms Molecular formula Molecule arrangement Chemical/physical characteristics Manufacturing process Impurities
Reported Harms	Systemic effects	Genotoxicity Carcinogenicity Reproductive/developmental toxicity
Derivation of TI	Point of departure Uncertainty factors Modifying factor TI value	Not applicable

The three types of toxicological profile information is organized into three distinct sections, which is illustrated in Figure 4, where explanatory information is included in bracketed text.

[color additive common name]

CAS # [xxxx-xx-x]

Toxicological Profile Summary

[summary appears here]

Section I. Substance Information

I.1 Substance Identity

	Descriptor
Synonyms	[alternative names]
Formula	[type and number of atoms]
Molecular Weight	[grams per mole]
Physical Form	[solid or liquid]
Type	[pigment or dye]
Physical / Chemical Characteristics	[solubility, lipophilicity, etc.]
Production	[description of typical manufacturing process]
Impurities	[chemical names]
Other	[miscellaneous information]
Surrogates	[chemical names if reported in the literature]

I.2 Molecular Descriptors (if available)

[image, particle size, etc.]

Section II. Hazard Identification

Hazard Identification Summary: *[*summary appears here*]*

II.1 Systemic, Genotoxicity, Carcinogenicity, Reproductive/Developmental Toxicity

[Study Type: Health Effect, Supporting, or Secondary Source]

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
Acute Toxicity							
[citation/link]	[yes/no]	[animal/cell line]	[oral, inhalation, intraperitoneal, intravenous]	[NOAEL, LOAEL]	[single or multiple]	[nature of harm or no effect]	[OECD or other]
Test article: [description of test article administered] ***Dose:*** [all doses (single or multiple) administered] ***Treatment Duration:*** [number of days, weeks, months, years and frequency if not treatment was not consecutive] ***Species (strain):*** [name of animal species and strain or cell line] ***Gender:*** Males and females. ***Number per Group:*** [number of animals, cells per group] ***Methods:*** [route and observations recorded] ***Adverse Effect(s) (brief):*** [description of reported change(s) in observable biological response(s) (i.e., harms)] ***Data Quality:*** [typically Klimisch score (i.e., 1 (reliable without restrictions), 2 (reliable with restrictions), 3 (not reliable, or 4 (not assignable) as reported by secondary source)] ***Note:*** [information pertinent to the method or findings pertinent to data interpretation] ***Reference:*** [bibliographic source information]							

Figure 4. Anatomy of a toxicological profile

II.2 Other Health Effects

Pigment	Endpoint	Outcome	Reference
[chemical name]	[name of biological endpoint]	[nature of harm or no effect]	[citation/link]

Test article: [description of test article administered] ***Dose:*** [all doses (single or multiple) administered ***Treatment Duration:*** [number of days, weeks, months, years and frequency if not treatment was not consecutive] ***Species (strain):*** [name of animal species and strain or cell line] ***Gender:*** Males and females. ***Number per Group:*** [number of animals, cells per group] ***Methods:*** [route and observations recorded] ***Adverse Effect(s) (brief):*** [description of reported change(s) in observable biological response(s) (i.e., harms)] ***Data Quality:*** [typically Klimisch score (i.e., 1 (reliable without restrictions), 2 (reliable with restrictions), 3 (not reliable, or 4 (not assignable) as reported by secondary source)] ***Note:*** [information pertinent to the method or findings pertinent to data interpretation] ***Reference:*** [bibliographic source information]

Section III. Dose-Response Assessment

III.1 Provisional Tolerable Intake Values

Contact Duration	Route of Exposure	Patient	pTI
Permanent	Parenteral	All	[numeric value] mg/kg

pTI is derived from [citation] [NOAEL or LOAEL] of [numeric value in mg/kg] ÷ MF. pTI does not apply to impurities, if present. Toxicological risk of an impurity may be addressed by deriving an impurity/chemical specific TI.

III.2 Modifying Factor for POD

Critical Study	Biological Endpoint	Exposure Route	POD Type	POD Value	UF_1	UF_2	UF_3	MF
[citation]	systemic toxicity, genotoxicity, carcinogenicity, reproductive/developmental toxicity	[oral, intraperitoneal inhalation, intravenous]	[NOAEL, LOAEL]	[numerical value] mg/kg/d	[numerical value]	[numerical value]	[numerical value]	[numerical value]

POD = point of departure; POD Type = no observable adverse effect level (NOAEL) or equivalent; POD is in mg/kg/day; TBD = tissue, bone, dentin; UF_1 = uncertainty among humans; UF_2 = intraspecies uncertainty between species; UF_3 = uncertainty due to route-to-route extrapolation and data quality; MF = modifying factor (UF_1 x UF_2 x UF_3); TI = tolerable intake in mg/kg/day

III.3 Toxicological Uncertainties Applied to POD

Uncertainty	Justification
UF_1	(10) Default value to account for susceptible individuals.
UF_2	(10) Default value to account for differences between species.
UF_3	(10) Default value to account for differences in exposure route and duration.

NA = not applicable; Study was analyzed by *[method and reference, if applicable)]*. UF1, UF2, and UF3, are used to extrapolate the POD to the general human population (including pediatric) exposed parenterally for a lifetime (see in III.2).

III.4 Critical Health Study Design and Outcome

[citation]			
Test Substance	[description of test article administered as reported]		
Species	[name of animal species and strain or cell line]	Frequency	[daily or number of day(s) per week]
Gender	[males, females or males and females]	Duration	[total number of treatment days, weeks, or months]
Age	[numerical value in days, weeks, etc.]	#/Group	[number of animals/cells]
Route	[oral, intraperitoneal inhalation, intravenous]	Protocol Guideline	[OECD or other]
Dose(s)	[numerical value] in mg/kg/d	Statistical Method(s)	[as reported]
Observed Responses	[description of reported change(s) in observable biological response(s) (i.e., harms)]		
Notes:	[information pertinent to the method or findings pertinent to data interpretation]		

Figure 4. Anatomy of a toxicological profile (continued)

ATTACHMENT B

Abbreviations

The following abbreviations are used throughout Attachment C Color Additive Toxicological Profiles.

Abbreviation	Term
8-oxo-dG	*8-Oxo*-2'-*deoxyguanosine*
ADME	absorption, distribution, metabolism, excretion
AHES	adverse health effect study
ALD	approximate lethal dose
ALP	*alkaline phosphatase*
avg	average
BAL	bronchoalveolar lavage
BMCL	benchmark concentration level
bw	body weight
CAT	catalase
CB	carbon Black
CHO	chinese hamster ovary
C.I.	color index
CMC	carboxymethyl cellulose
Cr	chromium
CuPc	copper(II) phthalocyanine
DE	diesel exhaust
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
ECHA	European Chemicals Agency
OECD	The Organisation for Economic Co-operation and Development
g	grams
GC–MS	gas chromatography–mass spectrometry
GLP	good Laboratory Practices

Abbreviation	Term
GOT	glutamate oxaloacetate
GPT	glutamic-pyruvic transaminase.
GR	glutathione reductase
GSH-Px	glutathione peroxidase
Hb	hemoglobin
HCl	hydrogen chloride
HPMC	hydroxypropylmethyl cellulose
HPRT	hypoxanthine phosphoribosyltransferase 1
IL1-β	*Interleukin 1 beta*
i.p.	intraperitoneal injection
KC	keratinocytes-derived chemokine
kg	kilogram
LC_0	lethal concentration 0%
LC–MS/MS	liquid chromatography–MS/MS
LD_0	lethal dose 0%
LDH	lactate dehydrogenase
LLNA	mouse local lymph node assay
LOAEC	lowest Observed Adverse Effect Concentration
LOAEL	lowest observed adverse effect level
m^3	meter cubed
MCV	mean corpuscular volume, or mean cell volume
MDM2	*mouse double minute 2* homolog
mg	milligram
MF	modifying factor
MIP2	Macrophage Inflammatory Protein 2
mo	month
mol	mole
MPc	metallo-organic phthalocyanine pigments
MN	micronuclei

Abbreviation	Term
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
ND	not determined
nitro-PAHs	nitro derivatives of PAHs
NOAEL	no-observable-adverse-effect-level
NOEL	no observable effect level
NR	not reported
OEL	occupational exposure level (time-adjusted chronic)
PAHs	aromatic hydrocarbons
PB29	C.I. pigment blue 29
PBPK	physiologically based pharmacokinetics
PcGn	phthalocyanine green
PCV	packed cell volume
PMN	polymorphonuclear neutrophils
ppb	part per billion
ppm	parts per million
pTI	provisional tolerable intake
QSAR	quantitative structure activity relationship
RBC	red blood cell
RT	room temperature
Sb	antimony
SCE	sister chromatid exchange assay
SOD	superoxide dismutase
SP-LC–MS/MS	solid phase extraction-LC–MS/MS
TBD	tissue, bone, dentin;
TE	tolerable exposure
TEM	transmission electron microscopy
TI	tolerable intake
TNF	*tumor necrosis factor*
TNF-α	Tumour necrosis factor alpha

Abbreviation	Term
TTC	threshold of toxicological concern
UF_1	uncertainty factor 1 (variation among humans)
UF_2	uncertainty factor 2 (interspecies variation)
UF_3	uncertainty factor 3 (route-to-route variation and data quality)
ufCB	ultrafine carbon black
UMB	ultramarine blue
U.S. EPA	United States Environmental Protection Agency
UVCB	Chemical Substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials
yr	year
µm	micron

ATTACHMENT C

Color Additive Toxicological Profiles

Pigments

CARBON BLACK – CAS # 1333-86-4

C.I. PIGMENT BLUE 29 – CAS # 57455-37-5

C.I. PIGMENT BROWN 24 – CAS # 57455-37-5

C.I. PIGMENT RED 101 – CAS # 1309-37-1

C.I. PIGMENT YELLOW 138 – CAS # 30125-47-4

METALLIC PHTHALOCYANINES (including):

COPPER(II) PHTHALOCYANINE (CuPc) – CAS # 147-14-8

MANGANESE(II) PHTHALOCYANINE (MnPc) – CAS # 14325-24-7

PHTHALOCYANINE GREEN (PcGn) – CAS # 1328-53-6

TITANIUM DIOXIDE – CAS # 13463-67-7

ZINC OXIDE – CAS # 1314-13-2

Dyes

SOLVENT VIOLET 13 – CAS # 81-48-1

Carbon Black

CAS # 1333-86-4

Toxicological Profile Summary

Carbon black (CAS # 1333-86-4) is an inorganic particle, which contains 95-99.5% elemental carbon; the particles are colloidal (*i.e.*, homogeneous mixture of microscopic particles) and near-spherical produced by incomplete combustion or thermal decomposition of gaseous or liquid hydrocarbons.[12, 13] Reported hazard information includes an adverse health effect study, supporting studies, and secondary sources. Carbon black, administered at high oral doses (up to ~ 28,000 mg/kg/d) to rodents, is reported to not elicit observable systemic toxic effects[14]. For assessing Carbon black exposure that will be without appreciable harm to health, the calculated provisional tolerable intake (pTI) value for parenteral systemic toxicity is 2 mg/kg/day after rounding down the NOAEL to 20,000 mg/kg/d. This value is based on the lowest reported point-of-departure (20,000 mg/kg/day oral, 18 months, rodent) with a modifying factor of 10,000 (i.e., 10 x 10 x 100), to address the following sources of uncertainty: variation among humans (10), variation between species (10), and data quality/route-to-route extrapolation (100).

NOTE: This pTI is protective for the color additive including any impurities listed in Section I.1 "Substance Identity," as the impurities likely will be present at a very low percentage of the color additive, and the quantity of the impurity will be at an even lower percentage in the final device (see Section III, Table III.1 footnote). Unknown impurities are addressed by the CHRIS calculator.

Section I. Substance Information

I.1 Substance Identity

	Descriptor
Synonyms[12, 15, 16]	Channel Black: CI: 77266; CI Pigment Black 7; impingement black Furnace Black: CI: 77266; CI Pigment Black 7; gas-furnace black; oil-furnace black
Formula[15]	(C), molecular formula unspecified
Molecular Weight[15]	12.011
Physical Form[15]	Solid at 20ºC and 1013 hPa; melting point is 3652 and 3697ºC
Type[15]	Element
Physical/Chemical Characteristics[15,16]	Carbon black is virtually pure elemental carbon in the form of colloidal particles. Its physical appearance is that of a black, finely divided pellets or powder. It is odorless. Insoluble in water and solvents. Furnace black can be produced with a wide range of properties. Channel black characteristics include small particle size and a high level of surface oxidation.
Production[16]	Several processes have been used to produce carbon black, including the oil-furnace (by which more than 95% of the total output of carbon black is produced), impingement (channel), lampblack, thermal (decomposition of natural gas) and acetylene (decomposition) processes.

[12] Auer, G., Griebler, W.-D., Jahn, B. (2005) White Pigments, in: Buxbaum, G., Pfaff, G. (Eds.), Industrial Inorganic Pigments. Wiley-VCH Verlag GmbH & Co. KGaA, pp. 51–97. https://doi.org/10.1002/3527603735.ch2

[13] Long, C.M., Nascarella, M.A., Valberg, P.A. (2013) Carbon black vs. black carbon and other airborne materials containing elemental carbon: Physical and chemical distinctions. Environmental Pollution 181, 271–286. https://doi.org/10.1016/j.envpol.2013.06.009

[14] Nau CA, Neal J, and Stembridge VA. (1958a) Study of the physiological effects of carbon black. I. Ingestion. Arch. Indust. Health. 17(1):21-28.

[15] ChemID. Carbon Black [WWW Document]. URL https://chem.nlm.nih.gov/chemidplus/name/carbon%20black

[16] IARC (2010) Carbon Black, Titanium Dioxide, and Talc. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. VOLUME 93. World Health Organization. International Agency for Research on Cancer.

	Descriptor
Impurities[16]	Polycyclic aromatic hydrocarbons (PAHs), nitro derivatives of PAHs (nitro-PAHs) and PAHs that contain sulfur (See Table 2). Benzo[ghi]perylene, coronene, cyclopenta[cd]pyrene, fluoranthene and pyrene are among the extractable PAHs.
Other	Black-colored
Surrogates	None

Physical Form = gas, liquid, solid (at room temperature); Type = gas, liquid, or solid (include temp & pressure if available)

I.2 Molecular Descriptors

The smallest molecular unit of carbon black is graphene with carbon atoms (no hydrogens) arranged in hexagonal planar structures (see Figure I.2a).

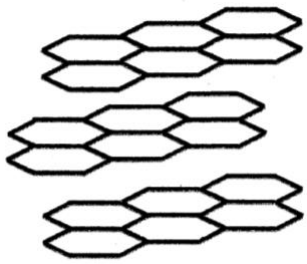


Figure I.2a. Planar structure of carbon atoms (image source: Pfaff G. 1977).[17]

The planar structures of carbon are layered randomly that forms a spherical, porous, insoluble, particle.

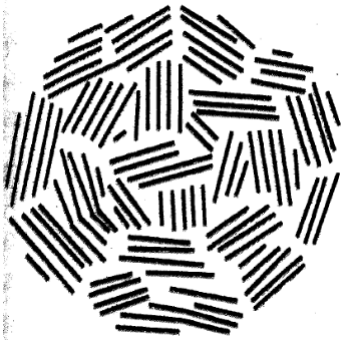
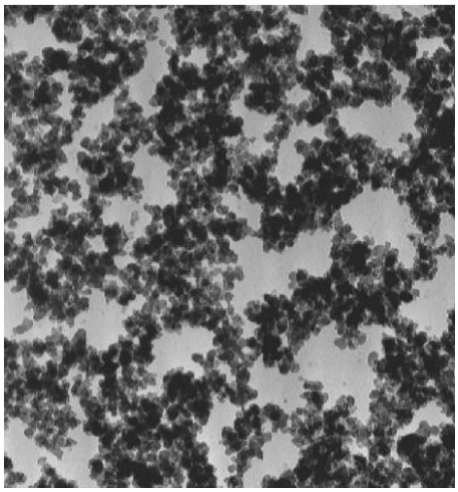


Figure I.2b. Spherical particle of carbon (image source: Pfaff G. 1977)[17]

Carbon black particles coalesce strongly with each other and eventually forms agglomerates visible by transmission electron microscopy (TEM).

[17] Pfaff, G. (2017) Chapter 5 Black pigments (carbon black), in: Inorganic Pigments. Walter de Gruyter GmbH, Berlin, Germany, pp. 167–189.



FigureI.2c. TEM-micrograph of a carbon black network obtained from an ultra-thin cut of a filled rubber sample (image source: Klueppel, 2003).[18]

I.3 Summary of Particle Size of Carbon Black

Carbon black	Surface area (m^2/g)	Approximate diameter of primary particle size (nm)	Diameter of aggregate (nm)	Size of agglomerate
Oil-furnace	12–240	10–400	50–400	Large (<2 mm)
Thermal	6–15	120–500	400–600	Large (<2 mm)
Impingement (channel)		10–30	50–200	Large (<2 mm)
Lampblack	15–25	60–200	300–600	Large (<2 mm)
Acetylene black	15–70	30–50	350–400	Pelletizes poorly

Data obtained from IARC compiled from the Working Group from Kuhlbusch et al. (2004) and Kirk-Othmer (2005).[16,19,20]

[18] Klüppel, M., 2003. The Role of Disorder in Filler Reinforcement of Elastomers on Various Length Scales, in: Capella, B., Geuss, M., Klüppel, M., Munz, M., Schulz, E., Sturm, H. (Eds.), Filler-Reinforced Elastomers Scanning Force Microscopy, Advances in Polymer Science. Springer, Berlin, Heidelberg, pp. 1–86. https://doi.org/10.1007/b11054

[19] Kuhlbusch, T.A.J., Neumann, S., Fissan, H., 2004. Number Size Distribution, Mass Concentration, and Particle Composition of PM1, PM2.5, and PM10 in Bag Filling Areas of Carbon Black Production. Journal of Occupational and Environmental Hygiene 1, 660–671. https://doi.org/10.1080/15459620490502242 (cited in IARC 2010)

[20] Kirk-Othmer (2005). Encyclopedia of Chemical Technology, 5th Ed. New York, John Wiley & Sons (cited in IARC 2010)

I.4 Impurities in Carbon Black Extracts[16]

Polycyclic aromatic hydrocarbons (PAHs)	Fluorene
(see also IARC, 1984, 2010)	Indeno[1,2,3-*cd*]pyrene
Acenaphthene	Naphthalene
Acenaphthylene	Perylene
Anthanthrene	Phenanthrene
Anthracene	Pyrene
Benz[*a*]acenaphthylene	*Nitro derivatives of PAHs* (nitro PAHs) (see also
Benz[*a*]anthracene	IARC, 1987)
Benzo[*b*]fluoranthene	1,3-Dinitropyrene
Benzo[*ghi*]fluoranthene	1,6-Dinitropyrene
Benzo[*j*]fluoranthene	1,8-Dinitropyrene
Benzo[*k*]fluoranthene	9-Nitroanthracene
Benzo[*a*]pyrene	3-Nitro-9-fluorenone
Benzo[*e*]pyrene	1-Nitronaphthalene
Benzo[*ghi*]perylene	1-Nitropyrene
Chrysene	1,3,6-Trinitropyrene
Coronene	*PAHs that contain sulfur*
4*H*-Cyclopenta[*def*]phenanthrene	Benzo[*def*]dibenzothiophene
Cyclopenta[*cd*]pyrene	Dibenzothiophene
Dibenz[*ah*]anthracene	Phenanthro[4,5-*bcd*]thiophene
Fluoranthene	Triphenyleno[4,5-*bcd*]thiophene

Extraction method, type and grade of carbon black and post-extraction treatments affect the type and quantity of impurities obtained. It is assumed some, but not all, of the above impurities are present in the grades of carbon black tested for adverse health effects.

Section II. Hazard Identification

Health effect data, supporting studies, and secondary sources were reviewed, see tables in sections II.1, and II.2 for details.

Acute exposure data oral LD50 > 15,400 mg/kg.[21], dermal > 3,000 mg/kg[21], inhalation > 2 000 mg/kg.[22] in rats, all exceeded 2000 mg/kg. In a two-day acute inhalation study, a dose of 100 mg/kg caused increased inflammation and mutation in rat alveolar cells after 15-months post exposure.[23] Results from another acute study showed lung inflammgenic response to 6.125 mg/rat carbon black particles (260 nm).[24].

Chronic dietary exposure (2.05 g/kg) for 52 weeks did not result in organ specific tumors in rats. Ingestion of carbon black did not increase tumor incidences in colon, kidney or mammary gland when compared with untreated controls in the 2-year study (3, 3, 24% versus 3, 3, 28% in untreated controls) or when compared with 1,2-dimethylhydrazine (DMH) treated animals in the 52-week study.[25]. Exposure to up to 40000 µg/mL did not result in direct genotoxic response in the ames assay, sister chromatid exchange test, mouse lympoma test, or the cell transformation assay.[26].Lung tumors are reported in animals exposed to carbon black (via inhalation route) long-term in which lungs are overloaded. In a chronic 30-month study, 326.95 g of carbon black (dose per surface area not reported) resulted in no observable adverse effects in mice, rats

[21] ChemIDplus_https://chem.nlm.nih.gov/chemidplus/rn/1333-86-4
[22] ECHA_acute_Inhalation_https://echa.europa.eu/registration-dossier/-/registered-dossier/16056/7/3/2/?documentUUID=a03fd97e-1806-4fb7-9dfe-c811851b08bf
[23] Driscoll, K.E., Deyo, L.C., Carter, J.M., Howard, B.W., Hassenbein, D.G., Bertram, T.A., 1997. Effects of particle exposure and particle-elicited inflammatory cells on mutation in rat alveolar epithelial cells. Carcinogenesis 18, 423–430. https://doi.org/10.1093/carcin/18.2.423
[24] Sager, T.M., Castranova, V., 2009. Surface area of particle administered versus mass in determining the pulmonary toxicity of ultrafine and fine carbon black: comparison to ultrafine titanium dioxide. Part Fibre Toxicol 6, 15.
[25] ECHA 1985_Repeatdose_toxicity_oral_https://echa.europa.eu/registration-dossier/-/registered-dossier/16056/7/6/2/?documentUUID=337ee0d8-de22-4ff1-8e7f-d9f9b1653186
[26] Kirwin, C.J., LeBlanc, J.V., Thomas, W.C., Haworth, S.R., Kirby, P.E., Thilagar, A., Bowman, J.T., Brusick, D.J., 1981. Evaluation of the genetic activity of industrially produced carbon black. Journal of Toxicology and Environmental Health, Part A Current Issues 7, 973–989.

or monkeys following skin contact of the "whole" carbon black extracts[27]. Chronic 18-month oral exposure at 200 g resulted in no significant change from the normal in any of the organ tissues of the mice treated with carbon black were reported[28]. Reproductive and developmental data show that oral exposure to 2733.5 mg/kg/d resulted in no difference in lung or liver tissue AHH activity among the younger group (litters), and non-dose dependent marginally detectable activity for the older breeder mice. At the highest dose tested, carbon black produced no evidence of mutagenic activity following oral exposure[29].

II.1 Systemic, Genotoxicity, Carcinogenicity, Reproductive/Developmental Toxicity

ADVERSE HEALTH EFFECT STUDIES

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
Systemic Toxicity (acute)							
Duffin et al. (2007)	Y	Animal	Intratracheal instillation	125 µg/mL **(LOAEL)**	single administration	Inflammation in lung	NR
Test article: Carbon black ***Dose:*** 125 µg in 0.5 mL of saline. ***Treatment duration:*** Single dose administration; sacrificed at 18-24hrs post treatment. ***Species (strain):*** Rats (Wistar) ~4 months old. ***Gender:*** Males. ***Number per Group:*** Not reported. ***Control***: 0.5 ml saline were used as controls. ***Methods:*** This study investigated the relationship between inflammation as indicated by neutrophil content of bronchoalveolar lavage of rats 18–24 h after particle instillation, the surface area, and the surface reactivity. Animals instilled with treatment or 0.5 ml saline were used as controls. Animals were sacrificed 18–24 hrs post exposure. bronchoalveolar lavage (BAL) was used to assess inflammation based on neutrophil content. ***Adverse Effect(s) (brief):*** **LOAEL = 125 µg/mL.** The extent of inflammation, observed by neutrophils content of BALF, was related with the surface area dose instilled. No other results related to CB were provided. ***Data Quality:*** NR. ***Note***: None: ***Reference:*** *Duffin, R., Tran, L., Brown, D., Stone, V., Donaldson, K., 2007. Proinflammogenic effects of low-toxicity and metal nanoparticles in vivo and in vitro: highlighting the role of particle surface area and surface reactivity. Inhal Toxicol 19, 849–856.*							
Li et al. (1999)	Y	Animal	Intratracheal instillation	643 mg/kg (FCB) **(LOAEL)**	single administration e	Inflammation, oxidative stress	NR
Test article: Fine (diameter = 260.2 ± 13.7 nm) carbon black (Huber 990); ***Dose:*** 643, 1607, and 4017 µg at 6, 24, 168h. ***Treatment Duration:*** Single administration up to 168hr ***Species (strain):*** Rats (Syngeneic Wistar-derived of the HAN strain) (~12wk and ~250g) Gender***:*** Males. ***Number per Group:*** 3-9 per experiment. ***Control:*** PBS is used as control. ***Methods:*** Carbon black (CB) was instilled intratracheally in rats at mass of 125 µg, and the bronchoalveolar lavage (BAL) profile at 6 h was assessed. To investigate time response, bronchoalveolar lavage was carried out at 6 h, 24 h, and 7 days after a single 125-µg instillation of ufCB. ***Adverse Effect(s) (brief):*** **LOAEL = 643 mg/kg (CB). S**ix-hour single administration, CB-treated group did not increase TNF or nitric oxide (NO) production by the leukocytes compared to control cells. CB increased epithelial permeability measured as total protein, increased LDH levels in BAL fluid. CB did not alter glutathione (GSH) in BAL fluid or lung tissue compared with PBS control. In the lung overload study, at high doses, CB triggered a marked inflammatory response evident as significantly increased numbers of BALF neutrophils, increased epithelial permeability, and lowered total lung GSH compared with their own controls. CB showed a dose-related increasing inflammatory response. CB caused a decrease in glutathione (GSH) in lung tissue compared to control. ***Data Quality:*** NR. ***Reference***: *Li, X.Y., Brown, D., Smith, S., MacNee, W., Donaldson, K., 1999. Short-Term Inflammatory Responses Following Intratracheal Instillation of Fine and Ultrafine Carbon Black in Rats. Inhalation Toxicology 11, 709–731. https://doi.org/10.1080/089583799196826*							
Nau, et al. (1960)	Y	Animal	Subcutaneous, Intraperitoneal injection	212 mg **(NOAEL)**	single administration	No observable adverse effect reported	NR
Test article: Various "channel" and/or "furnace" carbon blacks. ***Dose:*** 0-212 mg extract. Injections of the various carbon blacks were made, using water, cotton seed oil, or tricaprylin as the suspending vehicle. ***Treatment Duration:*** single administration, followed by up to 20 months observation period. ***Species (strain):*** Mice (C3H brown, CFW white) Rabbit (White); 8-10 weeks old ***Gender:*** Males. ***Number per Group:*** 10-20 per experiment (943 controls) ***Control:*** Vehicle ***Methods:*** Subcutaneous, intraperitoneal (i.p.) injection in rodents. adsorbed component (on furnace black) which could be removed in whole or in part by continuous extraction of the carbon black for 24 hours with redistilled benzene, using a Soxhlet Extractor. The amount of this extractable component varies with the type of carbon black extracted (up to 0.374% in the "blacks" we studied). Extensive studies							

[27] Nau, C.A., Neal, J., Stembridge, V., others, 1958. Study of the Physiological Effects of Carbon Black. II. Skin Contact. Arch. Indust. Health 18, 511–20.

[28] Nau, C.A., Neal, J., STUMBRIDGE, V., others, 1958. Study of the Physiological Effects of Carbon Black. I. Ingestion. Arch. Indust. Health 17, 21–8.

[29] Buddingh, F., Bailey, M.J., Wells, B., Haesemeyer, J., 1981. Physiological significance of benzo(α)pyrene adsorbed to carbon blacks: Elution studies, AHH determinations. American Industrial Hygiene Association Journal 42, 503–509. https://doi.org/10.1080/15298668191420152

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
of the benzene-extractable reveals the presence of various components -some of which have a carcinogenic effect when painted on the skin of C3H brown mice and CFW white mice. Consideration of these findings is made a part of our study on subcutaneous and intraperitoneal injections. When a diameter of 4 cm. for the mass (*i.e.*, 212 mg) was attained, the animal was weighed and killed, and all organs and tissues were subjected to gross and microscopic examination. All mice were kept for observation until an age of 20 months was reached. Injections of the various carbon blacks were made, using water, cotton seed oil, or tricaprylin as the suspending vehicle. ***Adverse Effect(s) (brief)***: **NOAEL = 212 mg extract.** Pathology generally reviewed mixed soft tumor in mice. However, there were no significant difference in tumor formation when compared to control group. No changes from the normal were noted in the test rabbits. The subcutaneous or intraperitoneal injection made, using water, cotton seed oil, or tricapryl in mice of channel or furnace carbon black leads to no significant changes from the normal regarding tumor formation. ***Data Quality:*** NR. ***Note***: None ***Reference:*** *Nau, C.A., Neal, J., Stembridge, V.A., 1960. A study of the physiological effects of carbon black: III. Adsorption and elution potentials; subcutaneous injections. Archives of Environmental Health: An International Journal 1, 512–533.*							
Sager and Castranova (2009)	Y	Animal	Intratracheal instillation	0.047 mg UFCB, 1.53 mg FCB **(LOAEL)**	single administration	Cytotoxic, Inflammation in lung	NR
Test article: ultrafine carbon black fine carbon black ***Dose:*** UFCB; (14 nm); 0.047, 0.094, 0.188 mg/rat; FCB; (260 nm); 1.53, 3.06, 6.125 mg/rat were suspended in rat BAL fluid. (0.3 mL of respective of dose). These mass doses resulted in identical surface area doses (0.0313, 0.0625, and 0.125 cm2/cm2) of UFTiO2 and UFCB ***Treatment Duration***: Single dose with post observation period of 1-, 7-, or 42-days post-exposure. ***Species (strain):*** Rat (Fischer CDF) (F344/DuCrl) (200–300 g). ***Gender:*** Males. ***Number per Group:*** 8 per dose. ***Control:*** Vehicle ***Methods:*** Intratracheal instillation. Rats were exposed by intratracheal instillation to various doses of ultrafine and fine carbon black. At 1-, 7-, or 42-days post-exposure, BAL inflammatory and cytotoxic potential of each particle type was compared on both a mass dosage (mg/rat) as well as an equal surface area dosage (cm^2 of particles per cm^2 of alveolar epithelium). Cytotoxicity induced by the instilled particles was determined by lactate dehydrogenase (LDH) activity in the BAL fluid. Albumin concentrations were also measured. The presences of inflammatory mediators present in the BAL fluid were analyzed by enzyme-linked immunosorbent assay (ELISA). Reactive oxygen species production was determined by measuring AM chemiluminescence. ***Adverse Effect(s) (brief):*** **LOAEL = 0.047 mg ufCB, 1.53 mg FCB.** Ultrafine carbon black particles caused a dose-dependent cytotoxicity, albumin levels, and polymorphonuclear neutrophils number in BAL observed but declined with time post exposure (1 > 7 > 42 days post intratracheal exposures). TNF-α, MIP-2, and IL-1β levels produced by ultrafine carbon black greater than fine carbon black. When doses were equalized based on surface area of particles given, the ultrafine carbon black particles were only slightly (non-significantly) more inflammogenic and cytotoxic compared to the fine sized carbon black. ***Data Quality:*** NR. ***Note***: None ***Reference:*** *Sager, T.M., Castranova, V., 2009. Surface area of particle administered versus mass in determining the pulmonary toxicity of ultrafine and fine carbon black: comparison to ultrafine titanium dioxide. Part Fibre Toxicol 6, 15.*							
Stoeger Tobias et al. (2006)	Y	Animal	Intratracheal instillation	5 µg **(NOAEL)**	24hrs	Inflammation in lung	NR
Test article: PrintexG, Printex90, flame soot particles with different organic content (SootL, SootH), spark-generated ultrafine carbon particles (ufCP), and the reference diesel exhaust particles (DEP) SRM1650a. ***Dose:*** 5, 20, 50 µg particles (DEP, PtxG, Ptx90, SootH, SootL,) per mouse in 50 µL of pyrogene-free distilled water, followed by 100 µL air. Control animals were not instilled, and sham animals received 50 µL pure distilled water. ***Treatment Duration:*** 24hrs**.** ***Species (strain):*** Mice (BALB/cJ) 10 – 12 weeks old (BW:19.6 – 23.1g). ***Gender:*** Females. ***Number per Group:*** 8/group, 22 experimental groups. ***Control:*** two groups served as control and sham exposed. ***Methods:*** Six different particle types (primary particle size 10–50 nm, specific surface area 30–800 m2/g, and organic content 1–20%) were instilled in mice. Twenty-four hours after instillation, mice were anesthetized. BAL was performed to determine inflammatory cells and the level of proinflammatory cytokines, lactate dehydrogenase (LDH), and total protein. ***Adverse Effect(s) (brief):*** **NOAEL = 5 µg; LOAEL = 5 µg (to a surface area of 22.1 cm^2**). Each particle type caused a dose-dependent inflammatory. All particles evoked a significant PMN influx into the alveolar space at a dose of 50 µg/mouse. Of all particles, ultrafine carbon black (50 µg) generated the highest impact. Significant dose-dependent increase in total protein levels, polymorphonuclear neutrophils (PMN), and cytokine levels (IL1-β, TNF-α, MIP2, and KC) observed after exposures to ultrafine carbon black. UFPs, ufCP, leading to BALF PMN contents of about 60% at a dose of 50 µg and of > 20% at 5 µg, respectively. None of the instilled materials at any dose caused an increase in LDH concentration or the number of dead cells in BALF. A significant association between the number of PMN and the particle size and particle surface area found. ***Data Quality:*** NR. ***Note***: None. ***Reference:*** *Stoeger Tobias, Reinhard Claudia, Takenaka Shinji, Schroeppel Andreas, Karg Erwin, Ritter Baerbel, Heyder Joachim, Schulz Holger, 2006. Instillation of Six Different Ultrafine Carbon Particles Indicates a Surface Area Threshold Dose for Acute Lung Inflammation in Mice. Environmental Health Perspectives 114, 328–333. https://doi.org/10.1289/ehp.8266*							
Systemic Toxicity (subacute, subchronic, or chronic)							
Creutzenberg et al. (1995)	Y	Animal	Intratracheal instillation	0.15 mg **(LOAEL)**	4 times	Chemotactic properties of alveolar macrophages	NR

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
Test article: Carbon black (type Printex 90, Degussa Co., Hanau, Germany) consists of a carbon core with less than 0.05 % extractable organic substances. The specific surface area was about 300 m^2/g. Carbon black was suspended by means of 0.25 % Tween in saline. ***Dose:*** 0, 0.15, 0.5, 1.5 mg/animal followed by 0 or 0.5 ppm ozone whole body chamber for 1 week or 2 months. ***Treatment Duration:*** The total dose was administered intratracheally in 4 weekly aliquots of a volume of each 0.3 ml. ***Species (strain):*** Rat. (Wistar) 7 weeks. ***Gender:*** Males. ***Number per Group:*** Not reported. ***Control:*** Control animals were given a sham exposure to clean air. ***Methods:*** The investigations included measurements on lung mechanics, surfactant, histopathology and cellular and biochemical parameters in the bronchoalveolar lavagate. Carbon black was intratracheally instilled over a period of 4 weeks followed either by a subsequent I-week ozone exposure (acute ozone experiment) or a subsequent 2-month ozone exposure (sub chronic ozone experiment). In a separate experiment chemotaxis was measured after an acute 2-day ozone exposure without carbon black treatment (experiment No.3). About 16 h after cessation of exposure, rats were sacrificed for BAL. Phagocytosis and oxygen radical formation assay was conducted. Chemotactic migration was measured by a modified under-agarose assay. ***Adverse Effect(s) (brief):*** **LOAEL = 0.15 mg.** Significant decrease of macrophage migration distances after carbon black exposures (impairment in chemotactic capability of the macrophages). No acute or subchronic ozone inhalation effects on rat alveolar macrophages or at the control level after 7 days and 2 months of exposure, respectively. The number of ingested particles per macrophage and the formation of superoxide anion radicals did not differ from control after a 7 -day exposure to ozone. Prolonged exposure to ozone increased ingested particles per macrophage and the formation of superoxide anion radicals after a 2-month ozone exposure. A synergistic effect of ozone was observed in the combined groups. Chemotactic migration was generally retarded in the CB-treated groups (1.5mg/ppm; 75%). From the results it can be concluded that ozone is able to stimulate the phagocytotic and chemotactic activity of alveolar macrophages whereas CB impairs these functions. ***Data Quality:*** NR. ***Note***: None. ***Reference:*** *Creutzenberg, O., Bellmann, B., Klingebiel, R., Heinrich, U., Muhle, H., 1995. Phagocytosis and chemotaxis of rat alveolar macrophages after a combined or separate exposure to ozone and carbon black. Exp Toxicol Pathol 47, 202–6.*							
Driscoll et al. (1996)	Y	Animal	Inhalation	7.1 mg/m^3 **(LOAEC)** 1.1 mg/m^3 **(NOAEC)**	≤ 13 weeks	Inflammation, tissue injury, hprt gene mutation	NR
Test article: Carbon black. ***Dose:*** 0, 1.1, 7.1, 52.8 mg/m^3 ***Treatment Duration:*** 6.5 or 13 weeks followed by 3 and 8 months of recovery. ***Species (strain):*** Rat (Fischer 344), (200-250 g). ***Gender:*** Males. ***Number per Group:*** 3-4 per experiment. ***Control:*** Vehicle ***Methods:*** Animals were exposed via inhalation (6 hours/day, 5 days/week, 13 weeks) or aerosols of carbon black (Monarch 880, particle diameter, 0.016 mm surface 220 m2/g) in flow whole-body chamber. The effects on the lung were characterized after 6.5 and 13 weeks of exposure and 3 and 8 months of recovery. The following parameter were examined; mutagenesis in alveolar epithelial cells, pulmonary inflammation, inflammatory cytokine/growth factor expression, and lung histopathology. Bronchoalveolar lavage (BAL) was performed. Histopathology was examined after 13 weeks of exposure and after 3- and 8-months recovery rats. Lungs were removed and fixed. mRNA was examined for MIP-2 and MCP-1 using PCR. The rat alveolar type II cell isolation and the *hprt* clonal selection assay were performed. ***Adverse Effect(s) (brief):*** **NOAEC= 1.1 mg/m^3; LOAEC = 7.1 mg/m^3** After 13 weeks of exposure to 1.1, 7.1, and 52.8 mg/m3 carbon black, lung burdens were 354, 1826, and 7861 micrograms carbon black, respectively. Lung clearance of carbon black appeared impaired after exposure to 7.1 and 52.8 mg/m3 carbon black. 7.1 and 52.8 mg/m3 carbon black exposure resulted in changes in bronchoalveolar lavage fluid markers of lung injury and inflammation, expression of mRNA for the chemokines, MIP-2 and MCP-1, and lung histopathology. MCP-1 mRNA remained increased at the end of the 13-week exposure, but not after 3 or 8 months of recovery. Lung tissue injury and inflammation, increased chemokine expression, epithelial hyperplasia, and pulmonary fibrosis were observed after exposure to 7.1 and 52.8 mg/m^3carbon black, with the effects being more pronounced at the higher exposure level. Carbon black treatment for 13 weeks resulted in dose-dependent increase in hprt gene mutation of alveolar epithelial cells in rats (i.e., increased in rats dosed with 7.1 or 52.8 mg/m^3), followed by time-dependent decrease in hprt gene mutation (hprt gene mutation returned to control after 12 weeks of recovery. No increase in hprt mutation frequency was observed for epithelial cells obtained from rats exposed to 1.1 mg/m^3 carbon black. The subchronic inhalation of 1.1 mg/m3 carbon black did not elicit any detectable adverse lung effects. Control rats intratracheally instilled with vehicle control suspension did show observable adverse effect. ***Data Quality:*** NR. ***Note***: The highest two doses are very high. hprt gene mutation may be secondary effect to chronic inflammatory response. ***Reference:*** *Driscoll, K.E., Carter, J.M., Howard, B.W., Hassenbein, D.G., Pepelko, W., Baggs, R.B., Oberdörster, G., 1996. Pulmonary inflammatory, chemokine, and mutagenic responses in rats after subchronic inhalation of carbon black. Toxicol. Appl. Pharmacol. 136, 372–380. https://doi.org/10.1006/taap.1996.0045*							
Gallagher et al. (2003)	Y	Animal	Inhalation	1 mg/m^3 **(NOAEC)** 7.1 mg/m^3 **(LOAEC)**	13 weeks	Inflammatory, 8-oxo-dG	NR
Test article: Printex-90 carbon black or Sterling V carbon black. ***Dose:*** 0, 1.2, 7.1, 52.8 mg/m^3 Printex-90 carbon black or 50 mg/m^3 Sterling V carbon black ***Treatment Duration:*** Exposed for 13 weeks with 44-week recovery group. (6 hours/day, 5 days/week, 13 weeks). ***Species (strain):*** Rat (Fischer 344), 200-250g. ***Gender:*** Females. ***Number per Group:*** (n=5 per treatment/exposure or recovery group). ***Control:*** Vehicle ***Methods:*** Rats were exposed for 6 h/day, 5 days/week for 13 weeks to 1, 7, and 50 mg/m3 of Printex-90 (16 nm; specific surface area 300 m2 /g) and to 50 mg/m3 of Sterling V CB (70 nm; surface area of 37 m2 /g). The formation of 8-oxo-dG in the lung DNA was assessed using a reverse phase HPLC system coupled with UV and							

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
electrochemical (EC) detection. Lung burdens of CB were determined at both time points as well as differential cell populations from bronchoalveolar lavage fluid (BAL). Animals were euthanized after the 13-week exposure period and after the 44-week recovery period, at which time lung burden for carbon black was determined. The exposure concentration of Sterling V was selected to be equivalent in terms of retained mass in the lung to the high dose of Printex-90 at the end of exposure. However, in terms of retained particle surface area, the retained lung dose of Sterling V was equivalent to the mid-dose of Printex 90. ***Adverse Effect(s) (brief):*** **LOAEC = 7.1 mg/m^3.; NOAEC = 1 mg/m^3.** There was a dose-dependent increase in carbon black accumulation in lungs compared to controls. Exposure resulted in significant increase in the 8-oxo-dG DNA base lesion in the 52.8 mg/m^3 group and in 7.1 and 52.8 mg/m^3 groups in the 44-week recovery group. Exposure caused significant increase in the percentage of neutrophils found after 7.1 and 52.8 mg/m^3 carbon black exposures. No adverse effects were observed in rats following exposure to 1 mg/m^3 or untreated control. ***Data Quality:*** NR. ***Note***: None. ***Reference:*** *Gallagher, J, 2003. Formation of 8-Oxo-7,8-Dihydro-2'-Deoxyguanosine in Rat Lung DNA Following Subchronic Inhalation of Carbon Black. Toxicology and Applied Pharmacology, 190 3, 224–31. https://doi.org/10.1016/S0041-008X(03)00187-X.*							
Borm et al. (2005)	Y	Animal	Inhalation	50 mg/m^3 **(NOAEC)**	13 weeks	No observable adverse effect reported	NR
Test article: Printex-90 carbon black or Sterling V carbon black. ***Dose:*** Control, 1, 7, and 50 mg/m^3 Printex-90 carbon black or 50 mg/m^3 Sterling V carbon black. ***Treatment Duration:*** 13-week ***Species (strain):*** Rats (F344). ***Gender:*** Not reported. ***Number per Group:*** 3 animals/group. ***Control:*** positive controls, B[a]P (0.03 AM) and a mixture of 16 PAHs (0.1 AM) were used ***Methods:*** Inhalation. 13 weeks were investigated for lipophilic DNA adducts in lung tissue using ^{32}P post labeling was performed using either nuclease P1 enrichment or butanol extraction to enhance DNA adduct detection. Both procedures are suitable to detect PAH-related DNA adducts with its own specificities. ***Adverse Effect(s) (brief):*** **NOAEC = 50 mg/m^3.** No polycyclic aromatic hydrocarbon (PAH)-DNA adducts found after 13-week inhalation of 50 mg/m3 Sterling V or Printex-90 exposures compared to sham-exposed rats. ***Data Quality:*** NR. ***Note***: None. ***Reference:*** *Borm, P.J.A., Cakmak, G., Jermann, E., Weishaupt, C., Kempers, P., van Schooten, F.J., Oberdörster, G., Schins, R.P.F., 2005. Formation of PAH–DNA adducts after in vivo and vitro exposure of rats and lung cells to different commercial carbon blacks. Toxicology and Applied Pharmacology 205, 157–167. https://doi.org/10.1016/j.taap.2004.10.020*							
Ernst et al. (2002)	Y	Animal	Intratracheal instillation	0.5 mg **(LOAEL)**	10 weekly instillations	Pulmonary inflammation	NR
Test article: Carbon black (Printex 90; average arithmetical diameter of primary particles 0.014 µm; BET surface area 300 m2 /g; density 1.8–1.9 g/m^2), quartz, amorphous SiO_2, coal dust. ***Dose:*** 0.5 mg Carbon black (10 instillations; 1-week intervals), 3 mg quartz (once), 0.5 mg amorphous SiO_2 (20 or 30 instillations; 2 weeks intervals), 1 mg coal dust (10 instillations; 1-week intervals). ***Treatment Duration:*** 10 weekly instillations. ***Species (strain):*** Rats (Crl:WI (WU) BR Wistar WU),8-weeks old ***Gender:*** Females. ***Number per Group:*** 5 rats/group. ***Control:*** Saline group/ The controls received medium. ***Methods:*** Rats were exposed to the test substance via intratracheal instillation. All animals were observed in their cages daily. Individual body weights were recorded once per week during the first three months, there after every 4 weeks up to the end of the study. Wet weights of lungs (including trachea) were recorded, and the relative weight of lungs was calculated as compared to final body weights. Histopathological examination was performed on the lungs and lung-associated lymph nodes (LALN) of the first two experiments, the 4- week and the 3-month study. ***Adverse Effect(s) (brief):*** **LOAEL=0.5 mg.** Multifocal, dose-dependent, moderate to severe alveolar and interstitial accumulations of particle accumulation, accompanied by an increase in wet lung weight. inflammation was observed with significant increases in BAL leukocytes and PMN. A decreased production of reactive nitrogen intermediate, reactive oxygen intermediate, and TNF-α levels after carbon black exposures. ***Data Quality:*** NR. ***Note***: None. ***Reference:*** *Ernst, H., Rittinghausen, S., Bartsch, W., Creutzenberg, O., Dasenbrock, C., Gorlitz, B.D., Hecht, M., Kairies, U., Muhle, H., Muller, M., Heinrich, U., Pott, F., 2002. Pulmonary inflammation in rats after intratracheal instillation of quartz, amorphous SiO2, carbon black, and coal dust and the influence of poly-2-vinylpyridine-N-oxide (PVNO). Exp Toxicol Pathol 54, 109–26.*							
Gallagher et al. (1994)	Y	Animal	Inhalation	11.3 mg/m^3 **(LOAEC)**	2 years	No observable adverse effect reported	NR
Test article: diesel emissions; carbon black. ***Dose:*** 7.5 mg/m^3 diesel emissions for 2 months, 6 months, or 2 years and for 2 years, 11.3 mg/m^3 carbon black. ***Treatment Duration:*** 2- years***. Species (strain):*** Rats (Crl: (WI) BR Wistar), ~ 7 weeks old. ***Gender:*** Females. ***Number per Group:*** 3- 5 per dose. ***Control:*** Filtered air ***Methods:*** Inhalation (18 hours/day, 5 days/week, 2 years). The current study was designed to test the commercial carbon blacks (CBs) as well as the ability of these PAHs to form bulky DNA adducts. ***Methods:*** In four commercial CBs particle were administered to rat for up to two years or filtered air (sham controls) for 18 h/day, 5 days/week for 2 years using whole body exposure chambers. The carbon black exposure was 7.5 mg/m3 for the first 4 months and 12 mg/m3 for the last 20 months***. Adverse Effect(s) (brief):*** **LOAEC = 11.3 mg/m^3.** No significant elevation in adduct levels in lung DNA isolated from any of the particle-exposed groups. DNA adduct levels for adduct 1 decreased > 2-fold compared to the filtered air controls in lung DNA isolated from rodents exposed for 2 years to diesel exhaust. A reduction of putative I-compound levels was measurable in response to all three particle types, despite the differences in the amount of adsorbed organic matter. The particle lung load for these three exposure groups were similar (19.9-23. 2 mg/hmg) 6 months after the start of the exposure. After 2 years, the diesel soot exposed animals had a higher particle lung load (63.9 mg) compared to carbon black (43.9 mg) exposed animals. ***Data Quality:*** NR. ***Note***: None. ***Reference:*** *Gallagher, J., Heinrich, U., George, M., Hendee, L., Phillips,*							

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
D.H., Lewtas, J., 1994. Formation of DNA adducts in rat lung following chronic inhalation of diesel emissions, carbon black and titanium dioxide particles. Carcinogenesis 15, 1291–9.							
Heinrich et al. (1995)	Y	Animal	Inhalation	11.6 mg/m3 **(LOAEC)**	2 years	Lung tumor	NR

Test article: diesel exhaust, carbon black (Printex 90, CAS no. 1333-86-4, primarily particle size 14 nm). ***Dose:*** Diesel particles: 6.98, 2.5, and 0.84 mg/m3 for 24 (13.5) months; Carbon black: 7.4 mg/m3 for 4 months, followed by 12.2 mg/m3 for 20 months; Control (particle-free diesel exhaust): 23 months (NMRI mice), or 24 months + 6 months clean (C57BL/6N mice). The average particle exposure concentrations for diesel soot and carbon black were 7 and 11.6, mg/m', respectively. ***Treatment Duration:*** Up to two years (18 hours/day, 5 days/week, 2 years)**.** ***Species (strain):*** Rats (Wistar Crl: (WI) BR); Mouse (NMRI), (C57BL/6) 7-weeks old. ***Gender:*** Females. ***Number per Group:*** 100 per group (rats); 80 per group (mice). ***Control:*** Clean air ***Methods:*** The animals were exposed 18 h/day, 5 days/ wk for up to 24 months (rats and C57BL/6N mice) in special whole-body exposure chambers. The body weight of the rats and mice was determined every 4 wk. The lung wet weight and lung retention of inhaled particles were determined at different time points during the study. The lung wet weight was determined 3, 6, 12, 18, 22, and 24 mo after starting the exposure. The measurement of the alveolar lung clearance was carried out with radioactively labeled test aerosols inhaled by the rats for about 30 min at 3, 12, and 18 months after the start of the experiment. A biochemical and cytological examination of lung lavage fluid was also carried out for the rats. Bronchoalveolar lavage was obtained by a twofold lavage with 4 ml saline. The lavage was analyzed for cytological and biochemical parameters (lactate dehydrogenase, beta-glucuronidase, total protein, hydroxyproline, total number of leukocytes, differential cell count). Histopathological investigations of the following organs were conducted for all animals: nasal and paranasal cavities (four sections; localization, larynx, trachea, and lung (five sections; localization: the left lobe, right caudal lobe, and right middle lobe were sectioned longitudinally, and the right cranial lobe and accessory lobe were sectioned transversely to the main bronchus). Graduation of the findings was done with four grades: very slight, slight, moderate, and high. ***Adverse Effect(s) (brief):*** **LOAEC = 11.6 mg/m^3**. After 24 months of exposure in rats, the mortality found was, 56% in the carbon black compared to 42% in the clean air control group. At the end of the 130 wk, the mortality reached 92% (carbon black) and 85% (control group). The body weight of the exposed animals was significantly lower from day 300 (carbon black) compared to the controls. At the end of the 2-yr exposure, the body weight (BW) of the rats exposed to carbon black (BW 325 g) was significantly lower compared to the control rats (BW 417 g). Carbon black caused significant increase in rat lung wet weight compared to controls. The exposure to carbon black led to a substantial increase in lung wet weight, progressing with study duration. The most severe increase in lung wet weight (0.8 g, five-fold compared to the controls) was found in the carbon black group after 18 months of exposure. During the second year of exposure, the particle lung load of carbon black exposed rats increased by only 15%, whereas the high diesel group showed an 80% increase in the particle lung load. After 18 months of exposure to carbon black, and 3 mo of recovery time without particle exposure, no reversibility of the alveolar lung clearance damage could be detected. After 6 month of exposure, slight bronchioloalveolar hyperplasia in the lung was found in serial sacrificed animals (20/20) of all groups exposed to high particle concentrations. Most of the exposed rats in the carcinogenicity study showed bronchioloalveolar hyperplasia in the lungs. The highest incidences were observed in the high diesel (98/100) and carbon black (96/100) groups. In these groups the bronchioloalveolar hyperplasia was more severe (moderate to high grade) compared to controls. There were no lung tumors observed in high diesel or carbon black satellite groups of about 20 animals each after 6 and 12 months of exposure. After an exposure time of 24 months followed by 6 mo of clean air, lung tumor rates of 39 % (were observed in rats exposed to carbon black). The following types of tumor were observed in rats at 30-month exposure: Benign squamous- cell tumor (20/100); Squamous-cell carcinoma (4/100); Adenoma (13/100); adenocarcinoma (13/100); Hemangioma (0/100). After 11 months in the carbon black group, up to 17 months the body weight of the NMRl mice was significantly (5-7%) lower compared to the clean air control group. During the last months of exposure there was no significant difference in the body weight between the control and exposed groups. The mortality rate was 20% in the carbon black, and 10% in the clean air control group 13.5 month after the start of exposure. A mortality rate of 50% was reached after 19 months in carbon black, and 20 in the control group. The lung wet weight was determined at 3, 6, 12, 18, and 21 months after the start of exposure. The measurements after 3 and 12 months of exposure to carbon black (0.3g,1.0g) showed a substantial increase in lung wet weight compared to the controls (0.2 g, 0.2 g), progressing with study duration. The most severe increase in lung wet weight (fivefold compared to the controls) was found in the carbon black group after 12 months of exposure. In the recovery phase, after 13.5 mo of exposure, a slight decrease in lung wet weight was found in the carbon black (0.8 g) compared to controls. In these groups, 11 (carbon black) and 4 (high diesel soot) animals each showed 2 tumors in their lungs. Bronchioloalveolar adenomas and adenocarcinomas were observed at a high frequency in carbon black-exposed animals. Only 1 lung tumor (adenocarcinoma) was found in 217 control animals. The only types of lung tumor observed in mice were adenomas and adenocarcinomas. The percentages of adenomas/adenocarcinomas in the various groups were 21.8%/15.4% (diesel), 11.3%/10% (carbon black) and 25%/15.4% (clean air). The lung tumor rates (adenomas and adenocarcinomas) of the diesel soot-exposed (32.1%) and carbon black-exposed (20%), -exposed animals were not significantly different from the tumor rate of the control animals (30%). Because the mean lifetime of the exposed and control mice was significantly different, the tumor rates were compared using the prevalence method. By means of this statistical approach, it was found that the lung tumor rate of the NMRl mice was not significantly influenced by the different types of exposure. Overall, rat and mice exposure to carbon black caused a significant decrease in mean lifetime and body weight compared to controls. Alveolar lung clearance rate changed significantly compromised, and adverse effects on BAL observed (e.g., the differential cell count, LDH concentration, β-glucuronidase). In rats, bronchioloalveolar hyperplasia and tumors in lungs, but in mice, adenomas and adenocarcinomas observed in lungs. ***Data Quality:*** NR. ***Note***: Because the exposure concentration was changed for carbon black during the exposure time, it was not possible to compare the exposure to lung tumor response relationships between the various exposure groups based on the exposure concentrations. Doses administered are very high. Mechanism of

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
tumorigenicity is lung overload. ***Reference:*** *Heinrich, U., Fuhst, R., Rittinghausen, S., Creutzenberg, O., Bellmann, B., Koch, W., Levsen, K., 1995. Chronic Inhalation Exposure of Wistar Rats and two Different Strains of Mice to Diesel Engine Exhaust, Carbon Black, and Titanium Dioxide. Inhalation Toxicology 7, 533–556. https://doi.org/10.3109/08958379509015211*							
Nau, et al. (1958b)	ND	Animal	Dermal	326.95 g **(NOAEL)**	up to 30 months (3 times a week)	No observable adverse effect reported	NR
Test article: whole carbon black in oil or whole carbon black in water. Benzene extracted carbon black and "free" benzene extract of carbon black, 3,4-benzpyrene or methylcholanthrene adsorbed carbon black. ***Dose:*** 7.44 – 326.95 g whole carbon black in oil or 5.54 – 947.93 g whole carbon black in 20% in water, using 1 % aqueous carboxymethylcellulose (CMC). The oil mixtures were either 20% in cottonseed oil or 20% in mineral oil. ***Treatment Duration:*** up to 30 months. (3 times a weekly) ***Species (strain):*** Mice (CFW white and C_3H brown), 6 – 10 weeks old; Rabbit (white); Monkeys (Phesus) (5-7 lbs). ***Gender:*** Not reported. ***Number per Group:*** (Number per group not applicable). ***Control:*** Untreated controls were used ***Methods:*** Mice were painted three each week. The test substance was applied in one stroke to unshaved back. The treated animals were observed twice daily. Complete gross and microscopic pathology were made of all organs and tissues. Rabbits were painted on shaven abdominal surface three time each week with test material determined by weight. Monkeys were painted in four areas (both arm pits and both groins) three times each week with amounts of the material. ***Adverse Effect(s) (brief):*** **NOAEL = 326.95 g.** 30-months post exposure, no observable adverse effects were reported in mice, rats or monkeys following skin contact of the "whole" carbon black doses up to of 326.95 g. The NOAEL of 326.95g was selected based on a 17.5-month monkey exposure study. However, carbon blacks with adsorbed component, when free, produces skin cancer. ***Data Quality:*** NR. ***Note***: None. ***Reference:*** *Nau, C.A., Neal, J., Stembridge, V., others, 1958. Study of the Physiological Effects of Carbon Black. II. Skin Contact. Arch. Indust. Health 18, 511–20.*							
Nau et al. (1958a)	ND	Animal	Oral (feed)	~ 28,000 mg/kg/d **(NOAEL)**	≤ 18 months	No observable adverse effect reported	NR
Test article: Whole carbon black (10% of feed) per mouse, extracted carbon black and "free" benzene extract of carbon black, methylcholanthrene adsorbed carbon black for 12 to 18 months. ***Dose:*** 182 – 229 g whole carbon black (10% of feed) per mouse, extracted carbon black and "free" benzene extract of carbon black, methylcholanthrene adsorbed carbon black ***Treatment Duration:*** 12 to 18 months ***Species (strain):*** Mice (CFW white and C_3H brown) 6-10 weeks old. ***Gender:*** Males and females. ***Number per Group:*** (Number per group not applicable). ***Control:*** Untreated group ***Methods:*** Oral (feed). Mice were fed a mixture of 90% dog chow and 10% carbon black wetted with 1% carboxymethycellulose /5% cotton seed oil. Controls received 85% dog chow with 15% wheat flour or dog chow alone. ***Adverse Effect(s) (brief):*** **NOAEL = ~ 200 g/mouse (= 10% feed; 28,000 mg/kg/d).** At 200 g (highest dose tested) no significant change from the normal in any of the organ tissues of the mice treated with carbon black were reported. The continuous oral exposure to carbon black resulted in no observable adverse effects compared to controls, but "free" benzene extract from carbon black or methylcholanthrene adsorbed carbon black resulted stomach tumors. ***Data Quality:*** NR. ***Note***: None. ***Reference:*** *Nau, C.A., Neal, J., STUMBRIDGE, V., others, 1958. Study of the Physiological Effects of Carbon Black. I. Ingestion. Arch. Indust. Health 17, 21–8.*							
Nau et al. (1962)	ND	Animal	Inhalation	NR	Life span (mice) >13000 hours (monkeys)	No observable adverse effect reported	NR
Test article: Channel or furnace carbon black. ***Dose:*** The dust concentration for channel black was 2.4mg per cubic meter, Furnace black was 1.6mg per cubic meter. The chamber size was ~ 6X8X10 ft. ***Treatment Duration:*** life span for mice; > 13,000 hours for monkeys. (7hrs per/day for 5/day per week). ***Species (strain):*** Mice (CFW white) and (C_3H brown),10-weeks old, monkeys. ***Gender:*** Males and females. ***Number per Group:*** Not reported. ***Control***: 60 untreated ***Methods:*** Inhalation occurred life span for mice and > 13,000 hours for monkeys. The mucosa, submucosa, or adjacent structures of the nasal, oral, pharyngeal, laryngeal, or tracheal air passages were examined by microscopic pathology. ***Adverse Effect(s) (brief):*** No changes in the mucosa, submucosa, or adjacent structures of the nasal, oral, pharyngeal, laryngeal, or tracheal air passages were observed in the treated animals. Carbon black was deposited within the wall of the alveoli but the channel dust more readily penetrated into the intersitices. Detectable changes were observed in chest x-ray films of mice and monkey exposed. There was increased lung weight (absolute and relative to body weight) due to particle deposition. In the lung there was minimal or no fibrous tissue proliferation and no malignancies. No significant effects were detected in exposed animals. ***Data Quality:*** NR. ***Note***: None. ***Reference:*** *Nau, C.A., Neal, J., Stembridge, V.A., Cooley, R.N., 1962. Physiological effects of carbon black: IV. Inhalation. Archives of Environmental Health: An International Journal 4, 415–431.*							
Nikula et al. (1995)	Y	Animal	Inhalation	2.5 mg/m^3 **(LOAEC)**	2 years	Lung neoplasm	NR
Test article: diesel exhaust or carbon black. ***Dose:*** 0, 2.5, 6.5 mg/m^3 diesel exhaust or carbon black ***Treatment Duration:*** Two-year; sacrificed 3, 6, 12, 18, 23 months after exposure. (16 hrs/day, 5 days/week) ***Species (strain):*** Rats (F344),5-7 weeks. ***Gender:*** Males and females. ***Number per Group:*** Approx. 100 rats of each gender/group/ ***Control***: untreated group. ***Methods:*** Rats were exposed via inhalation (16 hours/day, 5 days/week, 24 months) for up to two years. The animals were sacrificed 3, 6, 12, 18, 23							

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
months after exposure. Various parameters were examined. Blood was collected, lung weights was recorded, histopathology, animals were observed for clinical signs of toxicity, survival and body weights were recorded. The occurrence of tumors was examined. ***Adverse Effect(s) (brief):*** **LOAEC = 2.5 mg/m^3.** No clinical signs related to exposure other than darkened fur coats and decreased body weight gain were observed. CB-exposed males survival was shortened compared to females. CB exposure caused decreased body weight gain, but increased lung weight due to particle deposition. Lung burden was 90.6 mg of the high dose group males at 23 months. Particles represented 2.3% of lung weight. Neoplasms were found in 11.8% mice at 6.5 mg/m^3 , 3.8% at 2.5 mg/m^3 , and 0.9% in control. 6.5 mg/m^3 exposure resulted in a significant prevalence of lung neoplastic responses (adenoma and adenocarcinoma), in female rats compared to controls (20% and 0%, respectively). The incidences of neoplasms were lower in males at 6.5 mg/m^3 (3.8%). ***Data Quality:*** NR. ***Note***: None. ***Reference:*** *Nikula, K.J., Snipes, M.B., Barr, E.B., Griffith, W.C., Henderson, R.F., Mauderly, J.L., 1995. Comparative pulmonary toxicities and carcinogenicities of chronically inhaled diesel exhaust and carbon black in F344 rats. Fundam Appl Toxicol 25, 80-94 (cited in Kuempel and Sorahan, 2006). https://doi.org/S0272059085710421 [pii]*							
Nolte et al. (1993)	ND	Animal	Inhalation	NR	10 months	Squamous metaplasia	NR
Test article: Pyrolized pitch condensate and/or carbon black. ***Dose:*** 50 mg BaP and 6 mg carbon black/m^3 clean air ***Treatment Duration:*** 10 months followed by up to 20 months recovery. ***Species (strain):*** Rats. (Wistar). ***Gender:*** Females. ***Number per Group:*** Nor reported. ***Control***: human tissue with known positive reactivity as well as a variety of epithelial tissues of the rat were used as controls. ***Methods:*** Inhalation (18 hours/day, 5 days/week, 10 months)**,** followed by a clean air period of up to 20 months. The following parameters were observed in this study: routine histology, scanning and transmission electron microscopy, and by immunohistochemical detection of various cytokeratins (CKs). ***Adverse Effect(s) (brief):*** Squamous metaplasia occurs in the transitional region of terminal bronchioles and alveolar ducts. ***Data Quality:*** NR. ***Note***: None. ***Reference:*** *Nolte, T., Thiedemann, K.U., Dungworth, D.L., Ernst, H., Paulini, I., Heinrich, U., Dasenbrock, C., Peters, Ueberschar, S., Mohr, U., 1993. Morphology and histogenesis of squamous cell metaplasia of the rat lung after chronic exposure to a pyrolized pitch condensate and/or carbon black, or to combinations of pyrolized pitch condensate, carbon black and irritant gases. Exp Toxicol Pathol 45, 135–44.*							
Pence and Buddingh (1985)	Y	Animal	Oral (feed)	274 mg/kg/d (mice); 104.1 mg/kg/d (rats) **(NOAEL)**	2 years	No observable adverse effect reported	NR
Test article: Carbon black. For tumor induction, intraperitoneal injections of 1,2-dimethylhydrazine ***Dose:*** 100 g/kg body weight/year for mice and 38 g/kg body weight/year for rat. ***Treatment Duration:*** 2 years. For tumor induction, intraperitoneal injections of 1,2-dimethylhydrazine (rats 10 mg/kg body weight for 16 weeks and mice 20 mg/kg for 6 weeks). ***Species (strain):*** Rats (Sprague-Dawley); Mice (CF1). ***Gender:*** Females. ***Number per Group:*** 29 -45 rats/group and 26 – 33 mice/group. ***Control:*** Control animals did not have carbon black added to the feed ***Methods:*** Carbon black was added to the diet at 2.05 g/kg feed and fed for 52 weeks. Female rats and mice treated with 1,2-dimethylhydrine (DMH) to induce adenarcinomas of the colon. Colonic tumors were induced in rats by 16 weekly intraperitoneal (i.p.) injections of DMH at 10 mg/kg body weight. Mice treated with DMH received 6 weekly i.p. injections at 20 mg/kg body weight. At the end of this period all surviving animals were necropsied and examined for colon. At necropsy, all tissues were examined for gross pathology, and samples from lesions were fixed in 10% buffered formalin. ***Adverse Effect(s) (brief):*** **NOAEL = 100 g/kg/yr (= 274 mg/kg/d; mice); 38 g/kg/yr (= 104.1 mg/kg/d; rats).** There were no differences due to carbon black. Lung adenomas and mammary tumors prevalent in mice and rats, respectively, but no statistical significance. No enhancing effect of carbon black on gastrointestinal carcinogenesis. ***Data Quality:*** NR. ***Note***: None. ***Reference:*** *Pence, B.C., Buddingh, F., 1985. The effect of carbon black ingestion on 1,2-dimethylhydrazine-induced colon carcinogenesis in rats and mice. Toxicology Letters 25, 273–277. https://doi.org/10.1016/0378-4274(85)90207-3*							
Swafford et al. (1995)	Y	Animal	Inhalation	NR	2 years	Low frequency mutations	NR
Test article: *C*arbon black or diesel exhaust. ***Dose:*** Weekly mean concentrations from daily filter samples were 2.46 ± 0.03 and 6.55 ± 0.06 mg/m^3 carbon black or 2.44 ± 0.02 and 6.33 ± 0.04 mg/m^3 diesel exhaust. ***Treatment Duration:*** 2-years. (16 hours/day, 5 days/week, 24 months). ***Species (strain):*** Rats. (F344). 7-9 weeks old. ***Gender:*** Males and females. ***Number per Group:*** 1150 (divided in five groups) ***Control:*** filtered air (sham control). ***Methods:*** Chronic inhalation exposure was assessed in rats to diesel exhaust (DE) or carbon black (CB) at identical particle concentrations. DE contained 8.2% particle associated, extractable, organic compounds by weight. The CB, which contained only 0.12% extractable organics, served as a surrogate for the elemental carbon matrix of DE soot. Rats (7-9 weeks old) were divided into five groups and exposed chronically (16 h/day, 5 days/week, 24 months) by inhalation to either DE or CB at one of two particle concentrations or to filtered air (sham control). Means (± standard error) of weekly mean particle concentrations from daily filter samples were 2.44 ± 0.02 and 6.33 ± 0.04mg/m3 for the DE exposure chambers, and 2.46 ± 0.03 and 6.55 ± 0.06 mg/ m 3 for the CB chambers. Lungs were collected; lung neoplasms were fixed in 4% buffered paraformaldehyde or in 10% neutral buffered formalin. These samples were stained with H&E and examined by light microscopy for histologic diagnosis. Serial sections were also cut for immunohistochemical assays and DNA analysis, K-ras mutational analysis, and p53 immunohistochemistry. ***Adverse Effect(s) (brief):*** Histopathologic evaluation revealed that chronic exposure to CB produced pulmonary neoplasia (adenomas, adenocarcinomas (ACs), squamous cell carcinomas (SCCs) and adenosquamous carcinomas). However, p53 and K-ras genes are infrequently altered in lung adenocarcinomas induced in the rat by carbon black exposures. One G → T transversion in the K-ras gene observed after carbon black exposures. ***Data Quality:*** NR.							

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
Note: None. ***Reference*****:** *Swafford, D.S., Nikula, K.J., Mitchell, C.E., Belinsky, S.A., 1995. Low frequency of alterations in p53, K-ras, and mdm2 in rat lung neoplasms induced by diesel exhaust or carbon black. Carcinogenesis 16, 1215–21.*							
Von Haam and Mallette (1952)	Y	Animal	Skin	NR	315 days	No observable adverse effect reported (crude CB); tumors at site of contact (skin) (CB extracts)	NR
Test article: Crude and extracted carbon black ***Dose:*** Not reported. (0.06% to 2% extractable material) ***Treatment Duration:*** for 315 days. ***Species (strain):*** Mice (Swiss). ***Gender:*** Not reported. ***Number per Group:*** 212 totals animals. Grouping not specified. (20/ control) ***Control:*** Positive and negative groups ***Methods:*** An oval area of the back measuring approximately 2 cm by 1cm was clipped and the reagents were applied once a week with cotton applicators. The concentrated fraction of carbon black extracts was prepared by adding 100 times the volume acetone containing 0.5% of croton oil. Negative controls were painted with 0.5% solution croton oil in acetone. Positive controls were used. Animals underwent autopsy and the treated skin area was studies under the microscope upon death. ***Adverse Effect(s) (brief):*** **No POD reported.** Crude unfractionated extracts did not produce any tumors, but some extracts resulted tumors (squamous cell carcinoma and papilloma) and epithelial proliferation. ***Data Quality:*** NR. ***Note***: Tumors were at site of contact (i.e., local effect). ***Reference:*** *Von Haam, E., MALLETTE, 1952. Studies on the Toxicity and Skin Effects of Compounds used in the Rubber and Plastics Industries. III. Carcinogenicity of Carbon Black Extracts. Arch. Indust. Hyg. & Occupational Med. 6, 237–42.*							
Genotoxicity							
Don Porto Carero et al. (2001)	Y	A549, THP-1	Alamar blue assay, Comet assay	1600 ng/mL	DNA damage	NR	
Test article: carbon black (Vulcan M, furnace black). ***Treatment duration:*** 48-hour ***Dose:*** exposures at 16, 160, 800, 1600, 8000, and 16000 ng/mL for cytotoxicity tests or at 16, 10, and 1600 ng/mL for genotoxicity tests suspended in 5 ml complete tissue culture medium (89% RPMI 1640 1 10% FBS 1 1% P/S), supplemented with 0.1% Tween 80. ***Speciec (strain):*** A549 (human alveolar carcinoma) and THP-1 (human monocyte) cell lines. ***Gender:*** NA ***Control:*** nonexposed control cells-Paraquat, used as a positive control in the cytoxicity assay. ***Method:*** Extracts of CB were all prepared using the same procedure. Initially 8 mg of each particle type was suspended in 5 ml DCM followed by two washing steps. The two supernatants were mixed and evaporated, resuspended in DMSO, and further diluted in tissue culture medium. The extract and the washed particles were further diluted in tissue culture medium. Cytotoxicity studies, cells were seeded in 96-well plates. The cells were then exposed for 48 hr to different concentrations of paraquat (PQ) or carbon black (CB). A color indicator that detects the reduction of the tissue culture medium. This reduction results in a colorimetric or fluorometric signal of AlamarBlue, which is a measure for cell growth or death. For genotoxicity testing, cells were seeded in six-well plates. Cells were then exposed for 48 hr to different concentrations of the particles or their extracts. Comet assay cells are embedded in agarose on a microscopic slide and lysed with detergent. DNA was then denatured, subjected to alkaline electrophoresis, and stained with a fluorescent DNA-binding dye. Cells with increased DNA damage display increased migration of the DNA from the nucleus toward the anode. The amount of DNA migration indicated the amount of DNA breakage in the cell. ***Number per Group:***Three independent experiments, with six replicates per sample. The mean particulate diameter in tissue culture medium was approximately 100 nm for CB. ***Adverse Effects (s) (brief):*****N OAEL =1600 ng/mL.** No cytotoxicity observed for either cell line at all concentration tested. In A549 and mature THP-1 cells, original particle carbon black at the highest test concentration (1600ng/mL) induced DNA damage compared to control cells. The washed particles or extracts did not result in DNA damage at any dose. The dichloromethane extracts of carbon black did not cause any significant DNA damage. ***Data quality:*** NR. ***Note:*** Mechanism of DNA damage observed at the highest dose tested is unrelated to the extractable substances. ***Reference:*** *Don Porto Carero, 2001. Genotoxic Effects of Carbon Black Particles, Diesel Exhaust Particles, and Urban Air Particulates and Their Extracts on a Human Alveolar Epithelial Cell Line (A549) and a Human Monocytic Cell Line (THP-1). Environmental and Molecular Mutagenesis 37, 155–163.*							
Kirwin et al. (1981)	ND	Salmonella typhimurium, Chinese hamster ovary, L5178Y, C3H/10T$_{1/2}$, Drosophila melanogaster	Ames assay, Sister chromatid exchange test, Mouse lympoma test, Cell transformation assay, Genetic effects	40000 µg/mL	No observable adverse effect reported	NR	
Test article: Oil furnace carbon black (N-339) ***Dose:*** salmonella typhimurium were treated at concentrations, 0, 75, 375, 1875, 3750, and 7500 µg. Activated and non-activated Chinese hamster ovary (CHO) cells were treated at 0, 0.32, 1.6, 8, 40, 200, and 1000 µg/mL carbon black. Mouse lymphoma L5178Y TK+/- locus cells were treated with 10000, 15000, 20000, 25000, 30000,							

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
35000, and 40000 µg/mL carbon black ***Treatment duration:*** up to 35 days. ***Speciec (strain):*** Salmonella typhimurium cells (TA98, TA100, TA1535, TA1537, TA1538); Activated and non-activated Chinese hamster ovary (CHO) cells. Mouse lymphoma L5178Y TK+/- locus cells, C3H/10T1/2 cells, and Drosophila melanogaster ***Gender:*** NA. ***Number per Group:*** 2-4 replicates. ***Control:*** untreated/ DMSO; Salmonella Assay:The positive controls were 2- aminoanthracene (2AA), propane sultone (PS), and 9-aminoacridine (9AAD). SCE assay: Dimethylnitroamine (DMN); MLA: acetone ***Method:*** Ames Salmonella typhimurium reverse mutation test, sister chromatid exchange test in CHO cells, mouse lymphoma test, cell transformation assay in C3H/10T1/2 cells, and assay for genetic effects in Drosophila melanogaster ***Adverse Effects (s) (brief):*** **NOAEL= 40000 µg/mL.** N-339 carbon black was negative in salmonella typhimurium with or without metabolic activation. Two of six dose levels are associated with significant increases in sister chromatid exchange frequency, but the increases are again small, and there is no indication of a positive dose response. The compound did not cause significant increases in mutant frequencies in the L5178Y mouse lymphoma assay. The cell transformation assay N-339 carbon black did not cause morphological transformation of cells, while the positive control DMBA exhibited the expected positive results. The Drosophila assay indicate that the test product did not induce significant genetic damage under the test conditions. N-339 carbon black did not exhibit any significant genetic activity in five short-term genetic activity assays. ***Data quality:*** NR. ***Reference:*** *Kirwin, C.J., LeBlanc, J.V., Thomas, W.C., Haworth, S.R., Kirby, P.E., Thilagar, A., Bowman, J.T., Brusick, D.J., 1981. Evaluation of the genetic activity of industrially produced carbon black. Journal of Toxicology and Environmental Health, Part A Current Issues 7, 973–989.*							
Zhong et al. (1997)	ND	V79, Hel 299	Comet assay	137.9 µg/m^3		No observable adverse effect reported	NR
Test article: crystalline silica, amorphous silica, and carbon black. ***Dose:*** crystalline silica (Min-U-Sil 5; at 0,17.2, 34.4, 68.8, 103.4 µg/cm^2), amorphous silica (Spherisorb; at 0, 17.2, 34.4, 68.9, 137.9 µg/cm^2), carbon black (at 0, 17.2, 34.4, 68.9, 137.9 µg/cm^2), and glass fibers (AAA-10; at 0, 1.7, 3.4, 6.9, 13.8 µg/cm^2). ***Treatment duration:*** for 3 hours. ***Speciec (strain):*** Chinese hamster lung fibroblasts (V79 cells) and human embryonic lung fibroblasts (Hel 299 cells). ***Number per Group:*** two experiments ***Control:*** MEM was used as a concurrent solvent control ***Method:*** DNA damage induced by four different mineral dusts in V79 and Hel 299 cells using the alkaline SCG comet assay, and to compare DNA damage with other biological activities of occupationally related dusts particles. ***Adverse Effects (s) (brief):*** **NOAEL=137.9 µg/m3.** After carbon black exposures up to 137.9 µg/cm^2, no significant increase in tail length in V79 and Hel 299 cells were detected. ***Data quality:*** NR. ***Reference:*** *Zhong, B., Whong, W., Ong, T., 1997. Detection of mineral-dust-induced DNA damage in two mammalian cell lines using the alkaline single cell gel/comet assay. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 393, 181–187. https://doi.org/10.1016/S1383-5718(97)00094-6*							
Borm et al. (2005)	Y	A549	DNA addcut assay	NR		No observable adverse effect reported	NR
Test article: four commercial CBs (Printex 90, Sterling V, N330, Lampblack 101), leaching of PAH. ***Dose:*** epithelial cells were exposed at equivalent mass (100 mg/m^3) using equivalent volume (50 µL) of particle extracts, reaching different PAH levels (target concentration was 0.03 µM B[α]P or 30 – 300 µg carbon black particles per cm^2). ***Treatment duration:*** 24-h incubation. ***Species (strain):*** A549 human lung***.*** ***Number per Group:*** three different experiments, each in duplicate. ***Control:*** As positive controls, B[a]P (0.03 µM) and a mixture of 16 PAHs (0.1 µM) were used. ***Method:*** The current study was designed to test the possible release and bioavailability of polycyclic aromatic hydrocarbons (PAHs) from a set of commercial carbon blacks (CBs) as well as the ability of these PAHs to form bulky DNA adducts. In four commercial CBs (Printex 90, Sterling V, N330, Lampblack 101), leaching of PAH was examined through (1) release of parent PAHs in saline with or without surfactant, and (2) PAH adducts in lung epithelial cells (A549). In vitro experiments were done with original and extracted particles, as well as organic extracts of carbon blacks in DMSO. ***Adverse Effects (s) (brief):*** In vitro incubations with CB particles (30 – 300 Ag/cm^2) revealed no adduct spots except for Sterling V. However, the spot was not concentration dependent and remains unidentified. Lung DNA from rats after inhalation of Printex 90 or Sterling V showed no spots related to PAH –DNA adduct formation compared to sham-exposed rats. The cells were incubated with original and extracted carbon black particles in concentrations between 30 and 300 Ag/cm2 that were shown to be noncytotoxic using two independent assays (LDH, MTT). ***Data quality:*** NR. ***Reference:*** *Borm, P.J.A., Cakmak, G., Jermann, E., Weishaupt, C., Kempers, P., van Schooten, F.J., Oberdörster, G., Schins, R.P.F., 2005. Formation of PAH–DNA adducts after in vivo and vitro exposure of rats and lung cells to different commercial carbon blacks. Toxicology and Applied Pharmacology 205, 157–167. https://doi.org/10.1016/j.taap.2004.10.020*							
Driscoll et al. (1997)	Y	Animal	Intratracheal instillation	10 mg/kg **(NOAEL)**	2 days	Mutation; inflammation	NR
Test article: carbon black (diameter 515 nm; surface area 5 230 m^2/g) ***Dose:*** Two equal doses of 0, 5, 50 mg/kg. (a total of 0, 10, 100 mg/kg). 12 animals were administered saline as a vehicle control. ***Treatment Duration:*** Two consecutive days, followed by 15 months. ***Species (strain):*** Rats (F344, 180-200g) ***Gender:*** Females. ***Number per Group:*** 9/group. ***Control:*** Vehicle control***.*** ***Methods:*** Rats were exposed to carbon black particle via intratracheal instillation. A total particle dose of 10 and 100 mg/kg body weight equal doses of 5 and 50 mg/kg on consecutive days to groups of nine rats/ treatment. Controls received saline. The test substance was administered as two doses followed by 15-monhts after exposure bronchalveolar lavage (BAL) cells were characterized for number and type of lung cell. Histology was performed on lung tissues. The effect of in vivo particle exposure on hprt mutation frequency in rat alveolar epithelial cells were determined with Hprt mutation assay. Alveolar type II epithelial							

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
cells isolated from rat lungs. After 14–21 days in culture the cells were fixed and analyzed. The mutagenic effects were determined in BAL cells from saline or particle exposed rats on the rat lung epithelial cell line, RLE-6TN in a coculture. ***Adverse Effect(s) (brief):*** **NOAEL = 10 mg/kg.** Carbon black exposure were evaluated for their ability to cause neutrophilic inflammation, mutation in rat alveolar cells. Exposure resulted in significantly increased number of macrophages, neutrophils, and lymphocytes in BALF 15 months after exposures. Carbon black exposure at 100 mg/kg in rat significantly increased the number of hprt mutation frequency in lung alveolar Type II epithelial cells. Carbon black-exposed lungs consisted of dose-related minimal to slight centriacinar alveolitis and bronchiolitis and bronchoalveolar region type II cell hyperplasia ***Data Quality:*** Not reported. **Note:** Authors report the mutagenic activity appeared greatest for neutrophils compared to macrophages. ***Reference:*** *Driscoll, K.E., Deyo, L.C., Carter, J.M., Howard, B.W., Hassenbein, D.G., Bertram, T.A., 1997. Effects of particle exposure and particle-elicited inflammatory cells on mutation in rat alveolar epithelial cells. Carcinogenesis 18, 423–430. https://doi.org/10.1093/carcin/18.2.423*							
Carcinogenicity							
Belinsky et al. (1997)	Y	Animal	Inhalation	NR	NR	K-ras mutation	NR
Test article: diesel exhaust, carbon black. ***Dose:*** NR ***Treatment Duration:*** NR ***Species (strain):*** Rats (F344). ***Gender:*** Not reported. ***Number per Group:*** Not reported. ***Control:*** Sham group; and anti-p53 antibody CM1 and was used as a positive control in assay. ***Methods:*** Pulmonary adenocarcinomas or squamous cell carcinomas were induced by tetranitromethane (TNM), 4-methylnitrosamino-1-(3-pyridyl)-1-butanone (NNK), beryllium metal, plutonium-239, X-ray, diesel exhaust, or carbon black. These agents were chosen because the tumors they produced could arise via different types of DNA damage. Mutation of the K-ras gene was determined by approaches that included DNA transfection, direct sequencing, mismatch hybridization, and restriction fragment length polymorphism analysis. Lung neoplasms were fixed in 4% buffered paraformaldehyde or in 10% neutral buffered formalin. These samples were embedded in paraffin, cut at 5 pm, stained with hematoxylin and eosin, and examined by light microscopy for histologic diagnosis. Serial sections were also cut for immunohistochemical assays and DNA analysis. If lung tumor specimens were > 0.15 mg, the additional tissue was frozen in liquid nitrogen and stored at -80°C. DNA was isolated from fixed and frozen tissue for the analyses. ***Adverse Effect(s) (brief):*** In rats exposed to carbon black, 1/18 rat lung tumors was positive for K-ras activation. Mutations detected were present at similar frequencies (2/4) in adenocarcinomas and squamous cell carcinomas. ***Data Quality:*** NR. ***Note***: Authors posit the low frequency of gene mutation support tumorigenic effect by a nongenotoxic mechanism. ***Reference:*** *Belinsky, S A, Mitchell, C.E., Kelly, G., Nikulal, K.J., 1997. Alterations in the K-ras and p53 Genes in Rat Lung Tumors. Environmental Health Perspectives 105, 6.*							
Dasenbrock et al. (1996)	Y	Animal	Intratracheal instillation	15 mg **(NOAEL)**	17 weekly instillations	Lung tumors	NR
Test article: carbon black, diesel soot (original or toluene extracted). ***Dose:*** 0, 15 or 30 mg carbon black, diesel soot (original or toluene extracted)— Volumes of 0.2 – 0.3 mL/animal administered in saline/tween80 solution. ***Treatment Duration:*** 17-weeks. subdivided into 16 – 17 applications ***Species (strain):*** Rats (Crl: (WI) BR Wistar.) 7-weeks old ***Gender:*** Females. ***Number per Group:*** ~52 rats/group. ***Control:*** one vehicle: control and two groups were treated with a total dose of either 30 or 15 mg pure BaP as positive control. ***Methods:*** Animal were administered intratracheally (i.tr.) once per week for 16-17. A complete necropsy was performed on all animals which were killed or died during the study. All tissues were preserved in 10% neutral buffered formalin; the lungs were fixed by intratracheal instillation of formalin. Histopathological examinations were carried out on pharynx, larynx, trachea and lung (five sections). In addition, organ specimens from gross lesions observed during necropsy were histopathologically examined. ***Adverse Effect(s) (brief):*** **NOAEL= 15mg.** Treatment had no effect on survival. At the end of the instillation period, the weights of lungs in all particle groups were significantly increased. The CB retained mass of particles per lung was ~ 10.8 mg/lung. This result demonstrates a strong accumulation of the weekly i.tr. instilled dose of carbon particles. The histological finding of particle deposits in the lungs in nearly all animals exposed to carbon blacks or to diesel soot confirmed this effect. Different lung tumor types and the incidences of benign and malignant lung tumors observed after exposures. 21% (10/48) of CB exposed animals developed significant number of benign tumors compared to control. No lung tumor occurred in the control group. ***Data Quality:*** NR. ***Note***: Lung overload could have contributed to the formation of lung tumors. ***Reference:*** *Dasenbrock, C., Peters, L., Creutzenberg, O., Heinrich, U., 1996. The carcinogenic potency of carbon particles with and without PAH after repeated intratracheal administration in the rat. Toxicol Lett 88, 15–21.*							
Pence and Buddingh (1987)	Y	Animal	Oral (feed)	24 g/kg bw **(NOAEL)**	52 weeks	No observable adverse effect reported	NR
Test article: carbon black (ASTM N-375). ***Dose:*** 2.05 g carbon black (ASTM N-375) per kg (avg. consumption = 24 g/kg/yr). ***Treatment Duration:*** 52 weeks. ***Species (strain):*** Rats (Sprague-Dawley). ***Gender:*** Females. ***Number per Group:*** 23-25/group. ***Control:*** Control animals did not have carbon black added to the feed. ***Methods:*** Oral (feed). To simulate chronic CB ingestion, the CB was added to the corn oil/ground chow diet at 2.05 g/kg, which resulted in an average consumption of 24 g/kg body weight/year for the rats in this study. Control diets did not have CB added to the corn oil/chow mixture. Body weights were recorded weekly during DMH treatment and thereafter monthly for the duration of the experiment. Food consumption was recorded during week 10 of the experiment during active growth of the animals. Colonic tumors were induced in the rats by 16 weekly intraperitoneal injections of DMH at 10 mg/kg body weight. Control animals (groups 2 and 4) not receiving DMH were injected with the vehicle, 1 mM EDTA, 1 ml/kg body weight. At necropsy, all tissues were examined for gross pathology, and specimens							

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
from all lesions were fixed in 10% buffered formalin. The size, shape, and location of all lesions were noted. Histological sections were stained with hematoxylin and eosin for microscopic identification of all tumors. ***Adverse Effect(s) (brief):*** **NOAEL=24 g/kg bw**. No effects on survival rate, weight gain, food consumption, and colon tumor incidence after carbon black exposures. ***Data Quality:*** NR. ***Reference:*** *Pence, B.C., Buddingh, F., 1987. Co-carcinogenic effect of carbon black ingestion with dietary fat on the development of colon tumors in rats. Toxicology Letters 37, 177–182. https://doi.org/10.1016/0378-4274(87)90155-X*							
Steiner (1954)	Y	Animal	Subcutaneous	300 mg **(LOAEL)**	ND	No observable adverse effect reported (nonbenzpyrene extractable carbon black) Sarcoma (benzpyrene extractable carbon black)	NR
Test article: Benzpyrene-containing carbon black or nonbenzpyrene extractable carbon black ***Dose:*** 300 mg Benzpyrene-containing carbon black in 1 cc tricaprylin (either in liquid or pallet form) or 300 mg nonbenzpyrene extractable carbon black in 1.5 cc tricaprylin (either in liquid or pallet form). ***Species (strain):*** Mouse (C57BL/6) ***Gender:*** Males and females. (30, 26g. respectively) ***Number per Group:*** 50/group. ***Control:*** Untreated group ***Methods:*** All tests for carcinogenicity were made by subcutaneous injection in the interscapular region of mice. All tumors, all lesions suspected of being neoplastic, and many injection sites were examined by microscopical sections. At the termination of the experiments all survivors were sacrificed, postmortem examinations were made, and all question able lesions were subjected to microscopical examinations. Each experiment was started with 50 mice, and the total used. ***Adverse Effect(s) (brief):*** **NOAEL= 300mg**. 4.3% (2/50) of mice fed diets containing benzpyrene extractable carbon black at 300mg produced sarcomas. No significant sarcoma induced after exposures to nonbenzpyrene extractable carbon black. ***Data Quality:*** NR. ***Note***: Benzpyrene extractable carbon black is not pigment. ***Reference:*** *Steiner, P.E., 1954. The conditional biological activity of the carcinogens in carbon blacks, and its elimination. Cancer research 14, 103–110.*							
Reproductive and Developmental Toxicity							
Buddingh et al. (1981)	Y	Animal	Oral (feed)	2733.5 mg/kg/d **(NOAEL)**	ND	No observable adverse effect reported	NR
Test article: carbon black. ASTM, N-351, N-375, N-234 ***Dose:*** 0, 4.01, 99.77, and 997.73 g carbon black/kg/yr. ***Treatment Duration:*** up to 180 days ***Species (strain):*** Mouse (ICR). ***Gender:*** Males and females. ***Number per Group:*** 5 pair/group for breeder group. ***Control:*** 46 untreated. ***Methods:*** Mice were divided in to five groups and fed diets containing CB. Dams were observed up to 180days. Litters form the f_0,f_1, f_2 generation were sacrificed at day 29. Liver and lungs were collected. The benzo(a)pyrene hydroxylase (AHH) assay was performed. ***Adverse Effect(s) (brief):*** **NOAEL = 997.73 g/kg/yr (= 2733.5 mg/kg/d).** No demonstrable difference in lung or liver tissue AHH activity among the younger group (litters), and non-dose dependent marginally detectable activity for the older breeder mice. At the highest dose tested carbon black produced no evidence of mutagenic activity. ***Data Quality:*** NR. ***Reference:*** *Buddingh, F., Bailey, M.J., Wells, B., Haesemeyer, J., 1981. Physiological significance of benzo(α)pyrene adsorbed to carbon blacks: Elution studies, AHH determinations. American Industrial Hygiene Association Journal 42, 503–509. https://doi.org/10.1080/15298668191420152*							
Jackson et al. (2012)	Y	Animal	Intratracheal instillation	1.675 mg/mL **(LOAEL)**	4 times (GD 7, 10, 15, 18)	Inflammation, gene expression changes	NR
Test article: carbon black. ***Dose:*** 0, 2.75, 13.5, 67 µg carbon black in 40 µL water (11, 54, 268 µg/mice) at gestational days 7, 10, 15, and 18. ***Treatment Duration:*** 4 times (GD 7, 10, 15, 18) ***Species (strain):*** Mouse (C57BL/6 BomTac). ***Gender:*** Pregnant females; male and female pups. ***Number per Group:*** 17-24/group. ***Control:*** controls (24 mice weighing 23.0 ± 1.3 g) ***Methods:*** Mice were exposed by intratracheal instillation to vehicle (water) or one of three concentrations (2.75, 13.5 or 67 µg in 40µl Nanopure water) of carbon black Printex 90 (CB) on gestational days 7, 10, 15 and 18, to final cumulative doses of 11, 54 or 268 µg/animal. Samples from a subset of male and female newborns were collected on postnatal day 2 (4 days after the last maternal exposure) and from dams 26 to 27 days post-exposure (post-weaning period). Histopathology, DNA microarrays, pathway-specific RT-PCR arrays, focused RT-PCR, and tissue protein analysis were employed to characterize pulmonary response in dams exposed to CB during pregnancy. On GD 4 and 7, dams were weighed, and their health condition was checked. The body weight of each dam was also recorded on GD 10, 13, 15 and 18 immediately before the exposures. The expected day of delivery, GD 20, was assigned as postnatal day zero (PND 0) for the offspring. On PND 1, all mice were weighed, and the offspring were counted, and sex was determined. On PND 2 (4 days after the last instillation), one male and one female offspring per litter where possible ("new-borns") were euthanized and the liver was dissected. After weaning, at PND 24–25 (26–27 days after the last instillation) mice that had given birth ("dams") were anesthetized. Bronchoalveolar lavage (BAL) fluid was collected from lungs. Heart, liver, and lungs were dissected. All downstream analysis was carried out on at least 5 dams from each treatment group. Male and female offspring (n = 5/treatment group) chosen for microarray analysis were not necessarily derived from the litter set of dams analyzed.							

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
Adverse Effect(s) (brief): **LOAEL = 67 µg/40µL (= 1.675 mg/mL).** Histological analysis revealed retention of CB particles in mice exposed to both the medium and the high doses. Exposure caused maternal adverse effects on BAL fluid (e.g., increased neutrophils and dead cells). There were twice as many neutrophils observed in dams 26–27 days post-exposure c3–5 days post-exposure. Lung injuries at high dose (thickening of the alveolar septa and infiltration of macrophages and neutrophils). Significant changes in genes and proteins associated with inflammation in maternal lungs. There was no evidence for significant inflammation at the medium or low doses. Pups—significant hepatic response in male and female offspring exposed to the highest dose at the mRNA level. Hepatic response was much more pronounced in the female offspring. Majority of altered genes belonged to metabolic pathways. ***Data Quality:*** NR. ***Reference:*** *Jackson, P., Hougaard, K.S., Vogel, U., Wu, D., Casavant, L., Williams, A., Wade, M., Yauk, C.L., Wallin, H., Halappanavar, S., 2012. Exposure of pregnant mice to carbon black by intratracheal instillation: Toxicogenomic effects in dams and offspring. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, Genotoxic Potential of Nanomaterials 745, 73–83. https://doi.org/10.1016/j.mrgentox.2011.09.018*							
Carcinogenicity							
Maisel et al. (1959)	ND	Human	NR	NR	NR	Carcinoma in the parotid duct	NR
Test article: Carbon black and soot. ***Dose:*** Not reported ***Treatment Duration:*** NR ***Species (strain):*** Human. ***Gender:*** Males and females. ***Number per Group:*** (Number per group not applicable). ***Control:*** Control not defined ***Methods:*** Six cases of malignant disease of the parotid duct reported in the literature are summarized and discussed. The report uses case studies to outline exposure scenario for carbon black in which cancer resulted. ***Adverse Effect(s) (brief):*** The authors concludes that the presence of squamous metaplasia, a probable precancerous lesion in the left parotid gland possibly resulted from CB exposure. It was distinguished that in the parotid gland, a black material was noted in the gross specimen and in the histological study. This foreign material had produced a foreign body multinucleated giant-cell reaction. However, glandular tissue was not submitted for chemical analysis for conformation of substance identity. ***Data Quality:*** Not reported. ***Note***: This study lacks adequate reporting. ***Reference:*** *MAISEL, B., PEARCE, C., CONNOLLY, J., PEARCE, J., 1959. Carbon-black carcinoma of Stensen's duct: report of a case. AMA archives of surgery 78, 331–339.*							

SUPPORTING STUDIES

Source	Peer Review	Strain/Species	Dose	Duration	Response	Quality Guideline
Duffin et al. (2007)	Y	A549	125 µg/m^3 **(LOAEL)**	24hrs	Not cytotoxic, produce IL-8 protein	NR
Test article: various particle types, including carbon black, ***Treatment duration:*** 24-hour ***Dose:*** Concentrations of carbon black were 0, 125 µg/m^3. ***Speciec (strain):*** A549 (human alveolar carcinoma) cell line. ***Gender:***NA ***Number per Group:*** Three independent experiments, with six replicates per sample. ***Control:*** media control ***Method:*** MTT and LDH assays/ Cells were incubated with particles for 24 h, before adding MTT (20 µl/well of a filtered 5-mg/ml solution in PBS) and the plates incubated for 1 h at 37◦C. In the Elisa Control wells contained cells and medium alone. The first treatment was with 200 µl/well of blocking buffer (1% ovalbumin in 1× PBS) for 1 h before treating with 100 µl of either sample or standard for 4 h at room temperature while shaking. In the LDH assay, the samples were then incubated for 30 min in the dark and read at 490 nm on a Dynex MRX microplate reader. Data are expressed as means ± SEM and were analyzed using one-way analysis of variance (ANOVA). Multiple comparisons were analyzed using the Tukey-HSD method, unless otherwise stated. In all cases, $p < .05$ was considered significant. ***Adverse Effects (s) (brief):*** **LOAEL=125 µg/m^3** cytoxicity observed at 18h based on the MTT and LDH assays, but resulted in the production of IL-8 protein. ***Data quality:*** NR. ***Note:*** The gradient of the relationship between IL-8 protein production and low toxicity particle surface area was much less steep. The relative inflammatogenicity of the quartz surface suggesting that 1cm2 of quartz surface was approximately 60 times more inflammogenic that 1cm^2 of a LTLS particle surface. ***Reference:*** *Duffin, R., Tran, L., Brown, D., Stone, V., Donaldson, K., 2007. Proinflammogenic effects of low-toxicity and metal nanoparticles in vivo and in vitro: highlighting the role of particle surface area and surface reactivity. Inhal Toxicol 19, 849–856.*							

SECONDARY SOURCES

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
Systemic Toxicity (acute)							
ChemIDplus (n.d.)	ND	Animal	Skin	> 3,000 mg/kg **(LD$_{50}$)**	NR	NR	NR
Test article: Carbon black. ***Dose:*** 3,000 mg/kg ***Species*** 100 mg/kg bw ***strain):*** Rabbits. ***Gender:*** Not reported. ***Number per Group:*** Not reported. ***Control:*** NR ***Method:*** NR ***Adverse Effect(s) (brief):*** LD$_{50}$ > 3,000 mg/kg. ***Data Quality:*** NR. ***Note***: Acute Toxicity Data. Journal of the American College of Toxicology, Part B. Vol. 15 (Suppl,)							

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
ChemIDplus (n.d.)	ND	Animal	Oral	> 15,400 mg/kg **(LD_{50})**	NR	Behavorial: Somnolence (G)eneral depressed activity)	NR
Test article: Carbon black. ***Dose:*** 15,400 mg/kg. ***Species (strain):*** Rats. ***Gender:*** Not reported. ***Number per Group:*** Not reported. ***Control:*** NR ***Method:*** NR ***Adverse Effect(s) (brief):*** LD_{50} > 15,400 mg/kg. ***Data Quality:*** NR. ***Note***: Acute Toxicity Data. Journal of the American College of Toxicology, Part B. Vol. 15 (Suppl,)							
ECHA, 1997	ND	Animal	Inhalation	1 mg/m3 **(LOAEL)**	0≤ 24 months	neutrophil influx in lung, epithelial permeability, lung GSH	Non-guideline study; NR
Test article: carbon black ***Dose:*** 1 mg/m3 ***Treatment duration:***7hrs, followed by 48hr observation period. ***Species (strain):*** Rat. ***Gender:*** male/female. ***Number per Group:*** / per sex/ per dose. ***Control:*** NR ***Method:*** two kinds of carbon black particles: 20 nm ultrafine carbon black, and 200 nm fine carbon black (no further details given) ***Adverse Effect(s) (brief):*** **LOAEL= > 1 mg/m³ air** (carbon black (200 nm)); **LOAEL=** 1 mg/m³ air (ultrafine carbon black). After 7 hours inhalation exposure to 1 mg/m3 of ultrafine carbon black, there was a small (1%), but significant neutrophil influx in rat lung ($p<0.01$), compared with control animals. No such response occurred in animals exposed to 1 mg/m3 carbon black for 7 hours. Ultrafine carbon black inhalation resulted in a marked increase in epithelial permeability, measured as total protein in bronchoalveolar lavage (BAL) fluid, which peaked 16 hours after ultrafine carbon black inhalation, but not after carbon black inhalation. The increase in total protein was largely due to albumin. Immediately after 7 hours inhalation of ultrafine carbon black, there was 23.56 (+/- 5.37) % reduction of total lung GSH compared to control values ($p < 0.001$). However, 16 hours after inhalation of ultrafine carbon black total lung GSH was significantly higher than the control level ($p < 0.001$) and returned to control by 48 hours. Inhalation of carbon black, on the other hand, did not change total lung GSH significantly. ***Data Quality:*** ECHA Reliability 3 (not reliable) ***Note***: Only ultrafine carbon black particles (20nm) was associated with local inflammation of the respiratory tract.							
ECHA, 1978	ND	Animal	Oral (gavage)	> 8 000 mg/kg bw **(LD_{50})**	NR	NR	Similar to OECD 401; non-GLP
Test article: Carbon black. ***Dose:*** 8,000 mg/kg bw ***Treatment duration:*** Single administration, followed by a 14-day observation period. ***Species (strain):*** Rat (Sprague-Dawley). 100-105 g. ***Gender:*** male/female. ***Number per Group:*** 20/ per sex/ per dose. ***Control:*** None ***Method:*** 10 male and 10 female Sprague-Dawley rats, weight between 100 and 105 g. Age at study begin: 38 days (male animals), 42 days (female animals). Animals were fed standard diet; water was available ad libitum. Animals were held individually in Macrolon cages in an air-conditioned room at 22 +/- 0.5 deg centigrade, and a relative humidity of 55% +/- 5%. The test substance was applied via single gavage as suspension in 0.8% aqueous hydroxypropyl methyl cellulose E4M in a constant volume of 50 mL/kg bw. Food was withdrawn 15-16 hours before administration of the test substance. 4 weeks. The parameters were behavior, food consumption, body weight gain. All animals were necropsied and macroscopically evaluated at the end of the post-observation period ***Adverse Effect(s) (brief):*** LD50 > 8,000 mg/kg. Food consumption was reduced 0, 4, and 6% at days 1, 2, and 14, respectively. Body weight gain was reduced 2, 4, and 4% at days 1, 2, and 14, respectively. Findings at necropsy were unremarkable. The acute oral LD50 value in rats is greater than 8000 mg/kg bw (maximum achievable concentration). ***Data Quality:*** ECHA Reliability 1 (reliable without restriction).							
ECHA, 1977	ND	Animal	Oral	> 10,000 mg/kg bw **(LD_{50})**	NR	NR	Similar to OECD 401; non-GLP
Test article: Dose: 6350, 7900, 10000 mg/kg bw***. Treatment duration:*** Single administration, followed by a four week observation period. ***Species (strain):*** Rat (Sprague-Dawley). ***Gender:*** male/female. ***Number per Group:*** 10 / per sex/ per dose. ***Control:*** None Method: 10 male and 10 female Sprague-Dawley rats per group, weight between 100 and 105 g. Age at study begin: 38 days (male animals), 42 days (female animals). Animals were fed diet; water was available ad libitum. Animals were held individually in macrolon cages in an air-conditioned room at 22 +/- 0.5 deg centigrade, and a relative humidity of 55% +/- 5%. The test substance was applied via single gavage as suspension in 0.8% aqueous hydroxypropyl methyl cellulose E4M in a constant volume of 50 mL/kg bw. Food was withdrawn 15-16 hours before administration of the test substance. There was a 4 weeks observation period. The parameter observed included behavior, food consumption, body weight gain. All animals were necropsied and macroscopically evaluated at the end of the post-observation period. ***Adverse Effect(s) (brief):*** **LD_{50}>0,000 mg/kg bw.** Food consumption was reduced by 0, 8, and 4% at days 1, 2, and 7 for the 6350 mg/kg bw group; 2, 6, and 8% at days 1, 2, and 7 for the 7900 mg/kg bw group; and 3, 0, and 6% at days 1, 2, and 7 for the 10000 mg/kg bw group, respectively. Body weight gain was reduced 0, 6, and 2% at days 1, 2, and 7 for the 6350 mg/kg bw group; 4, 4, and 7% at days 1, 2, and 7 for the 7900 mg/kg bw group; and 0, 6, and 6% at days 1, 2, and 7 for the 10000 mg/kg bw group, respectively. Findings at necropsy were unremarkable. The acute oral LD50							

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
value in rats is greater than 10,000 mg/kg bw (maximum technically feasible dose). ***Data Quality:*** ECHA Reliability 1 (reliable without restriction)							
ECHA, 2003	ND	Animal	Inhalation	> 2 000 mg/kg bw **(LD_{50})**	4 hours	No observable adverse effect reported	OECD Guideline 403
Test article: Carbon black ***Dose:*** 2000 mg/kg bw. ***Treatment duration:*** up to two exposures, followed by a 14 -day observation period. ***Species (strain):*** Rat (Sprague-Dawley). ***Gender:*** female. 176-200 g ***Number per Group:*** 3/per dose. ***Control:*** None ***Method:*** A single group of 3 female animals were dosed at 2000 mg/kg bw. A second group of 3 female rats were dosed again at 2000 mg/kg bw. Animals were sacrificed on day 15. Clinical signs including body weight: Animals were observed for clinical signs immediately upon dosing, approx. 30 min, 2 h and 4 h after dosing and daily thereafter for a total of 14 days. clinical signs, body weight, organ weights, histopathology, other: Gross necropsy examination was performed for all animals. Cranial, thoracic and abdominal cavities were opened. Larger organs were sectioned. Both the stomach and representative sections of the GIT were opened for examination of the mucosal surfaces. ***Adverse Effect(s) (brief):*** **LD_{50} > 2 000 mg/kg bw.** Clinical signs were limited to reduced activity which was noted on day 1 after dosing. No clinical signs were observed during the 14 day observation period. Changes of the body weight were within the range expected for this strain and age of animals. No abnormalities were found on necropsy examination of the animals on termination of the study. ***Data Quality:*** ECHA Reliability 2 (reliable with restrictions)							
ECHA, 2005	ND	Animal	Oral (gavage)	10 000 - 10 000 mg/kg bw **(LD_0)**	NR	NR	OECD 420
Test article: NR ***Dose:*** NR ***Treatment duration: Species (strain):*** Rat. ***Gender:*** NR. ***Number per Group:*** NR. ***Control:*** NR ***Method:*** NR ***Adverse Effect(s) (brief):*** LD50 (oral, rat) = 10000 mg/kg (no mortality, no clinical signs of toxicity). ***Data Quality:*** ECHA Reliability 2 (reliable with restrictions)							
ECHA, 2001	ND	Animal	Inhalation (nose-only)	4.6 mg/m3 **(LD_0)**	NR	NR	OECD 403
Test article: Carbon black ***Dose:*** 4.6 mg/m^3 ***Treatment Duration:*** 4 h ***Species (strain):*** Rat (Wistar). ***Gender:*** male/female. ***Number per Group:*** NR. ***Control:*** NR ***Method:*** Nose only inhalation of dust. ***Adverse Effect(s) (brief):*** LC0 (4-hour)=4.6 mg/m3. no mortality, no signs of acute toxicity were observed. ***Data Quality:*** ECHA Reliability 2 (reliable with restrictions).							
ECHA, 1997	ND	Animal,	intratracheally	125 µg **(LOAEL)**	NR	Respiratory system	Non-guideline; NR
Test article: carbon black (Degussa Huber NG90, diameter 200-250 nm and 260 nm), and ultrafine carbon black (Degussa Printex 19, diameter 20 nm; and Printex 90, 14nm). ***Dose:*** 0, 50 µg (ultrafine carbon black), 125 µg (ultrafine carbon black, carbon black) ***Treatment duration:*** Single administration, followed by up to seven-day observation period. ***Species (strain):*** Rat (Wistar). ***Gender:*** male. ***Number per Group:*** 3-6 / per dose. ***Control:*** The controls for these experiments were animals that did not receive any instillation and animals instilled with 0.2 mL PBS alone. ***Method:*** BAL profile at 6h, 24h and 7d after intratracheal instillation of 125 µg. The animals were anesthetized with pentobarbitone, and 0.2 mL of PBS- particle suspension (containing 50-125 ug of PM10, or 125 ug of either carbon black or ultrafine carbon black particles) was instilled intratracheally. The controls for these experiments were animals that did not receive any instillation and animals instilled with 0.2 mL PBS alone. Six hours after intratracheal instillation of particle suspensions, rats were sacrificed. and 4 mL PBS at 37 deg C was instilled and withdrawn from the lungs. After centrifugation this solution was referred to as bronchoalveolar lavage fluid (BALF). To obtain broncholalveolar lavage (BAL) leukocytes, 4x 8 mL PBS was used to wash the lungs and then collected in a universal tube. The cell suspension was spun and cell pellets were resuspended in Dulbecco´s minimum essential medium (DMEM) plus 0.2% low endotoxin-bovine serum albumin in which the cells from the first lavage were combined. The total number and differential count of BAL leukocytes were obtained. BAL leukocytes from control animals consisted of greater than 99% macrophages. Statistical analysis: differences between mean values were assessed by analysis of variance. ***Adverse Effect(s) (brief): LOAEL*=125 µg.** Compared with animals that had instillations of PBS, carbon black produced a small but significant neutrophil influx. The greatest inflammatory cell influx occurred after instillation of ultrafine carbon black (control 1.5%, carbon black 3.5%, ultrafine carbon black 49.6% neutrophils, $p < 0.05$). The neutrophil influx was still present 7 days after instillation of ultrafine carbon black, and also occurred following instillation of 50 ug of ultrafine carbon black. As with the influx of inflammatory leukocytes, the greatest increase in airspace epithelial permeability occurred after instillation of ultrafine carbon black ($p< 0.01$). Carbon black produced a lesser increase in epithelial permeability ($p< 0.05$) than PM10 ($p< 0.001$) or ultrafine carbon black. Ultrafine carbon black instillation also resulted in a marked increase in LDH levels in BAL fluid. Ultrafine carbon black, compared with carbon black also produced a significant depletion of total lung GSH (control lung GSH 50.4 +/- 9.7, ultrafine carbon black 29.0 +/- 3.7 nmoles/g lung, $p < 0.05$). Alveolar leukocytes in both carbon black and ultrafine carbon black instilled animals produced significantly increased amounts of tumors necrosis factor (TNF) compared with control animals, with leukocytes from ultrafine carbon black animals producing most TNF. ***Data Quality:*** ECHA Reliability 3 (not reliable) ***Note***: In a rat instillation model ultrafine carbon black at 125 µg produces more lung inflammation and oxidant stress than carbon black at 125 µg. The threshold dose for neutrophil influx occurred at 50 µg.							

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
ECHA, 2007	ND	Animal,	intratracheal	3.5 mg/m³ **(TWA)**	single administration	Pulmonary, cardiovascular	Non-guideline; NR
Test article: Carbon black ***Dose:*** 4 mg/rat were instilled intratracheally; the dose was suspended in 0.2 mL vehicle, using a specially designed aeroliser (Vehicle; 10% propylene glycol and 1% sodium carboxymethylcellulose in saline)***. Treatment duration:*** single intratracheal instillation of 0.2 mL ***Species (strain):*** Rat (Fischer 344/DuCrj). ***Gender:*** male. ***Number per Group:*** 14/ per dose. ***Control:*** 14 animals were used as vehicle controls; 10 animals were used as untreated control. ***Method:*** 14 animals/group; subgroups of 7 animals were sacrificed on day 1 and on day 28 of the study. histopathology, bromodeoxyuridine (BrdU) labelling indices, inducible nitric oxide (iNOS) levels, and matrix metalloproteinase-3 (MMP-3) levels performed. Groups of 14 rats were treated by single intratracheal instillation. Five different test materials were used: quartz, hydrotalcite, potassium octatitanate, palladium oxide, or carbon black. Vehicle controls received a single intratracheal instillation of 0.2 mL of 10% propylene glycol and 1% sodium carboxylmethyl cellulose in saline (vehicle control for the carbon black group) or of 0.2 mL saline (vehicle control for all other groups). ***Adverse Effect(s) (brief):*** Lungs of rats sacrificed on days 1 and 28 and treated with carbon black or palladium oxide showed surface discoloration and were partially black on day 1. This change was found to be diminished on day 28. The bronchi, liver, adrenal glands, spleen, pulmonary lymph nodes, and kidneys demonstrated no remarkable changes. organ weights: On day 1, weights of lungs from quartz, hydrotalcite or potassium octatitanate treated groups were increased significantly. On day 28, increase was seen in all five particle treated groups. Other findings: Lungs of rats sacrificed on days 1 and 28 and treated with carbon black or palladium oxide showed surface discoloration and were partially black on day 1. This change was found to be diminished on day 28. The bronchi, liver, adrenal glands, spleen, pulmonary lymph nodes, and kidneys demonstrated no remarkable macroscopic or histopathological changes. Main histopathological changes of lungs in rats treated with the test materials were neutrophil infiltration in the walls and spaces of the alveoli, pulmonary edema, pulmonary fibrosis, histiocyte infiltration in the alveoli, restructuring of alveolar walls and microgranulation. These effects were most marked in the group treated with quartz and mildest in the carbon black treated group. Cell proliferation was enhanced in all treated groups on day 1 as evidenced by BrdU labelling indices but returned to normal values at day 28. MMP-3 and iNOS levels were not significantly altered in the carbon black treated group. ***Data Quality****: ECHA Reliability* 3 (not reliable) ***Note***: Only quartz was classified as highly toxic; hydrotalcite, potassium octatitanate and palladium oxide demonstrated slight toxicity. Carbon black was of only low toxicity.							
ECHA, 2013	ND	Animal	Inhalation	10 mg/m³ air **(NOAEC)**	30hrs	No observable adverse effect reported	Short-Term Inhalation Toxicity protocol (STIS); GLP
Test article: Carbon Black ***Dose:*** 0.5, 2.5, or 10 mg/m3. ***Treatment duration:***30hr**s**, followed by up to 28-day observation period. (6h/day for 5 days***) Species (strain):*** Rat (Sprague-Dawley). ***Gender:*** male ***Number per Group:*** 11/ per dose. ***Control:*** A concurrent control group was exposed to conditioned air. ***Method:*** Groups of 11 male Wistar rats were head-nose exposed to respirable dusts on 6 hours per day, on 5 consecutive days (days 0 to 4). The target concentrations were 0.5, 2.5, or 10 mg/m3. A concurrent control group was exposed to conditioned air. On study day 4 (after the last exposure) and 25 (21 days after the last exposure), 6 animals per group were sacrificed and designated for histopathological examinations. On study day 7 (3 days after last exposure) and 28 (24 days after last exposure), the remaining 5 animals per group were sacrificed. The lungs of these animals were lavaged, and BALF was analyzed for markers indicative for injury of the bronchoalveolar region. ***Adverse Effect(s) (brief): NOAEC*=10 mg/m³ air.** There were no clinical effects, nor any changes found at necropsy, in hematology, acute phase proteins, histology (only the lung was investigated), or BAL fluid. No mortality was observed. No compound-related adverse effects were found in hematology and histology. On study day 4, black particles (considered to be Carbon Black) were observed within´ alveolar macrophages in animals exposed to 10 mg/m3. In three of the six treated animals there was a minimal increase in numbers of alveolar macrophages***. Data Quality:*** ECHA Reliability 1 (reliable without restriction)							
ECHA, 1997	ND	Animal	Inhalation	1 mg/m3	0≤ 24 months	neutrophil influx in lung, epithelial permeability, lung GSH	Non-guideline study; NR
Test article: carbon black ***Dose:*** 1 mg/m3 ***Treatment duration:***7hrs, followed by 48hr observation period. ***Species (strain):*** Rat. ***Gender:*** male/female. ***Number per Group:*** / per sex/ per dose. ***Control:*** NR ***Method:*** two kinds of carbon black particles: 20 nm ultrafine carbon black, and 200 nm fine carbon black (no further details given) ***Adverse Effect(s) (brief):*** **LOAEL= > 1 mg/m³ air** (carbon black (200 nm)); **LOAEL=** 1 mg/m³ air (ultrafine carbon black). After 7 hours inhalation exposure to 1 mg/m3 of ultrafine carbon black, there was a small (1%), but significant neutrophil influx in rat lung ($p<0.01$), compared with control animals. No such response occurred in animals exposed to 1 mg/m3 carbon black for 7 hours. Ultrafine carbon black inhalation resulted in a marked increase in epithelial permeability, measured as total protein in bronchoalveolar lavage (BAL) fluid, which peaked 16 hours after ultrafine carbon black inhalation, but not after carbon black inhalation. The increase in total protein was largely due to albumin. Immediately after 7 hours inhalation of ultrafine carbon black, there was 23.56 (+/- 5.37) % reduction of total lung GSH compared to control values ($p < 0.001$). However, 16 hours after inhalation of ultrafine carbon black total lung GSH was significantly higher							

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
than the control level ($p < 0.001$) and returned to control by 48 hours. Inhalation of carbon black, on the other hand, did not change total lung GSH significantly. ***Data Quality:*** ECHA Reliability 3 (not reliable)							
Systemic Toxicity (subacute, subchronic, or chronic)							
ECHA, 2013	ND	Animal	Inhalation	13 mg/m³ air **(LC0)**	Up to 14-days		Non-guideline study; NR
Test article: carbon black ***Dose:*** 1.5 g in 300 ml of distilled water ***Treatment duration:*** 6 hours/day for 3 days or for 2 weeks. ***Species (strain):*** Rat (Sprague-Dawley). ***Gender:*** male. ***Number per Group:*** / per sex/ per dose. ***Control:*** normal control. ***Method:*** Aerosols were prepared either with or without sonication to verify the effects of agglomeration on the toxicity and lung deposition of Printex 90. Male Sprague-Dawley rats were exposed to carbon black aerosols 6 hr a day for 3 days or for 2 weeks. The median mass aerodynamic diameter of carbon black aerosols averaged 2.08 µm (for aerosol prepared without sonication; group N) and 1.79 µm (for aerosol prepared with sonication; group S). The average concentration of carbon black during the exposure period for group N and group S was 13.08 ± 3.18 mg/m3 and 13.67 ± 3.54 mg/m3 respectively, in the 3-day experiment. The average concentration during the 2-week experiment was 9.83 ± 3.42 mg/m3 and 9.08 ± 4.49 mg/m3 for group N and group S, respectively. Five-week-old male Specific pathogen-free (SPF) Sprague-Dawley (SD) rats were obtained from Central Lab Animal Inc. (Seoul, Korea) and were acclimatized at least 1 week prior to carbon black exposure. During the acclimation and experimental period, rats were housed at polycarbonate cages in a room with controlled temperature (23 ± 2°C), humidity (55 ± 7%), and a 12-hr light/dark cycle. Rats were fed filtered water and a rodent diet ad libitum. The study was approved by an animal ethics committee to ensure appropriate animal care for research. ***Adverse Effect(s) (brief):*** **LC0= 13 mg/m³ air.** Male Sprague-Dawley rats were exposed to carbon black aerosols 6 hours/day for 3 days or for 2 weeks. The median mass aerodynamic diameter of carbon black aerosols averaged 2.08 µm (for aerosol prepared without sonication; group N) and 1.79 µm (for aerosol prepared without sonication; group S). The average concentration of carbon black during the exposure period for group N and group S was 13.08 ± 3.18 mg/m3 and 13.67 ± 3.54 mg/m3, respectively, in the 3-day experiment. The average concentration during the 2-week experiment was 9.83 ± 3.42 mg/m3 and 9.08 ± 4.49 mg/m3 for group N and group S, respectively. The amount of carbon black deposition in the lungs was significantly higher in group S than in group N in both 3-day and 2-week experiments. The number of total cells, macrophages and polymorphonuclear leukocytes in the bronchoalveolar lavage (BAL) fluid, and the number of total white blood cells and neutrophils in the blood in the 2- week experiment were significantly higher in group S than in normal control. However, differences were not found in the inflammatory cytokine levels (IL-1β, TNF-α, IL-6, etc.) and protein indicators of cell damage (albumin and lactate dehydrogenase) in the BAL fluid of both group N and group S as compared to the normal control. No mortality occurred and there was an overall absence of adverse toxic effects. ***Data Quality:*** ECHA Reliability 2 (reliable with restrictions). ***Note***: After 7 hours of inhalation (1mg/m3), ultrafine (20 nm) carbon black was more biologically active in rats than 200 nm carbon black under the same exposure conditions; the effects were limited to an increase in neutrophil influx, protein increase in BAL and reductions in total lung GSH.							
ECHA, 2018	ND	Animal	oro-pharyngeal aspiration	70 µg/animal	14-day	No observable adverse effect reported	Non-guideline study; NR
Test article: carbon black (Printex 90) ***Dose:*** 70 µg/animal ***Treatment duration:*** Single or repeated (7times with intervals of 2 weeks each) applications ***Species (strain):*** Mouse (Balb/c). ***Gender:*** male/female. ***Number per Group:*** / per sex/ per dose. ***Control:*** Controls were used. ***Method:*** Single or repeated (7times with intervals of 2 weeks each) applications of carbon black (Printex 90). ***Adverse Effect(s) (brief):*** **LOEC=70 µg/animal.** carbon black (Printex 90) did not reveal toxic effects. Only very slight effects were noted (reduction of IL-6 expression). ***Data Quality:*** ECHA Reliability 3 (not reliable) ***Note***: No conclusions can be drawn from this study with regard to acute inhalation toxicity. The non-physiological route of administration via oro-pharyngeal aspiration is not in accordance with the guideline and not suitable to assess acute inhalation toxicity.							
ECHA, 2006	ND	Animal	Inhalation	7.1 mg/m³ **(LOAEC)**	≤ 13 weeks	Pulmonary effects, inflammation	NR
Test article: Carbon black. ***Dose:*** 5, 20, 50 µg in 50 µL distilled water. ***Treatment duration: Species (strain):*** Mouse (Balb/c). ***Gender:*** male/female. ***Number per Group:*** / per sex/ per dose. ***Control:*** Controls not defined. ***Method:*** 6 different carbonaceous particles were tested, including Printex G and Printex 90; BAL analysis 24h after instillation. ***Adverse Effect(s) (brief):*** **threshold for inflammation=20 cm².** Mice were instilled with 5, 20, and 50µg of 6 different carbonaceous particle types, and bronchoalveolar lavage (BAL) was analyzed 24 hours after instillation for inflammatory cells and the level of proinflammatory cytokines. At identical mass-doses, particle-caused detrimental effects ranked in the following order: ufCP > Soot L≥Soot H > Printex 90 > Printex G > DEP. Relating the inflammatory effects to the particle characteristics - organic content, primary particle size, or specific surface area - demonstrated the most obvious dose response for particle surface area. The existence of a threshold for the particle surface area at an instilled dose of approximately 20 cm2 was established below which no acute pro-inflammatory responses could be detected in mice. Wittmaack (2007) disagreed with the suggestion of a threshold based on particle surface area. In a review using the published dose-response data of Stoeger et al. (2006) he suggests that the physical characterization of particles and the methods to determine surface toxicity have to be improved significantly before the appropriate dose metric for lung inflammation can be							

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
identified safely. ***Data Quality:*** ECHA Reliability 3 (not reliable); significant methodological deficiencies. ***Note***: The authors claim that surface area is the most important dose metric and suggest a threshold of 20 cm2 BET surface area for inflammatory effects.							
ECHA, 2006	ND	Animal	Inhalation	1 mg/m3 **(NOAEL)**	3 and 11 months	indirect genotoxic response	Similar to 476; NR
Test article: Carbon Black ***Dose:*** 1 mg/m³ air, 7 mg/m³ air, 50 mg/m³ air ***Treatment duration:*** 3 and 11 months (6h/day for 5 days***) Species (strain):*** Rat, mouse, hamster (F344, B6C3F1, F1B Syrian golden). ***Gender:*** Female. 5 weeks ***Number per Group:*** 5/ per dose. ***Control:*** sham-exposed. ***Method:*** This study investigated pro- and anti-inflammatory mediators underlying species specific mechanisms in particle-induced lung inflammation using ex vivo mutational analysis of inflammatory cells co-incubated with lung epithelial cells. 13 weeks with or without post-exposure periods of 3 and 11 months. Bronchoalveolar lavage (BAL) was performed and fluid and cells were collected. Lavage cells were pelleted and the supernatant was used for biochemical and immunologic analyses. Bronchoalveolar lavage (BAL) fluid was used for cellular and biochemical analysis (superoxide, hydrogen peroxide, nitric oxide, TNF-alpha, macrophage inflammatory protein-2 (MIP-2), IL-10); superoxide dismutase, glutathione reductase, and glutathione peroxidase levels in BAL fluid and lung tissue, gamma-glutamylcysteine synthetase and manganese superoxide dismutase mRNA expression. Ex vivo mutational analysis of inflammatory cells was evaluated by co-incubating BAL cells with RLE-6TN lung epithelial cells. Lung tissue was evaluated for gene expression of various anti-inflammatory mediators. one-way analysis of variance (ANOVA) and Tukey multiple comparisons; significance level set at 0.05 ***Adverse Effect(s) (brief):*** **NOAEL=1 mg/m3.** This study compared inflammatory responses and ex vivo hprt mutation frequencies in rat, mouse and hamster after subchronic inhalation of carbon black (1, 7 or 50 mg/m3). Rats demonstrated greater propensity for generating a proinflammatory response and hprt mutations, whereas mice and hamsters demonstrated an increased anti-inflammatory response. No effects on hprt mutation frequencies were found at a dose level of 1 mg/m3, indicating a secondary indirect genotoxic response at levels at which chronic inflammation exists. ***Data Quality:*** ECHA Reliability 1 (reliable without restriction). ***Note***: The differences in pro- and anti-inflammatory responses between rat, mouse and hamster may contribute to species differences in inflammation, genotoxicity and tumorigenesis. The inflammatory response (PMN/neutrophil count in bronchoalveolar lavage fluid (BALF)) was the highest and the response more protracted in the rat versus the mouse or hamster and increased during recovery for the mid- and high-dose groups. Mice and hamsters demonstrated an increased anti-inflammatory response. Hamster bronchoalveolar lavage (BAL) cells did not significantly increase the mutation frequency in the hprt gene for any dose at any time point. Both the rat and mouse showed dose-related effects in the hprt mutation frequency. Although the mouse response significantly decreased by the 11-month postexposure time point, the rat response was sustained throughout the course of the study. The authors conclude that secondary indirect genotoxic response exists at levels at which chronic inflammation exists; at levels below this, no hazard should be anticipated.							
ECHA, 2015	ND	Animal	Oral (gavage)	>1000 mg/kg/day **(NOAEL)**	90-day	No observable adverse effect reported	OECD 408; GLP
Test article: Carbon Black ***Dose:*** 0, 100, 300, or 1000 mg/kg/day. ***Treatment duration:***30hr***s***, followed by up to 28-day observation period. (6h/day for 5 days***) Species (strain):*** Rat (Wistar). ***Gender:*** male/female ***Number per Group:*** 11/ per dose. ***Control:*** Controls not defined. ***Method:*** Groups of 11 male Wistar rats were head-nose exposed to respirable dusts on 6 hours per day, on 5 consecutive days (days 0 to 4). The target concentrations were 0.5, 2.5, or 10 mg/m^3. A concurrent control group was exposed to conditioned air. On study day 4 (after the last exposure) and 25 (21 days after the last exposure), 6 animals per group were sacrificed and designated for histopathological examinations. On study day 7 (3 days after last exposure) and 28 (24 days after last exposure), the remaining 5 animals per group were sacrificed. The lungs of these animals were lavaged, and BALF was analyzed for markers indicative for injury of the bronchoalveolar region. ***Adverse Effect(s) (brief):*** **NOAEL >1000 mg/kg/day** Following daily administration of carbon black, there were no deaths, no adverse clinical signs, no ocular findings or changes in body weight gain and food intake when compared to controls. Dark colored feces were observed in animals given carbon black at all dose levels. This finding is related to the colored nature of the test item and hence considered to be non-adverse. There were no relevant changes in laboratory parameters. Some hematology and blood clinical chemistry parameters showed minimal changes when compared to controls. However, as they remained minor and showed no dose-relationship, these changes were considered to be unrelated to the administration of carbon black. There were no changes attributed to carbon black in any of the urinary parameters evaluated. Dosing with carbon black did not produce any changes in organ weights. The only finding at necropsy consisted of black gastro-intestinal tract contents in all animals given carbon black, without any associated gross or microscopic changes in the gastro-intestinal mucosa apart from black discoloration. These findings were also considered to be related to the colored nature of the test item and hence non adverse. There were no other histopathological findings attributed to carbon black***. Data Quality:*** ECHA Reliability 1 (reliable without restriction). ***Note***: There were no adverse effects found in a modern 90-day GLP and guideline study after repeated oral administration by gavage. The study authors concluded that the repeated daily oral administration to rats of carbon black for 13 weeks at 100, 300 or 1000 mg/kg/day was well tolerated, some no adverse findings related to the staining properties of carbon black. Accordingly, under the conditions of this study, the No Observed Adverse Effect Level (NOAEL) was 1000 mg/kg/day							

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
ECHA, 1985	ND	Animal	Oral (feeding)	2.05 g/kg	2 years	No observable adverse effect reported	Non-guideline study; non-GLP
Test article: Carbon Black ***Dose:*** 2.05 g/kg feed (137 mg/kg/day) ***Treatment duration:*** 52 weeks or 2 years. (6h/day for 5 days***) Species (strain):*** Rat (Sprague-Dawley). ***Gender:*** male ***Number per Group:*** 29-45/ per dose. ***Control:*** A concurrent control group was exposed to plain diet. 29 animals for the corresponding untreated control group. ***Method:*** Groups of 29-45 female Sprague-Dawley rats were fed either 0 or 2.0 g carbon black (ASTM N-375) per kg of ground lab chow diet for 2 years. This dietary level was calculated to amount to an average consumption of 38 g/kg/year. (The average fat content of this rodent chow was later reported by these authors (1986) to be approximately 5% by weight.) Simultaneously, groups of rats were exposed to carbon black for 52 weeks with or without the administration of 1,2-dimethylhydrazine (DMH) via 16 weekly i.p. injections at a dose level of 10 mg/kg bw. Control animals were given the solvent (1 mM EDTA) by injection. After 52 weeks or 2 years, the animals were killed, gross necropsies performed, and all lesions examined microscopically. ***Adverse Effect(s) (brief):*** There was no significant difference in survival between rats treated with carbon black for 2 years (38%) and untreated controls (45%). There were no weight differences due to carbon black effects. The average compound intake was 38 g/kg/year. Ingestion of carbon black did not increase tumor incidences in colon, kidney or mammary gland when compared with untreated controls in the 2-year study (3, 3, 24% versus 3, 3, 28% in untreated controls) or when compared with 1,2-dimethylhydrazine (DMH) treated animals in the 52-week study. No gastric tumors were seen in any of the animals. Ingestion of carbon black did not increase tumors incidences in colon, kidney or mammary gland when compared with untreated controls in the 2-year study (3, 3, 24% versus 3, 3, 28% in untreated controls) or when compared with 1,2-dimethylhydrazine (DMH) treated animals in the 52-week study ***Data Quality:*** ECHA Reliability 2 (reliable with restrictions) ***Note***: There were no tumorigenic effects in rats that received carbon black in their diets (2.05 g/kg diet) for two years							
ECHA, 2013	ND	Animal	Inhalation	9.83 mg/m³ air **(NOAEC)** 9.08 mg/m³ air. **(LOEC)**	Up to 14-days	No observable adverse effect reported	Non-guideline study; GLP
Test article: Carbon Black ***Dose:*** 0.5, 2.5, or 10 mg/m3. ***Treatment duration:*** 6 hr a day for 3 days or for 2 weeks (6h/day for 5 days***) Species (strain):*** Rat (Sprague-Dawley). ***Gender:*** male ***Number per Group:*** 11/ per dose. ***Control:*** A concurrent control group was exposed to conditioned air. ***Method:*** Pathology, hematology, cytokine levels, cell damage and inflammatory parameters in bronchoalveolar fluid. ***Adverse Effect(s) (brief): NOAEC*= 9.83 mg/m³ air; LOEC =9.08 mg/m³ air.** increases in white blood cells and neutrophils in BALF only in the group treated with the sonicated test material, and only in the group treated for 2 weeks. There were no clinical effects, nor any changes found at necropsy, in hematology, acute phase proteins, histology (only the lung was investigated), or BAL fluid. No mortality was observed. No compound-related adverse effects were found in hematology and histology. On study day 4, black particles (considered to be Carbon Black) were observed within´ alveolar macrophages in animals exposed to 10 mg/m3. In three of the six treated animals there was a minimal increase in numbers of alveolar macrophages**.** Carbon black concentrations in lungs was increased in the groups treated with sonicated carbon black as compared with the group treated with non-sonicated carbon black (both after 3 days and 2 weeks of treatment). The number of total cells, macrophages and polymorphonuclear leukocytes in the bronchoalveolar lavage (BAL) fluid and the number of total white blood cells and neutrophils in the blood in the 2- week experiment were significantly higher in the group treated with sonicated carbon black than in normal control. However, in none of the treated group were differences found in the inflammatory cytokine levels (IL-1β, TNF-α, IL-6, etc.) or protein indicators of cell damage (albumin and lactate dehydrogenase) in the BAL fluid compared to the normal control. ***Data Quality:*** ECHA Reliability 1 (reliable without restriction) ***Note***: Carbon black aerosol generated by sonication has smaller particles as compared to aerosol formulated without sonication. Test material preparation with sonication might therefore affect experimental results.							
Genotoxicity							
ECHA, 1998	ND	Ames assay	In vitro	5,000.0 µg/plate	48hrs	No observable Adverse effect reported	OECD 471; GLP
Test article: Carbon Black ***Dose:*** 33.3; 100.0; 333.3; 1,000.0; 2,500.0; 5,000.0 ug/plate. ***Treatment duration:*** At least 48 hours ***Species (strain):*** Salmonella typhimurium TA 1535, TA 1537, TA 98, TA 100, Escherichia coli WP2uvrA ***Number per Group:*** triplicate per dose. ***Control***: 4-NOPD (10-40 µg, for TA 1537, 98), MMS (1 uL; for E. coli WP2uvrA); for assays with metabolic activation: 2-AA (2.5 ug; for TA 1535, 1537, 98, 100 and E. coli WP2uvrA**).** ***Method:*** The test material was suspended in dimethylsulphoxide (DMSO) and diluted prior to treatment to the following test concentrations: 33.3; 100.0; 333.3; 1,000.0; 2,500.0; 5,000.0 ug/plate. The toxicity of the test material was determined with strains TA98 and TA100 in a pre-experiment. 8 concentrations (3.3, 10, 33.3, 100, 333, 1000, 2500, 5000 ug/plate) were tested for toxicity and mutation induction with each 3 plates both in the presence and the absence of metabolic activation. The experimental conditions in this pre-experiment were the same as for the main plate incorporation test. No toxicity was observed with the highest test concentration. ***Adverse Effect(s) (brief): NOAEL=5,000.0 ug/plate.*** In order to investigate the potential of Printex 90 to induce gene mutations in bacteria, the plate incorporation test (experiment I) and the pre-incubation test (experiment II) were performed in Salmonella typhimurium strains							

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
TA1535, TA1537, TA98, TA100 and in E. coli WP2uvrA. Printex 90 was suspended in DMSO and tested with and without metabolic activation (S9 mix) in triplicate at the following concentrations: 33.3, 100.0, 333.3, 1000.0, 2500.0 and 5000.0 ug/plate. Cytotoxicity was only observed in experiment II with Salmonella typhimurium TA 100 and E. coli WP2 uvrA at concentrations equal or higher than 2500 ug/plate. No increases in revertant frequencies were detected in any of the tester strains at any dose level either with or without metabolic activation. (increases in revertant frequencies were always below a factor of 2 as compared to the controls). The positive controls were functional. ***Data Quality:*** ECHA Reliability 3 (not reliable);							
ECHA, 2010	ND	RAW 267.4 mouse macrophage cell line.	Cell culture	100mg/L	48hrs	No observable adverse effect reported	Similar to OECD 471
Test article: carbon black. ***Dose:*** 0.01-100mg/L ***Treatment duration:*** 48h in total (20 hours without CytoB and 28h with cyto B) The test material was suspended in 10*%* serum***.*** ***Species (strain):*** RAW 267.4 mouse macrophage cell line. ***Gender:*** NA. ***Number per Group:*** two paired, independent cultures for each concentration were prepared. ***Control:*** CB. Mitomycin C was used as a positive control***.*** ***Method:*** Macrophages were treated with CB. Mitomycin C was used as a positive control. Forty-four hours after the initial preparation of the cultures, cytochalasin B was added to each test well to a final concentration of 4 mg/L to block cytokinesis of dividing cells. Macrophage cultures were then harvested after 28 hours. After 24 hr, the macrophages were treated with CB. Mitomycin C was used as a positive control. Forty-four hours after the initial preparation of the cultures, cytochalasin B was added to each test well to a final concentration of 4 mcg/ml to block cytokinesis of dividing cells. Macrophage cultures were then harvested after 28 hr: cells were treated with a 0.075 M. ***Adverse Effect(s) (brief):*** **NOAEL=100mg/L**. No increase in micronucleus frequency was found with carbon black. In addition, a comet assay was performed using H2O2 as positive control. Treatment with CB did not induce significant increases of primary DNA damage along the whole range of doses tested (0.208–20.83 mcg/cm2). ***Data Quality:*** ECHA Reliability 2 (reliable with restrictions) ***Note***: No increase in micronucleus frequency was found with carbon black							
ECHA, 2011	ND	Animal cell	Cell culture	1mg/L	48hr	No observable adverse effect reported	Similar to OECD 487: NR
Test article: Carbon black ***Dose:*** 1,3,10 mg/L ***Treatment duration:*** 48h ***Species (strain):*** RAW 264.7. ***Number per Group:*** NR ***Control: Method:*** The cyto- and genotoxic effects of single and multi-walled CNTs (SWCNTs, MWCNTs) and carbon black (CB) on the mouse macrophage cell line RAW 264.7 was investigated, including inflammatory response, release of tumor necrosis factor-(TNF-), intracellular reactive oxygen species (ROS) production, cell death (both necrosis and apoptosis), chromosomal aberrations and cellular ultrastructural alteration. in vitro mammalian cell micronucleus test. 1,3,10 mg/L, 48h; cytokinesis-block method for MNT, but no details reported; chromosome aberrations at 24, 48 and 72h post-exposure; ROS production at 50mg/L after 5 and 24h. ***Adverse Effect(s) (brief):*** **NOAEL =1mg/L.** Micronuclei frequencies were slightly increased at test concentrations of 3 and 10 mg/L; there were chromosome fragments present at all concentrations tested. Accumulation in phagolysosomes, causing acute necrosis; increased intracellular ROS production, similar at 5 and 24h**.** ***Data Quality:*** ECHA Reliability 2 (reliable with restrictions). ***Note***: Historical control data and absence of concentration-dependent response were not reported							
Reproductive/Developmental Toxicity							
ECHA, 2015	ND	Animal	Oral (gavage**)**	≥1000 mg/kg/day **(NOAEL)**	90-day	No observable adverse effect reported	OECD 414; GLP
Test article: Carbon Black ***Dose:*** 0, 100, 300, or 1000 mg/kg/day. In CMC (carboxymethyl cellulose) ***Treatment duration:*** Daily through days 5 to 19 of gestation. ***Species (strain):*** Rat (Wistar). ***Gender:*** male/female ***Number per Group:*** 14/ per dose. ***Control:*** concurrent vehicle. ***Method:*** Pregnant female rats were dosed through daily oral gavage at 0, 100, 300 or 1 000 mg/kg/day (24/group) during the sensitive period of organogenesis [day 5 through day 19 of gestation]. Following dosing on gestation days 5 through 19, the dams were killed on gestation day 20 and subjected to macroscopic examination. Usual litter parameters were recorded, and fetuses were sexed, weighed and submitted to external examination. About one half of the fetuses were also examined for soft tissue anomalies, and remaining fetuses were examined for skeletal anomalies. ***Adverse Effect(s) (brief):*** **maternal animals & fetuses NOAEL >1000 mg/kg/day** Discoloration of feces was observed. There were no adverse maternal changes or any effects on embryo-fetal development. Accordingly, under the conditions of this study, the No Observed Adverse Effect Level (NOAEL) for maternal toxicity and the No Observed Effect Level (NOEL) for developmental toxicity were both set at 1000 mg/kg/day. ***Data Quality:*** ECHA Reliability 1 (reliable without restriction). ***Note***: The oral No Observed Adverse Effect Level (NOAEL) for maternal toxicity and the No Observed Effect Level (NOEL) for developmental toxicity were both set at 1000 mg/kg/day in a study evaluated by the SCCS related to the staining properties of carbon black. Accordingly, under the conditions of this study, the No Observed Adverse Effect Level (NOAEL) was 1000 mg/kg/day. This study is used in a weight of evidence assessment for the developmental toxicity assessment. Together with modelled data on deposition fractions in the lungs of rats and humans it becomes evident that a significant amount of carbon black particles inhaled by rats and humans will be cleared from the lungs into the gastrointestinal tract; making this route of exposure of relevance for the human risk assessment.							

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
ECHA, 2019	ND	Animal	Inhalation (whole body)	34 mg/m³ air ***(NOEC)***; > 34 mg/m³ air ***(NOAEC)***.	30hrs	No observable adverse effect reported	Non-guideline study; non-GLP
Test article: Carbon Black ***Dose:*** 4.6 and 37 mg/m3. ***Treatment duration:*** 45 min per day; daily from GD 4 to 18 ***Species (strain):*** Mice (NMRI). ***Gender:*** male ***Number per Group:*** 11/ per dose. ***Control:*** A concurrent control group was exposed to conditioned air. ***Method:*** Time-mated females were exposed to a mean total suspended particle mass concentration of 4.79 ± 1.86 or 33.87 ± 14.77 mg/m3 for the low and high exposure, respectively. In this study, male fertility parameters were assessed following in utero inhalation exposure to carbon black in four generations of mice. One male offspring per litter was used to generate the subsequent generation and assessed for hereditary male reproductive parameters, but two F1 males were bred with a naïve female from some litters. Parameters examined in all male parental generations: testis weight, epididymis weight, daily sperm production, sperm chromatin structure. body and reproductive organ weights. Gestation length, number and loss of implantations. offspring weight, litter size, sex ratio. ***Adverse Effect(s) (brief): NOEC=34 mg/m³ air; NOAEC*** **> 34 mg/m³ air.** Time-mated females were exposed to a mean total suspended particle mass concentration of 4.79 ± 1.86 or 33.87 ± 14.77 mg/m3 for the low and high exposure, respectively. Exposure did not affect gestation and litter parameters in any generation. No significant changes were observed in body and reproductive organ weights, epididymal sperm parameters, daily sperm production, plasma testosterone or fertility. ***Data Quality:*** ECHA Reliability 2 (reliable with restrictions). ***Note***: In utero exposure to carbon black via maternal whole-body inhalation did not affect male-specific reproductive, fertility and litter parameters in four generations of mice.							

II.2 Other Health Effects

Pigment	Endpoint	Outcome	Reference
Dermal irritation			
Carbon Black	Dermal irritation	No observable adverse effect reported	ECHA, 1977
Test article: Carbon Black ***Dose:*** 0.5 g of the test substance were dissolved in distilled water at a concentration of 20%, and 0.5 mL. ***Treatment duration:*** 24 hrs, followed by a 14-day observation period. ***Species (strain):*** Rabbit (New Zealand White). ***Gender****:* male/ female; 2.3 and 2.8 kg. ***Number per Group:*** 6/ per sex. ***Control:*** not required ***Method:*** 0.5 g of the test substance were dissolved in distilled water at a concentration of 20%, and 0.5 mL of this solution was applied to the intact and scarified back skin of three rabbits, respectively. The skin was evaluated for edema and erythema at removal of the test substance, and again at daily intervals until study end at 14 days after exposure. The skin was evaluated by two independent investigators. In addition, clinical signs, behavior, general condition, and food consumption was recorded. Body weights were determined daily. ***Adverse Effect(s) (brief):*** None of the animals exhibited any signs of skin irritation (no edema, no erythema at any of the observations). No signs of systemic toxicity were noted. Behavior, general condition, and body weight gain were not affected by treatment. ***Data Quality:*** ECHA Reliability 1 (reliable without restriction).			
Carbon Black	Dermal irritation	No observable adverse effect reported	ECHA, 1978
Test article*:* Carbon Black ***Dose****:* 0.5 g of the test substance were dissolved in distilled water at a concentration of 20%, and 0.5 mL. ***Treatment duration****:* 24 hrs, followed by a 14-day observation period***.*** ***Species (strain):*** Rabbit (New Zealand White). ***Gender:*** male/ female; 2.3 and 2.8 kg. ***Number per Group:*** 6/ per sex. ***Control:*** not required. ***Method:*** 1) test performed under occlusive conditions (instead of semi-occlusive conditions) 2) exposure period was 24 hours (instead of 4 hours). 0.5 g of the test substance were dissolved in pharmaceutical grade olive oil (the maximum attainable concentration was 27%) and applied to the intact and scarified back skin of six rabbits, respectively. The test substance was applied on linen patches and held in contact with the clipped skin under occlusive conditions. In 6 animals, the skin was superficially scarified avoiding deeper injury and bleeding. the skin was evaluated for edema and erythema at removal of the test substance, and again at daily intervals until study end at 14 days after exposure. The skin was evaluated by two independent investigators. In addition, clinical signs, behavior, general condition and food consumption was recorded. Body weights were determined daily. ***Adverse Effect(s) (brief):*** None of the animals exhibited any signs of skin irritation (no edema, no erythema at any of the observations). No signs of systemic toxicity were noted. Behavior, general condition, and body weight gain were not affected by treatment. *Data Quality:* ECHA Reliability 1 (reliable without restriction).			
Carbon Black	Dermal irritation	No observable adverse effect reported	ECHA, 2003
Test article: Carbon Black ***Dose:*** 0.5 g of the test substance were dissolved in distilled water at a concentration of 20%, and 0.5 mL. ***Treatment duration:*** 4 hrs, followed by a 7-day observation period. ***Species (strain):*** Rabbit (New Zealand White). 9 - 11 weeks. ***Gender****:* male/ female; 3.7 kg, 2.8 kg and 3.8 kg. ***Number per Group****:* 3/ per sex. ***Control:*** yes, concurrent no treatment. ***Method:*** 0.5 g of the test substance was mixed with 1 mL of sterile water to form a paste. The paste was spread evenly over a gauze square measuring 2.5 x 2.5 cm. The gauze was then placed on the animal's skin with the test item in direct contact with the clipped skin. A strip of aluminum foil was placed over the treated site and the whole assembly held in place			

Pigment	Endpoint	Outcome	Reference
Dermal irritation			
by encircling the trunk of the animal with a length of elastic adhesive bandage. ***Adverse Effect(s) (brief):*** NOAEL= *0.5 g* None of the animals exhibited any signs of skin irritation (no edema, no erythema at any of the observations). Black coloration of the treatment site was recorded for all animals at the 1 -72-hour examinations. No coloration was noted on day 7. No signs of systemic toxicity were noted. Changes in body weights during the course of the study were not remarkable. ***Data Quality:*** ECHA Reliability 1 (reliable without restriction).			
Eye irritation			
Carbon Black	Eye irritation	No observable adverse effect reported	ECHA, 1984
Test article: Carbon Black ***Dose:*** 100 mg, undiluted *Treatment duration:* 24hrs. ***Species (strain):*** Rabbit (New Zealand White) 2.3-2.8 kg. ***Gender:*** NR ***Number per Group****:* 5/per dose. ***Control:*** other eye. ***Method:*** 100 mg of the test substance was instilled into the left eye. The other eye served as control. In 5 animals, the test substance was washed out of the eye after 5 minutes. In 3 animals the test substance was washed out of the eye after 24 hours. eyes were evaluated for erythema, edema, and effects on cornea and iris according to the Draize score 5 minutes before instillation, and at 24, 48, 72 and 96 hours after instillation. The eyes were evaluated independently by two examiners. ***Adverse Effect(s) (brief):*** No irritant effects were found in any of the animals at any observation (scores for cornea, iris and conjunctivae: 0.0 ,0.0 ,0.0). ***Data Quality:*** ECHA Reliability 1 (reliable without restriction)			
Carbon Black	Eye irritation	No observable adverse effect reported	ECHA, 2003
Test article: Carbon Black ***Dose:*** 100 mg. ***Treatment duration:*** continuous (Day 1 - Day 4) ***Species (strain):*** Rabbit (New Zealand White). ***Gender:*** male ***Number per Group:*** 3/per dose**.** ***Control:*** A concurrent control group; untreated. ***Method:*** 1, 24, 48, 72 hours after instillation. ***Adverse Effect(s) (brief):*** No eye irritation. One animal died. Necropsy examination revealed a spontaneous pathology. This death is therefore not recorded as being treatment related. Information from this animal were excluded. There was no indication of a systemic effect in any of the surviving animals. Dark skin/fur staining of the muzzle was seen in the 3 animals up to termination of the study. Body weights change was not remarkable through the study: Day 1 (3.7 kg, 3.7 kg and 4.1 kg) and on the termination of the study (Day 4: 3.7 kg, 3.7 kg and 4.2 kg, respectively*).* ***Data Quality****:* ECHA Reliability 1 (reliable without restriction)			
Skin Sensitization			
Carbon Black	Skin Sensitization	No observable adverse effect reported	ECHA, 2015
Test article: Carbon Black ***Dose****:* 0, 0.25, 0.5, 1.0, 2.5, or 5.0% in propylene glycol. ***Treatment duration:***30hr*s*, followed by up to 28-day observation period. (6h/day for 5 days*)* ***Species (strain):*** Mice (CBA:J). ***Gender:*** female 11-12 weeks old. *Number per Group:* 4/ per dose. ***Control:*** *(Positive control)* hexyl cinnamic aldehyde (CAS No 101-86-0). ***Method****:* LLN. ***Adverse Effect(s) (brief): NOAEL=5.0%*** Slight erythematic response at the site of application was seen in positive control animals. No significant increase in ear thickness in treated animals in comparison to vehicle and positive control groups. Acceptance criteria for the study was met i.e. 70% cell viability in negative control group and the SI for the positive control was greater than 3. SI value for the treated groups showed a less than 3-fold increase compared to negative control animals, hence, under experimental conditions, carbon black is not considered a contact sensitizer in the LLNA. ***Data Quality:*** ECHA Reliability 2 (reliable with restrictions)			
Carbon Black	Skin Sensitization	No observable adverse effect reported	ECHA, 2003
Test article: Carbon Black ***Dose:*** 50% of the test material in sterile water. ***Treatment duration:*** NR ***Species (strain):*** guinea pig (Dunkin-Hartley). ***Gender:*** female ***Number per Group:*** 20/ per dose. ***Control:*** 10/alpha-hexylcinnamaldehyde *(positive control)* **Method:** Buehler test. ***Adverse Effect(s) (brief):*** Carbon Black XPB 295 was not sensitizing in guinea pigs (Buehler test performed according to OECD guideline 406) ***Data Quality****:* ECHA Reliability 1 (reliable without restriction)			

Section III. Dose-Response Assessment

III.1 Provisional Tolerable Intake Values

Contact Duration	Exposure Route	Patient	POD	MF	pTI
Permanent	Parenteral	All	20,000 mg/kg/d	10,000	2.0 mg/kg/d

pTI = POD ÷ MF

Note: When a supplier-specific color additive safety data sheet (SDS) does not report the presence of a carcinogen or non-carcinogen impurity, and SDS complies with Globally Harmonized System (GHS) hazard communication limits (i.e., <0.1% and <1%, respectively), then this pTI is low enough to be protective for naturally occurring impurities that could be present in the color additive. Processes used to color a polymer system involve diluting the pigment and its impurities by at least 50x (i.e., the CHRIS

calculator is valid when the maximum concentration of pigment in a colored polymer is 2%, i.e., 1 ÷ 2% = 50); therefore, the concentration of naturally occurring impurities in the finished colored polymer will be significantly lower compared to the concentration of these substances in the pigment (i.e., 0.002% (i.e., 0.1% ÷ 50) for carcinogens and 0.02% (i.e., 1% ÷ 50) for non-carcinogens). Unknown impurities are addressed by the CHRIS calculator.

III.2 Modifying Factor for POD

Critical Study	Biological Endpoint	Exposure Route	POD Type	POD Value	UF_1	UF_2	UF_3	MF
Nau, Neal, and Stembridge (1958a)	Systemic Toxicity	Oral	NOAEL	20,000 mg/kg/d	10	10	100	10,000

MF = modifying factor (UF_1 x UF_2 x UF_3); POD is rounded down from 28,000 mg/kg/d.

III.3 Toxicological Uncertainties Applied to POD

Uncertainty	Justification
UF_1 Error! Bookmark not defined.	(10) Default value to account for susceptible individuals.
UF_2 Error! Bookmark not defined.	(10) Default value to account for differences between species.
UF_3 Error! Bookmark not defined.	(100) Account for differences in exposure route and reports of polycyclic aromatic hydrocarbon impurities in some, but not all, carbon blacks (e.g., lamp black)

UF_1, UF_2, and UF_3, are used to extrapolate the POD to the general human population (including pediatric) exposed parenterally for a lifetime. Polycyclic aromatic hydrocarbon impurities occur naturally. The presence of these impurities depends on the raw material and associated process used to produce carbon black (e.g., carbon black produced by the high purity furnace process results in the lowest quantity of polycyclic aromatic hydrocarbon impurities compared to other processes used to manufacture carbon black).

III.4 Critical Health Study Design and Outcome

Nau CA, Neal J, and Stembridge VA. (1958a) Study of the physiological effects of carbon black. I. Ingestion. Arch. Indust. Health. 17(1):21-28.			
Test Substance	Whole carbon black, extracted carbon black (extracted with hot benzene for 48 hours), "free" benzene extract from carbon black, or methylcholanthrene adsorbed carbon black		
Species	CFW white mice, C_3H brown mice	Frequency	Daily
Gender	Males and females	Duration	12 – 18 months
Age	6 – 10 weeks	#/Group	Not reported
Route	Oral (feed)	Protocol Guideline	Not reported
Dose(s)	10% of feed (~ 28,000 mg/kg/d)	Statistical Method(s)	Not reported
Observed Responses	No effects were observed after whole carbon black and extracted carbon black treatments. Benzene-extracts ("free") from carbon black and methylcholanthrene adsorbed carbon black resulted in stomach tumors.		
Notes:	Repeated dose toxicity Data. Arch Indust Health. Vol. 17, Issue 1		

* Technical grade sample produced by Bayer AG under the trade name Light Yellow 3R. (CAS number and purity not reported.)

C.I. Pigment Blue 29

CAS #57455-37-5

Toxicological Profile Summary

C.I. Pigment Blue 29 (a.k.a., ultramarine blue or PB29) is an inorganic pigment with a deep blue color [30]. PB29 size, shape, and surface characteristics indicate low reactivity potential. Reported hazard information includes an adverse health effect study, supporting studies, and secondary sources. C.I. Pigment Blue 29 pigment, administered at high oral doses (up to 1000 mg/kg/day) to rodents is reported to elicit local toxicity of the forestomach (no observable systemic toxicity was reported). Based on secondary reports of oral systemic toxicity in animals, no observable local or systemic toxicity occurred at doses below 300 mg/kg/day (~800 ppm in food) in an up to 42-day study[30]. For assessing C.I. Pigment Blue 29 exposure that will be without appreciable harm to health, the calculated provisional tolerable intake (pTI) value for parenteral systemic toxicity is 3.3 mg/kg/day based on the lowest reported point-of-departure (NOAEL of 300 mg/kg/day, oral, 28-day, rodent) with a modifying factor of 90 (i.e., 3 x 1 x 30) to address the following sources of uncertainty: variation among humans (3), variation between species(10), and data quality/route-to-route extrapolation (30).

NOTE: This pTI is protective for the color additive including any impurities listed in Section I.1 "Substance Identity," as the impurities likely will be present at a very low percentage of the color additive, and the quantity of the impurity will be at an even lower percentage in the final device (see Section III, Table III.1 footnote). Unknown impurities are addressed by the CHRIS calculator.

Section I. Substance Information

I.1 Substance Identity

	Descriptor
Synonyms[31]	C.I. Pigment 29, Pigment Blue 29, Ultramarine (pigment), azure blue
Formula[31]	$Al_6Na_8O_{24}S_3Si_6$
Molecular Weight[31]	994.473 g/mol
Physical Form[32,33]	Solid dark-blue powder (room temperature)
Type	UVCB-inorganic
Physical/Chemical Characteristics[33]	Melting point is >1000°C Boiling point is unavailable.
Production[31,33]	Grinding synthetic or naturally occurring lapis lazuli, a semi-precious stone, into a powder or calcining a mixture of kaolin, sulfur, sodium carbonate, and a source of carbon at temperatures above 700°C
Impurities[32]	Moiety of concern: Al (Al^{+3}).
Surrogates	None
Note	None

[30] EPA (2004). Ultramarine Blue: Exemption From the Requirement of a Tolerance. US Fed. Regist. Wash. DC 4, 11672.
31 ChemID [WWW Document] https://chem.nlm.nih.gov/chemidplus/rn/57455-37-5
32 OECD (2017). Categorization Results from the Canadian Domestic Substance List. CAS Number: 57455-37-5. Organisation for Economic Co-operation and Development. Accessed on: August 11, 2017. [WWW Document] http://webnet.oecd.org/ccrweb/ChemicalDetails.aspx?ChemicalID=CB5B2390-EE70-4902-8C59-7547D557E700
33 EPA (2004). Ultramarine Blue: Exemption From the Requirement of a Tolerance. US Fed. Regist. Wash. DC 4, 11672. [WWW Document] https://www.govinfo.gov/content/pkg/FR-2004-05-26/pdf/04-11672.pdf

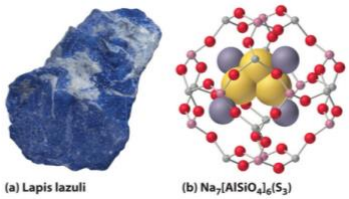


Figure I.1a. "*(a) The rich blue color of lapis lazuli is due to small amounts of the normally unstable S3− anion. (b) The aluminosilicate cages of the minerals (zeolites) that make up the matrix of blue ultramarine stabilize the reactive anion; excess Na+ ions in the structure balance the negative charges on the zeolite framework and the S3− anion*", source: Averill and Eldredge 1977).[34]

Section II. Hazard Identification

Acute exposure resulted in no observable adverse effects. Acute toxicity data from a single dose oral toxicity test at 10000 mg/kg resulted in no mortality.[35]; only bluish feces, derived from the color of the test article were observed.[36].

In a 90-day sub-chronic repeated dose toxicity study in rats, local forestomach toxicity (slight squamous hyperplasia at the limiting ridge of the forestomach in both sexes) and local inflammation of the GI tract are reported. In this sub-chronic study, the only effect of concern (i.e., organ pathology; siliceous stones in kidney and bladder) occurred in animals fed a diet that contained 10,000 or 100,000 ppm ultramarine blue. Moreover, the observable effects are reported to be consistent with siliceous earth materials, which describes ultramarine blue, a sulfurized sodium aluminum silicate.[37]. This change demonstrated reversibility or a tendency for reversibility in the male recovery group and the female satellite group. Ultramarine blue is reported to be non-mutagenic (via Ames assay) using two strains each of *Salmonella typhimurium* and *Escherichia coli.* For reproductive and developmental toxicity, exposure durations were not provided. However, the reported details indicate the parental animals exhibited no alterations in reproductive parameters or malformations in offspring at treatment doses at 100,000 ppm.

Reported methodological information and additional results from adverse health effect studies and secondary sources are presented in the following tables.

II.1 Systemic, Genotoxicity, Carcinogenicity, Reproductive/Developmental Toxicity

ADVERSE HEALTH EFFECT STUDIES

None reported, see Section III.4 for the critical adverse health effect study.

SUPPORTING STUDIES

None reported.

SECONDARY SOURCES

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
Systemic Toxicity (acute)							
Holliday Pigments 2008	ND	Animal	Oral	10,000 mg/kg (**LD_{50}**)	NR	No observable adverse effect reported	NR
Test article: Ultramarine blue. ***Dose:*** NR ***Treatment Duration:*** Single***. Species (strain):*** Rat and mouse. ***Gender:*** Not reported. ***Number per Group:*** Not reported. ***Control***: NR ***Adverse Effect(s) (brief):*** **LD_{50} ≥ 10,000 mg/kg**. No deaths were observed in							

34 Averill BA and Eldredge P. Periodic Trends and the s-Block Elements. Chemistry: Principles, Patterns and Applications. Prentice Hall; 1st edition. 2007. Available at: https://chem.libretexts.org/?title=Textbook_Maps/General_Chemistry_Textbook_Maps/Map:_Chemistry_(Averill_and_Eldredge)/21:_Periodic_Trends_%26_the_s-Block_Elements/21.3:_The_Alkali_Metals_(Group_1)
35 Holliday Pigments (2009). Ultramarine Pigments Safety and Regulatory Status (Holliday Pigments).
36 Hashima Laboratory (2006). C.I. Pigment Blue 29 CAS No. 57455-37-5 Ultramarine Blue.
37 EPA (2004). Ultramarine Blue: Exemption From the Requirement of a Tolerance. US Fed. Regist. Wash. DC 4, 11672.

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
either species over a period of 15-days. ***Data Quality:*** Not reported. ***Reference:*** *Hashima Laboratory, Japan. Accessed on August 11, 2017. Available at: http://dra4.nihs.go.jp/mhlw_data/home/file/file57455-37-5.html*							
Holliday Pigments 2009	ND	Animal	Oral (feed)	10,000 mg/kg/day **(NOAEL)**	NR	No observable adverse effect reported	NR
Test article: Ultramarine blue. ***Dose:*** NR ***Treatment Duration:*** NR***. Species (strain):*** NR. ***Gender:*** NR. ***Number per Group:*** NR. ***Control***: NR ***Methods:*** NR. ***Adverse Effect(s) (brief):*** **NOAEL = 10,000 mg/kg**. ***Data Quality:*** Not reported. ***Reference:*** *Holliday Pigments (2009). Ultramarine Pigments Safety and Regulatory Status (Holliday Pigments).*							
Hashima Laboratory 2006	ND	Animal	Oral (gavage)	2,000 mg/kg (**LD_{50}**)	Single administration	Bluish feces	OECD 423; GLP
Test article: C.I. Pigment Blue 29 (SiO_2 39.60 %, Al_2O_3 23.76 %, Fe_2O_3 0.45 %, S 12.08 %, Na_2O 22.59 %, unknown 1.52 %). ***Dose:*** 2000 mg/kg in 1 % Sodium carboxymethylcellulose solution (Vehicle). ***Treatment Duration:*** Single***. Species (strain):*** Rat (Sprague Dawley (Crj:CD(SD)IGS)). ***Gender:*** Female. ***Number per Group:*** 6. ***Control***: NR ***Method: Adverse Effect(s) (brief):*** **LD_{50} = 2,000 mg/kg**. Only effect seen was bluish feces derived from the color of the test article. The chemical was thus classified into category 5 of the GHS regarding acute toxicity. ***Data Quality:*** OECD Test Guideline 423. ***Reference:*** *Hashima Laboratory (2006). C.I. Pigment Blue 29 CAS No. 57455-37-5 Ultramarine Blue.*							
Systemic Toxicity (subacute, subchronic,							
EPA 2004	ND	Animal	Oral (feed)	10,000 mg/kg/day **(NOAEL)**	90-day repeat dose	No observable adverse effect reported	NR
Test article: Ultramarine blue. ***Dose:*** 10,000 mg/kg/day ***Treatment Duration:*** 90-day repeat dose ***Species (strain):*** Rat. ***Gender:*** Not reported. ***Number per Group:*** Not reported. ***Control***: NR ***Methods:*** 90-day feeding study. ***Adverse Effect(s) (brief):*** **NOAEL = 10,000 mg/kg/day**. No adverse effects. Effects were much like inert clay. ***Data Quality:*** Not reported. ***Reference:*** *EPA (2004). Ultramarine Blue: Exemption From the Requirement of a Tolerance. US Fed. Regist. Wash. DC 4, 11672.*							
EPA 2004	ND	Animal	Oral (feed)	100 ppm (**LOAEL**)	90-day repeat dose	Inflammation of the GI tract and the presence of siliceous stones in kidney and bladder	NR
Test article: Ultramarine blue. ***Dose:*** 0, 100, 1,000, 10,000, and 100,000 parts per million (ppm) ***Treatment Duration:*** 90 days Species ***(strain):*** Rat. ***Gender:*** Not reported. ***Number per Group:*** Not reported. ***Control:*** not reported ***Methods:*** NR. ***Adverse Effect(s) (brief):*** **LOAEL = 100 ppm.** Exposure resulted in inflammation of the GI tract and the presence of siliceous stones in kidney and bladder at all dose levels. Histologically, no pathological effects haven been observed in rats after ingestion of 100 or 1,000 ppm ultramarine blue. At higher concentrations, 10,000 and 100,000 ppm, there were increased excretion of silica and sodium, and pathological effects in the kidneys, stomach, intestine and bladder, which could be associated with high and prolonged intake of siliceous earth. ***Data Quality:*** Not determined since it is a secondary source. ***Reference:*** *EPA (2004). Ultramarine Blue: Exemption From the Requirement of a Tolerance. US Fed. Regist. Wash. DC 4, 11672.*							
Hashima Laboratory, n.d.	ND	Animal	Oral (gavage)	2,000 mg/kg bw **(NOAEL)**	3-day repeat administration	Bluish feces	OECD 422; GLP
Test article: C.I. Pigment Blue 29 (SiO_2 39.60 %, Al_2O_3 23.76 %, Fe_2O_3 0.45 %, S 12.08 %, Na_2O 22.59 %, unknown 1.52 %). ***Dose:*** Vehicle: 1.0 % Methylcellulose. ***Treatment Duration:*** NR ***Species (strain):*** Rat (Sprague Dawley (Crl:CD(SD)). ***Gender:*** Males and female. ***Number per Group:*** 2. ***Control***: NR ***Methods****:* 3-day repeat administration of exposure via oral gavage. Rats were observed once a day for 14 days. ***Adverse Effect(s) (brief):*** **NOAEL = 2,000 mg/kg bw**. Two males and females were repeatedly administered 2000 mg/kg of the test substance for 3 days, and as a result of examining the effect on general condition and body weight, no change was observed except for blue colored stools in both males and females. Only effect seen was bluish feces derived from the color of the test article. ***Data Quality:*** OECD Test Guideline 422. ***Reference*** GLP Hashima Laboratory (n.d.). Single Dose Oral Toxicity Study of C.I. Pigment Blue 29 in Rats. Hashima Laboratory Japan. http://dra4.nihs.go.jp/mhlw_data/home/file/file57455-37-5.html							
Genotoxicity							
EPA 2004	ND	Bacteria	In vitro	NR	NR	No observable adverse effect reported	NR
Test article: Ultramarine blue. **Dose:** NR. **Treatment Duration:** NR **Species (strain):** Salmonella typhimurium and Escherichia coli. **Gender:** Not reported. **Number per Group:** Not reported. **Methods:** Ames assay. **Adverse Effect(s) (brief):** Ultramarine blue was also shown to be non-mutagenic (via) using two strains. **Data Quality:** Not determined since it is a secondary source.							

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
Developmental and Reproductive Toxicity							
EPA 2004	ND	Animal	Oral (feed)	100,000 ppm **(Offspring; NOAEL)/** 1000 ppm **(Maternal; NOAEL)**	NR	No observable adverse effect reported	NR
Test article: Ultramarine blue. ***Dose:*** 0, 100, 1,000, 10,000, and 100,000 ppm in diet. ***Treatment Duration:*** NR***. Species (strain):*** Not reported. ***Gender:*** Not reported. ***Number per Group:*** Not reported. ***Control:*** Control animals received vehicle diets ***Methods:*** Histological evaluation of the kidneys, stomach, intestine and bladder. The reproductive/developmental parameters examined included litter size, fetal weights, resorptions, malformations. ***Adverse Effect(s) (brief):*** **(Maternal) NOAEL= 1000 ppm; (Reproductive/developmental toxicity) NOAEL = 100,000 ppm.** At higher concentrations (10,000 and 100,000 ppm), there were pathological effects in the kidneys, stomach, intestine and bladder which could be associated with high and prolonged intake of siliceous earth. Histologically, no pathological effects were observed at 100 or 1,000 ppm. There was no significant difference in litter size, fetal weights, or resorptions between controls and dose level groups. No malformations were observed in controls or the highest dose group (100,000 ppm). No maternal deaths at any dose. ***Data Quality:*** Not reported.						
Hashima Laboratory, 2006	ND	Animal	Oral	300 mg/kg/day **(NOAEL)** 1,000 mg/kg day **(LOAEL)**	Repeat dose	Stomach toxicity	OECD 422; GLP
Test article: C.I. Pigment Blue 29. ***Dose:*** 0, 100, 300, and 1,000 mg/kg day. ***Treatment Duration:*** 42 Days for males;14 Days before mating to day 4 of lactation for females; 42 Days for the satellite group for recovery of females ***Gender:*** Male and female. ***Number per Group:*** 12 / per dose/ per sex. ***Methods:*** OECD 422. ***Adverse Effect(s) (brief):*** **NOAEL = 300 mg/kg; LOAEL=1000 mg/kg**. Slight squamous cell hyperplasia in the forestomach of both sexes was observed at 1,000 mg/kg day. This change demonstrated reversibility or a tendency for reversibility in the male recovery group and the female satellite group. ***Data Quality:*** Not determined since it is a secondary source.							

II.2 Other Health Effects

Sensitization & Irritation			
Pigment	**Endpoint**	**Outcome**	**Reference**
UMB	Sensitization (dermal)	No observable adverse effect reported	EPA 2004
Test article: Ultramarine blue. ***Dose:*** 12.5% and 25% ***Treatment Duration:*** NR***. Species (strain):*** Guinea pig. ***Gender:*** Not reported. ***Number per Group:*** Not reported. ***Adverse Effect(s) (brief):*** Non-sensitizer. ***Data Quality:*** Not reported.			
UMB	Sensitization (dermal)	No observable adverse effect reported	EPA 2004
Test article: C.I. Pigment Blue 29. ***Dose:*** NR. ***Treatment Duration:*** NR***. Species (strain):*** Rabbit. ***Gender:*** Not reported. ***Number per Group:*** Not reported. ***Control:*** NR ***Methods:*** Used the maximization test of Magnusson and Kligman. In the induction phase, the injection concentrations were 6.25% without Complete Freund's Adjuvant and 12.5% with Complete Freund's Adjuvant; the topical application was a 50% concentration. In the challenge phase, 12.5% and 25% concentrations were used. ***Adverse Effect(s) (brief):*** Non-sensitizer. ***Data Quality:*** Not reported.			
UMB	Irritation (skin)	No observable adverse effect reported	EPA 2004
Test article: ultramarine blue. ***Dose:*** 0.5 g ***Treatment Duration:*** 24hr. ***Species (strain):*** Albino rabbits. ***Gender:*** Not reported. ***Number per Group:*** 6. ***Control:*** NR ***Methods:*** 24 h on abraded and intact skin. ***Adverse Effect(s) (brief):*** No irritation was found and no reactions. ***Data Quality:*** Not reported. ***Note***: The procedure is described in the US Federal Register 1973, Vol. 38, No. 187, Sect 1500:41.			

Section III. Dose-Response Assessment

III.1 Derivation of Provisional Tolerable Intake

Contact Duration	Exposure Route	Patient	POD	MF	pTI
Permanent	Parenteral	All	300 mg/kg/d	300	3.3 mg/kg/d

pTI = POD ÷ MF

Note: When a supplier-specific color additive safety data sheet (SDS) does not report the presence of a carcinogen or non-carcinogen impurity, and SDS complies with Globally Harmonized System (GHS) hazard communication limits (i.e., <0.1% and <1%, respectively), then this pTI is low enough to be protective for naturally occurring impurities which could be present in the color additive. Processes used to color a polymer system involve diluting the pigment and its impurities by at least 50x (i.e., the CHRIS calculator is valid when the maximum concentration of pigment in a colored polymer is 2%, i.e., 1 ÷ 2% = 50); therefore, the concentration of naturally occurring impurities in the finished colored polymer will be significantly lower compared to the concentration of these substances in the pigment (i.e., 0.002% (i.e., 0.1% ÷ 50) for carcinogens and 0.02% (i.e., 1% ÷ 50) for non-carcinogens). Unknown impurities are addressed by the CHRIS calculator.

III.2 Derivation of a Modifying Factor

Critical Study	Critical Health Effect	Exposure Route	POD Type	POD Value	UF_1	UF_2	UF_3	MF
Hashima Laboratory 2006	Stomach toxicity	Oral	NOAEL	300 mg/kg/d	3	1	30	90

MF = modifying factor (UF_1 x UF_2 x UF_3)

III.3 Toxicological Uncertainties Applied to POD

Uncertainty	Justification
UF_1	(3) No species differences based on elemental composition and physico-chemical properties (i.e., innocuous elements covalently bound into aluminosilicate cages and insoluble and chemically unreactive particles).
UF_2	(1) No species differences based on elemental composition and physico-chemical properties (i.e., innocuous elements covalently bound into aluminosilicate cages and insoluble and chemically unreactive particles).
UF_3 **Error! Bookmark not defined.**	(30) To account for differences in exposure route, low data quality, and low bioavailability because of extremely low water solubility and low absorption rate.

UF_1, UF_2, and UF_3, are used to extrapolate the POD to the general human population (including pediatric) exposed parenterally for a lifetime.

III. 4 Critical Health Study Design and Outcome

Reference: Hashima Laboratory (2006). C.I. Pigment Blue 29 CAS No. 57455-37-5 Ultramarine Blue.			
Test Substance	C.I. Pigment Blue 29 (SiO_2 39.60%, Al_2O_3 23.76%, Fe_2O_3 0.45%, S 12.08%, Na_2O 22.59%, unknown 1.52 %)		
Species	Rat/Crl:CD(SD)	Frequency	Daily
Gender	Male and female	Duration	14 days before mating to day 4 of lactation for females and 42 days for males. Euthanized on day 43 for males and day 5 of lactation for females.
Age	No age reported	#/Group	12 per sex per dose
Route	Oral gavage	Protocol Guideline	OECD Test Guideline 422; GLP compliant
Dose(s)	100, 300 and 1,000 mg/kg/day (vehicle is 1.0% methylcellulose)	Statistical Method(s)	Not reported
Observed Responses	In the 1,000 mg/kg group, the histopathological examination revealed slight squamous hyperplasia at the limiting ridge of the forestomach in both sexes. This change demonstrated reversibility or a tendency for reversibility in the male recovery group and the female satellite group. The NOAEL for repeated dose toxicity is considered to be 300 mg/kg/day for parental animals of both sexes. No changes in reproductive parameters were observed in the parental or F1 generation. The NOAELs for reproductive toxicity and offspring development are considered to be greater than 1000 mg/kg/day.		
Notes:	Test performed by the Research Institute for Animal Science in Biochemistry and Toxicology in Japan.		

C.I. Pigment Brown 24

CAS # 68186-90-3

Toxicological Profile Summary

C.I. Pigment brown 24 (PB24) is an inorganic pigment constiting of titanium oxide with small quantities of chromium (III) and antimony. PB24 pigment is manufactured by calcination, at high temperature, with titanium (IV) oxide, chromium (III) oxide and antimony oxide in varying amounts, homogeneously and ionically interdiffused to form a crystalline matrix of rutile.[38] Reported hazard information includes an adverse health effect study, supporting studies, and secondary sources. PB24, administered at high oral doses (up to 500 mg/kg/day) to rodents, is reported to not elicit observable systemic effects. The calculated provisional tolerable intake (pTI) value for parenteral systemic toxicity is 0.5 mg/kg/day based on the lowest reported point-of-departure (500 mg/kg/day, oral, 90-day, rodent) with a modifying factor of 1000 (e.g., 10 x 10 x 10) to address the following sources of uncertainty: variation among humans (10), variation between species (10), and data quality/route-to-route extrapolation (10).

NOTE: This pTI is protective for the color additive including any impurities listed in Section I.1 "Substance Identity," as the impurities likely will be present at a very low percentage of the color additive, and the quantity of the impurity will be at an even lower percentage in the final device (see Section III, Table III.1 footnote). Unknown impurities are addressed by the CHRIS calculator.

Section I. Substance Information

I.1 Substance Identity

	Descriptor
Synonyms[39, 40]	Chrome Antimony Titanate Chrome antimony titanium oxide rutile Chrome rutile yellow C. I. 77310 Titanium, Antimony, Chromium III oxide rutile
Formula[39, 41]	(Ti, Cr, Sb) O_2 (Unspecified); complex inorganic colored pigment based on titanium oxide; in the rutile lattice, titanium ions are partially replaced by 2-6% chromium (III) and 8.5-14% antimony (V) ions.
Molecular Weight[42]	Not available (no data)
Physical Form[42]	Solid
Type[40,42]	Inorganic; Mono constituent substance; UVCB-inorganic
Physical/Chemical Characteristics[40,42]	Extremely low solubility in water >1000°C melting point

[38] ECHA 2017. Chrome Antimony Titanium Buff Rutile. Updated on June 7, 2017. Accessed on June 28, 2017. https://echa.europa.eu/brief-profile/-/briefprofile/100.062.756

39 ChemID [WWW Document] https://chem.nlm.nih.gov/chemidplus/rn/68186-90-3

40 ECHA 2017. Chrome Antimony Titanium Buff Rutile. Updated on June 7, 2017. Accessed on June 28, 2017. https://echa.europa.eu/brief-profile/-/briefprofile/100.062.756

41 US EPA. C.I. Pigment 24. Substances Registry Services (SRS). United States Environmental Protection Agency. Last updated on June 1, 2017. Accessed on July 10, 2017. https://iaspub.epa.gov/sor_internet/registry/substreg/searchandretrieve/substancesearch/search.do?details=displayDetails&selectedSubstanceId=72520

42 OECD SIDS 2002. C.I. Pigment Brown 24. CAS No.: 68186-90-3. SIDS Initial Assessment Report. http://webnet.oecd.org/Hpv/UI/handler.axd?id=d2341afe-af11-478c-aaed-ddce1e8b4ecf

	Descriptor
	Density reached 4-5 g/cm³ Negligible vapor pressure assumed Flammable liquid
Production[40,42]	Doped rutile pigments are manufactured by reacting finely divided metal oxides, hydroxides or carbonates in the solid state at a temperature of 1,000 to 1,200 °C. The production is based on reactive anatase, or titanium dioxide hydrolysate containing sulfuric acid, and on the oxidation of trivalent antimony with oxygen in the form of nitric acid or air. For the production of C.I. Pigment Brown 24, trivalent chromium raw materials are used.
Impurities[42]	The pigment is typically >99% w/w pure. Acid-soluble impurities are not fixed in the lattice (i.e., extractable with HCl). Acid-soluble impurities include antimony (Sb at 10 –20 ppm) and trivalent chromium (Cr(III) at 10–20 ppm). After 2 extractions with HCl, acid soluble impurities are reduced to amounts below the detection limit (<1 mg/kg for Sb and <1 mg/kg for Cr).
Other[39,42]	Mixed phase dye based on titanium dioxide (rutile) where chromium and antimony ions partially replace titanium ions in the lattice. According to the intended use, medium particle size varies between 0.5 and 1.5 µm. TSCA Definition 2008: An inorganic pigment that is the reaction product of high temperature calcination in which titanium (IV) oxide, chromium (III) oxide and antimony oxide in varying amounts are homogeneously and ionically interdiffused to form a crystalline matrix of rutile. Its composition may include any one or a combination of the modifiers Al_2O_3, MnO, NiO, WO_3, or ZnO. This substance is identified in the COLOR INDEX by Color Index Constitution Number, C.I. 77310.
Surrogates[42]	C.I. Pigment Yellow 53 (CAS No. 8007-18-9, a nickel antimony doped rutile)

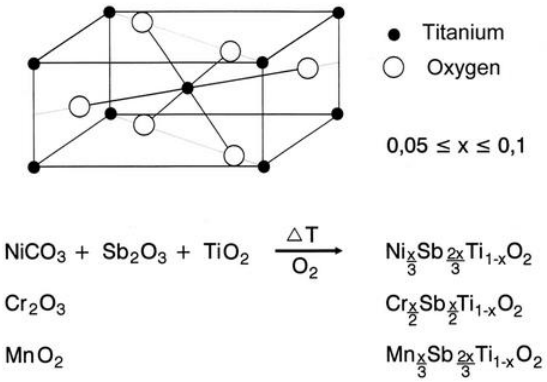


Figure I.1. Schematic of C.I. Pigment Brown 24 molecule (ECHA Registered Substances Database)

Section II. Hazard Identification

Health effect data, supporting studies, and secondary sources were reviewed, see tables in sections II.1, and II.2, for details.

Acute oral exposure doses, as high as 10,000 mg/kg bw, did not result in observable adverse effects[43].

A 90-day feeding study of rats dosed up to 500 mg/kg/day (NOAEL, highest dose tested) resulted in no adverse effects compared to controls. In a reproductive/developmental study, animals were exposed to the test material once daily on gestation days (6-19)/14 and doses of 0 (vehicle control), 100, 300 and 1000

[43] OECD SIDS 2002. C.I. Pigment Brown 24. CAS No.: 68186-90-3. SIDS Initial Assessment Report. http://webnet.oecd.org/Hpv/UI/handler.axd?id=d2341afe-af11-478c-aaed-ddce1e8b4ecf

mg/kg bw resulted in no adverse effect in the parental or offspring at the highest dose tested[44]. No mutagenic effects were observed in Ames assay studies in doses up to 5000 µg/plate. Repeat dose reproductive/ developmental toxicity data from rats resulted in no adverse effects in offspring exposed to doses as up to 500 mg/kg/d (NOAEL, highest dose tested)[45]. Toxicokinetic studies resulted in no adverse effects in rats[46].

There were no measurable effects on chromium content of liver and kidney at any dose level and exposure duration in rats.

II.1 Systemic, Genotoxicity, Carcinogenicity, Reproductive/Developmental Toxicity

ADVERSE HEALTH EFFECT STUDIES

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
Systemic Toxicity(subchronic)							
Bomhard E. et al., 1982	NR	Animal	Oral (Feeding)	500 mg/kg b.w./day **(NOAEL)**	90 day	No observable adverse effect reported	Similar to OECD guideline 408; Non-GLP
Test article: C.I. Pigment Brown 24. ***Dose:*** 0, 10, 100, 1000, 10000 mg per kg diet (corresponding to 0, 0.5, 5, 50, 500 mg/kg b.w./day) ***Treatment Duration:*** 90 days. ***Species (strain):*** Rat. (NR) **Gender:** Male and female. ***Number per Group:*** 15 per sex/ per dose. **Method:** Oral (Feeding). 15 animals per dose per gender were used for toxicological investigations (30 animals per gender in the control group) and additionally 10 animals per dose and gender for analytical investigations (control 20 animals per gender). Rats were observed daily; food consumption and body weight gain were determined once per week. Haematology, urinalysis and clinical and biochemical investigations were conducted after one month and at the end of the study (no post exposure observation period). Organ weights were determined at necropsy (thyroid gland, thymus, heart, lung, liver, spleen, kidneys, adrenal glands, and gonads). Complete histopathological investigations (above mentioned organs studied plus aorta, eyes, intestine, femur, brain, urinary bladder, pituitary, cervical lymph nodes, stomach, oesophagus, epididymides, pancreas, prostate, seminal vesicle, bone marrow of sternum, trachea, uterus, and skeletal muscles) were performed on 5 rats per gender of control and the high dose group. ***Adverse Effect(s) (brief):*** **NOAEL = 500 mg/kg b.w./day (highest dose tested).** No deaths, no overt signs of reactions to the treatment, no effects on body weight gain (similar food consumption in all groups) or organ weights, no treatment related findings from haematological or biochemical investigations and urinalysis were detected. No macroscopic pathological changes attributable to treatment and no treatment related effects in histopathology were observed. Since no substance related effects were observed up to the highest dose tested the NOEL and NO(A)EL of this study is defined to be 500 mg/kg body weight. The study followed good scientific principles as to experimental design and reporting and is therefore considered valid (validity 2). In a 90-day feeding study on rats doses up to 500 mg/kg/day resulted in no adverse effects in clinical observations, haematology, urine analysis, clinical chemistry and macro- and microscopical pathology. ***Data Quality:*** ECHA Reliability (ECHA) score of 2 (with restrictions). ***Reference***: *Bomhard, E., Loser, E., Dornemann, A., Schilde, B., 1982. Subchronic oral toxicity and analytical studies on nickel rutile yellow and chrome rutile yellow with rats. Toxicology Letters, 14, 189–194.*							

SUPPORTING STUDIES

None reported.

SECONDARY SOURCES

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
Systemic Toxicity (acute)							
OECD SIDS, 2002 (BASF AG, 1978)	NR	Animal	Oral	> 10,000 mg/kg bw **(LD_{50})**	Single oral administration	No observable adverse effect reported	Similar to OECD guideline 408; Non-GLP
Test article: C.I. pigment brown 24. ***Method:*** Oral. Rats received a single oral administration of the test substance, 10000 mg/kg bw, gavage in 10000 mg/kg bw, gavage. Following treatment rats were observed during the 14 days post observation period. The suspension							

[44] ECHA 2017_ Repro/Developmental:https://echa.europa.eu/registration-dossier/-/registered-dossier/15427/7/9/3
[45] Bomhard, E., Loser, E., Dornemann, A., Schilde, B., 1982. Subchronic oral toxicity and analytical studies on nickel rutile yellow and chrome rutile yellow with rats. Toxicology Letters, 14, 189–194.
[46] ECHA, 2017

was prepared in 0.5% carboxymethyl cellulose prior to application, stirred during application period. The volume administered was 31.6 ml/kg bw. Method: Rat body weight was measured prior to application and 7 and 14 d after dosing. The clinical symptoms were determined 15, 30 min and 1, 2, 4, 5 and 24 h after application, then daily. Post-exposure observation period was 14 days. The rats were sacrificed on day 14 and an autopsy was performed. ***(strain):*** Rat (Sprague Dawley). ***Gender;*** male and female. ***Number per Group:*** 10 per sex/ per dose. ***Adverse Effect(s) (brief):*** The test followed in principle the procedure described in OECD guideline 401 and is valid with restrictions to judge the acute oral toxicity (validity 2). No deaths occurred after administration and during the 14 days post observation period. No substance related effects were found on body weight development and no adverse effects were reported after necropsy. ***Data Quality:*** (4) not assignable; Data quality cannot be verified because information is from a secondary source. ***Reference:*** *OECD SIDS 2002. C.I. Pigment Brown 24. CAS No.: 68186-90-3. SIDS Initial Assessment Report. http://webnet.oecd.org/Hpv/UI/handler.axd?id=d2341afe-af11-478c-aaed-ddce1e8b4ecf*

OECD SISD, 2002	NR	Animal	Oral	> 10,000 mg/kg bw **(LD_{50})**	Single oral administration	Lethality	NR

Test article: C.I. pigment brown 24. ***(strain):*** Rat (NR). ***Method:*** Inhalation. For enrichment of the atmosphere 200 l air per h conducted through a layer of the product (height 5 cm); test at room temperature. Rats sacrificed. ***Gender:*** *NR*. ***Number per Group:*** 10 per sex/ per dose. ***Adverse Effect(s) (brief):*** No mortality during the exposure period, no symptoms of poisoning; autopsy revealed no macroscopical effects. ***Data Quality:*** (4) not assignable; Data quality cannot be verified because information is from a secondary source. ***Reference:*** *OECD SIDS 2002. C.I. Pigment Brown 24. CAS No.: 68186-90-3. SIDS Initial Assessment Report. http://webnet.oecd.org/Hpv/UI/handler.axd?id=d2341afe-af11-478c-aaed-ddce1e8b4ecf*

Genotoxicity

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
ECHA, 1995	ND	Bacteria	In vitro	5000 µg/plate.	Once	No effect	Similar to OECD 471; GLP

Test article: Chrome antimony titanium buff rutile; ***Dose:*** 100, 250, 500, 1000, 2500 and 5000 µg/plate ***Treatment Duration:*** NR ***Species (strain):*** S. typhimurium TA98, 100, 1535, 1537, 1538 and E. coli WP2uvrA ***Gender:*** ND. ***Number per Group:*** ND ***Method:*** Ames Assay ***Adverse Effect(s) (brief):*** The results were negative for mutagenic activity with and without metabolic activation in S. typhimurium ***Data Quality:*** ECHA Reliability 2 (with restrictions).

ECHA, 2017	ND	Animal	Dermal	10000 µg/plate.	3 consecutive applications	No effect	OECD 429; GLP

Test article: Chrome antimony titanium buff rutile; ***Dose:*** 0.3125, 0.6250, 1.2500, 2.5000, 5.0000, 10.0000 and 15.0000 µg/mL (24 h exposure, 24 h harvest time, -/+S9) ***Treatment Duration:*** NR ***Species (strain):*** Chinese hamster lung fibroblasts (V79) ***Age: ND***. ***Gender:*** ND. ***Number per Group:*** ND ***Method:*** Mixed Population Method ***Adverse Effect(s) (brief):*** Negative for genotoxicity in Chinese hamster lung fibroblasts (V79) cells with and without metabolic activation. ***Data Quality:*** ECHA Reliability 1 (with restrictions).

OECD SIDS, 2002	ND	Bacteria	In vitro	>= 5000 ug/plate	NR	No effect	NR; GLP

Test article: C.I. Pigment Brown 24 96.5 %. Bacteria was dosed at >= 5000 ug/plate. ***Dose:*** NR ***Treatment Duration:*** NR ***(strain):*** Salmonella typhimurium TA 1535, TA 100, TA 1537, TA 98 ***Gender:*** NA. ***Number per Group:*** NR. ***Methods:*** Ames Assay. **Adverse *Effect(s) (brief):*** Negative for genotoxicity in Salmonella typhimurium with and without metabolic activation. ***Data Quality:*** (4) Data quality cannot be verified because information is from a secondary source ***Reference:*** *OECD SIDS 2002. C.I. Pigment Brown 24. CAS No.: 68186-90-3. SIDS Initial Assessment Report. http://webnet.oecd.org/Hpv/UI/handler.axd?id=d2341afe-af11-478c-aaed-ddce1e8b4ecf*

OECD SIDS, 2002	ND	Animal cells	In vitro	100 µg/ml	NR	No effect	Similar to OECD 476; GLP

Test article: C.I. Pigment Brown 24 96.5 %. ***Dose:*** 0, 3.13, 6.25, 12.5, 25, 50, 100 µg/ml in DMSO ***Treatment Duration:*** NR ***(strain):*** L5178Y mouse lymphoma cells ***Gender:*** NA. ***Number per Group:*** NR. ***Method:*** Mouse lymphoma assay ***Adverse Effect(s) (brief):*** No cytotoxicity at any concentration tested. Results were negative in L5178Y mouse lymphoma cells with and without metabolic activation. ***Data Quality:*** (2) valid with restrictions. Data quality cannot be verified because information is from a secondary source; ***Reference:*** *OECD SIDS 2002. C.I. Pigment Brown 24. CAS No.: 68186-90-3. SIDS Initial Assessment Report. http://webnet.oecd.org/Hpv/UI/handler.axd?id=d2341afe-af11-478c-aaed-ddce1e8b4ecf*

OECD SIDS, 2002	ND	Bacteria	In vitro	>= 5000 ug/plate	NR	No effect	Similar to OECD 471; GLP

Test article: Chrome Antimony Titanate. ***Dose:*** 0, 100, 250, 500, 1000, 2500, 5000 µg/plate ***Treatment Duration:*** NR ***Species*** (strain): Salmonella typhimurium TA 1535, TA 100, TA 1537, TA 98 ***Gender:*** NA. ***Number per Group:*** NR. ***Method:*** Ames assay. ***Adverse Effect(s) (brief):*** NOAEL >= 5000 ug/plate. Negative for genotoxicity in Salmonella typhimurium with and without metabolic activation. Data Quality: No 2nd independent trial. Data quality cannot be verified because information is from a secondary source ***Note:*** Critical study for SIDS endpoint ***Reference:*** *OECD SIDS 2002. C.I. Pigment Brown 24. CAS No.: 68186-90-3. SIDS Initial Assessment Report. http://webnet.oecd.org/Hpv/UI/handler.axd?id=d2341afe-af11-478c-aaed-ddce1e8b4ecf*

OECD SIDS, 2002	ND	Animal cells	In vitro	25 µg/ml	NR	No effect	Similar to OECD 476; GLP
Test article: Pigment Brown 24 obtained from BASF AG, purity 99.4%. ***Dose:*** 0, or 0.78-25 µg/ml were administered DMSO used as vehicle. ***Treatment Duration:*** NR ***Method:*** Micronucleus test in vitro ***Species(strain):*** L5178Y mouse lymphoma cells ***Gender:*** NA. ***Number per Group:*** NR. ***Adverse Effect(s) (brief):*** No cytotoxicity at any concentration tested. Results were non-clastogenic in L5178Y mouse lymphoma cells with and without metabolic activation. ***Data Quality:*** (1) valid without restriction. Data quality cannot be verified because information is from a secondary source; ***Note***: Critical study for SIDS endpoint ***Reference:*** *OECD SIDS 2002. C.I. Pigment Brown 24. CAS No.: 68186-90-3. SIDS Initial Assessment Report. http://webnet.oecd.org/Hpv/UI/handler.axd?id=d2341afe-af11-478c-aaed-ddce1e8b4ecf*							
Reproductive/ Developmental Toxicity							
ECHA, 2017	Animal	Oral (gavage)	1000 mg/kg bw **(NOAEL)**	Once daily (GD 6-19)/14	NR	No observable adverse effect reported	OECD 414; GLP
Test article: chrome antimony titanium buff rutile ***Dose:*** *0 (*vehicle; control), 100, 300 and 1000 mg/kg bw in CMC (carboxymethyl cellulose) ***Treatment Duration:*** Once daily (GD 6-19)/14. ***Species (strain):*** Rat. (Wistar) 11-13 weeks. **Gender:** Male and female. ***Number per Group:*** 25 per sex/ per dose. **Method:** On GD 20, all surviving dams will be sacrificed and examined. During the administration period (GD 6 - 19) all animals will be checked daily for any abnormal clinically signs before the administration as well as within 2 hours and within 5 hours after the administration. Food consumption, body weights were recorded. The ovaries and uterine content were examined after termination (Gravid uterus weight, Number of corpora lutea, Number of implantations, Number of resorptions). Examination of the fetus included external examinations, soft tissue examinations, and skeletal examinations. ***Adverse Effect(s) (brief):*** **NOAEL=1000 mg/kg bw.** Yellowish discolored feces were recorded for all females of the high-dose group (1000 mg/kg/d) from GD 14 onwards until terminal sacrifice (GD 20). This feces discoloration mirrors the presence of the test substance (or its metabolites) in the gastrointestinal tract. It is not considered as an adverse toxic effect. The mean body weights and average body weight gain of the low-, mid- and high-dose dams (100, 300 or 1000 mg/kg/d) were in general comparable to the concurrent control group throughout the entire study period. This includes the slightly higher weight gain of the mid-dose dams on GD 19-20. The mean gravid uterus weights of the animals of test groups 1-3 (100, 300 and 1000 mg/kg/d) were not influenced by the test substance. The differences between these groups and the control group revealed no dose-dependency and were assessed to be without biological relevance. A yellow discolored content of the stomach was recorded in 9 out of 25 mid-dose females (36%) and in 14 high-dose females (56%), while a yellow discolored content of the small intestine was seen in 2 mid-dose (8%) and 5 high-dose females (20%). These yellow discolorations mirror presence of the test substance (or its metabolites) in the gastrointestinal tract. They are not considered as adverse, toxic effects by themselves. There were lower numbers of (early) resorptions than control in all treatment groups, producing higher live litter sizes in return. The conception rate reached 88% in the control and the mid-dose groups (0 and 300 mg/kg/d) and 100% in the low- and high-dose groups (100 and 1000 mg/kg/d). With these rates, enough pregnant females were available for the purpose of the study. There was no test substance-related and/or biologically relevant differences between test groups 0-3 in conception rate, in the mean number of corpora lutea and implantation sites or in the values calculated for the pre- and the post implantation losses, the number of resorptions and viable fetuses. severity). External malformations were detected in test groups 1 and 2 (100 and 300 mg/kg/d). In each case, these external malformations were associated with either soft tissue or skeletal malformations. None of these malformations are related to the treatment. The total incidence of external malformations in treated animals did not differ significantly from the concurrent control group and was covered by the historical control data. The rate of affected fetuses per litter with skeletal variations was statistically significantly higher in the low- and mid-dose groups (100 and 300 mg/kg/d, however, the incidences of all test groups were well within the historical control range and the variations neither showed a specific pattern nor a dose-response. The rate of affected fetuses per litter with skeletal variations was statistically significantly higher in the low- and mid-dose groups (100 and 300 mg/kg/d, however, the incidences of all test groups were well within the historical control range and the variations neither showed a specific pattern nor a dose-response. ***Data Quality:*** ECHA Reliability 1 (with restrictions).							

II.2 Other Health Effects

Pigment	Endpoint	Outcome	Reference
Irritation (dermal)			
PB24	Irritation (dermal)	Irritation	ECHA 1978c; non-GLP
Test article: Chrome antimony titanium buff rutile. Characterized as TiO_2 79,9 %, Sb_2O_5 13.4 %, Cr_2O_3 5.2 %, SiO_2 1.5 % (cross-contamination with material from ball mill) including 40 ppm As, 140 ppm Pb, 7 ppm Cu, 40 ppm Zn, and 2 ppm Ni. ***Dose:*** NR ***Treatment Duration:*** NR ***Species (strain):*** rabbit (Vienna White). ***Gender:*** Male/female. ***Number per Group:*** 6 (2 males, 4 females). ***Dose:*** 50% ***Adverse Effect(s) (brief):*** (Intact skin) Evaluation of erythema after 24 and 72 h not possible due to treatment related staining of the skin (no histological examination of the epidermis in full thickness has been conducted, thus conclusion of dermal penetration of the test substance cannot be drawn from this study. In another study, however, it was reported that the staining of the skin could be removed by washing with soap, thereby corroborating that the staining was only superficial; no erythema after 8 d. Slight edema in 3/6 animals after 24 h, no edema after 72 h or 8 d. (Abraded skin) Evaluation of erythema impossible after 24 and 72 h due to coloring of skin, no erythema after 8 d but scaling in 3/6 rabbits. Edema in 4/6 rabbits after 24 h, no edema after 72 h or 8 d. ***Data Quality:*** The assessment of the findings was based on the Federal Reg. 38, No. 187, Para. 1500.41, page 27029 (1973).			

Irritation (eye)			
PB24	Irritation (eye)	Irritating	ECHA 1987
Test article: Chrome antimony titanium buff rutile (solid Batch No.: 41635-12108). ***Dose:*** NR ***Treatment Duration:*** NR ***Species (strain):*** Rabbit (New Zealand White). ***Gender:*** (gender not reported). ***Number per Group:*** 3. ***Dose:*** 100mg. ***Frequency/Duration:*** Observed for 7 days. ***Adverse Effect(s) (brief):*** Cornea opacity, conjuctivae, chemosis and iris score revealed no effects. ***Data Quality:*** Data quality cannot be verified because information is from a secondary source; however, secondary source (ECHA) has assigned a reliability (Klimisch) score of 4 (not assignable) due to documentation insufficient for assessment. ***Notes:*** Equivalent or similar to US EPA §163.81-5, Federal Register 43, 163, 22. Aug. 1978. No GLP compliance.			

Sensitization			
Pigment	**Endpoint**	**Outcome**	**Reference**
PB24	Skin Sensitization	No observable adverse effects reported	ECHA, 2017
Test article: C.I. pigment brown 24, characterized as TiO_2 79,9 %, Sb_2O_5 13.4 %, Cr_2O_3 5.2 %, SiO_2 1.5 % (cross-contamination with material from ball mill) including 40 ppm As, 140 ppm Pb, 7 ppm Cu, 40 ppm Zn, and 2 ppm Ni. ***Dose:*** NR ***Treatment Duration:*** NR ***Species (strain):*** Rabbit Vienna White ***Gender:*** males/ females. ***Number per Group:*** 6. ***Dose:*** 50 % ***Adverse Effect(s) (brief):*** No effects on cornea and iris. After 24 h, clear reddening of conjunctivae in 1/6 rabbits, slight reddening in 5/6; slight secretion in 4/6 rabbits. After 48 h, slight reddening of conjunctivae in 5/6 of animals; one rabbit without symptoms; slight secretion in 1/6 rabbits. After 72 h, three of six animals without symptoms; slight reddening of conjunctivae in 3/6 rabbits, no secretion. No substance related staining has been observed. The result indicates a mechanically mediated slight, reversible irritation of the mucous membrane due to the instillation of test substance particles into the eye. ***Data Quality:*** Data quality cannot be verified because information is from a secondary source; however, secondary source (ECHA) has assigned a reliability (Klimisch) score of 3 (not reliable) due to significant methodological deficiencies (observation period too short). ***Notes:*** Before OECD Guideline 405 was established, eye irritation was tested according to Federal Register 38, No. 187, § 1500.42, S. 27019, 27. Sept. 1973			

Toxicokinetics, Metabolism and Distribution			
PB24	Oral	Irritation	OECD SIDS, 2002
Test article: technical grade chrome rutile yellow (C.I. pigment brown 24); ***Dose:*** Characterized on a molar base as (Ti0.94 Sb0.03 Cr0.03)O2 and as 85% TiO2, 10% Sb2O5, 5% Cr2O3 on weight% base. Rats were dosed at, males: 10, 100, 1000, 10000 mg/kg diet (0.5, 5, 50, 500 mg/kg/day) Doses, females: 10, 100, 1000, 10000 mg/kg diet (0.5, 5, 50, 500 mg/kg/day). ***Treatment Duration:*** NR ***Species (strain):*** Rat. ***Age:*** 4-5 weeks. ***Gender:*** Male/ Female ***Number per Group:*** 5/per sex per dose / concentration. ***Method:*** Rats observed daily; food consumption and body weight gain determined once per week. Haematological, clinical and biochemical investigations: RBC, reticulocytes, platelets, haemoglobin, haematocrit, total and differential WBC, MCV, ALP, GOT, GPT, creatinine, urea, glucose, cholesterol, total plasma proteins and urine proteins, urinalysis conducted after one month and at the end of the study on 5 males and 5 females of each group; in addition, thromboplastin time and glutamate dehydrogenase activity measured after three months. Organ weight determined for thyroid gland, thymus, heart, lung, liver, spleen, kidneys, adrenal glands, and gonads and histopathology performed together with aorta, eyes, intestine, femur, brain, urinary bladder, pituitary, cervical lymph nodes, stomach, oesophagus, epididymides, pancreas, prostate, seminal vesicle, bone marrow of sternum, trachea, uterus, skeletal muscles from 5 animals per gender of control and top dose group. After 1-, 2- and 3-months liver and kidneys from 5 animals per gender and dose group analyzed for their chromium and antimony contents by AAS. ***Adverse Effect(s) (brief):*** No deaths, no overt signs of reactions to the treatment, no effects on body weight gain (similar food consumption in all groups) or organ weight, no treatment related findings from haematological or biochemical investigations and urinalysis. No macroscopic pathological changes attributable to treatment. No treatment related effects observed in histopathology. In males and females the Sb concentrations in liver and kidney were below the detection limit at doses up to 1000 ppm. In the high dose groups the Sb levels slightly increased with exposure duration and reached max. 27 ppb in the liver (3 mo) of males (range 15-40 ppb) and 17 ppb in females (kidney 14 ppb in males and 15 ppb in females). Chromium No measurable effect on chromium content of liver and kidney at any dose level and exposure duration. ***Data Quality:*** ECHA Reliability 2 (reliable with restrictions). ***Note***: Critical study for SIDS endpoint ***Reference:*** *OECD SIDS 2002. C.I. Pigment Brown 24. CAS No.: 68186-90-3. SIDS Initial Assessment Report. http://webnet.oecd.org/Hpv/UI/handler.axd?id=d2341afe-af11-478c-aaed-ddce1e8b4ecfst article*			
PB24	Oral	No Observable adverse effect	ECHA, 1982
Test article: Chrome antimony titanium buff rutile; Batch No.: Pt 8817; - Composition of test material (percentage of components, weight based): TiO2 85%, Sb2O5 10%, Cr2O3 5% ***Dose:*** NR ***Treatment Duration:*** NR ***Species (strain):*** Rat. ***Age: Gender:*** Male/ Female. 4-5 weeks. ***Number per Group:*** 5/per sex per dose / concentration ***Adverse Effect(s) (brief):*** In males and females the Sb concentrations in liver and kidney were below the detection limit at doses up to 1000 ppm. In the high dose groups the Sb levels slightly increased with exposure duration and reached max. 27 ppb in the liver (3 mo) of males (range 15-40 ppb) and 17 ppb in females (kidney 14 ppb in males and 15 ppb in females). Chromium: No measurable effect on chromium content of liver and kidney at any dose level and exposure duration. ***Data Quality:*** ECHA Reliability 2 (reliable with restrictions)			

Section III. Dose-Response Assessment

III.1 Derivation of Provisional Tolerable Intake

Contact Duration	Exposure Route	Patient	POD	MF	pTI
Permanent	Parenteral	All	500 mg/kg/d	1,000	0.5 mg/kg/d

pTI = POD ÷ MF

Note: When a supplier-specific color additive safety data sheet (SDS) does not report the presence of a carcinogen or non-carcinogen impurity, and SDS complies with Globally Harmonized System (GHS) hazard communication limits (i.e., <0.1% and <1%, respectively), then this pTI is low enough to be protective for naturally occurring impurities which could be present in the color additive. Processes used to color a polymer system involve diluting the pigment and its impurities by at least 50x (i.e., the CHRIS calculator is valid when the maximum concentration of pigment in a colored polymer is 2%, i.e., 1 ÷ 2% = 50); therefore, the concentration of naturally occurring impurities in the finished colored polymer will be significantly lower compared to the concentration of these substances in the pigment (i.e., 0.002% (i.e., 0.1% ÷ 50) for carcinogens and 0.02% (i.e., 1% ÷ 50) for non-carcinogens). Unknown impurities are addressed by the CHRIS calculator.

III.2 Derivation of a Modifying Factor

Critical Study	Biological Endpoint	Exposure Route	POD Type	POD Value	UF_1	UF_2	UF_3	MF
Bomhard et al. 1982	Histopathological analysis of organs	Oral	NOAEL	500 mg/kg/d	10	10	10	1,000

MF = modifying factor (UF_1 x UF_2 x UF_3)

III.3 Toxicological Uncertainties Applied to POD

Uncertainty	Justification
UF_1 Error! Bookmark not defined.	(10) Default value to account for susceptible individuals.
UF_2 Error! Bookmark not defined.	(10) Default value to account for differences between species.
UF_3 Error! Bookmark not defined.	(10) Default value to account for differences in exposure route and data quality. Bioavailability of PB24 is not expected because of the extremely low water solubility, rodent study data and structure-related inert properties of the rutil.

UF_1, UF_2, and UF_3, are used to extrapolate the POD to the general human population (including pediatric) exposed parenterally for a lifetime.

III.4 Critical Health Study Design and Outcome

Bomhard, E. et al. (1982) Subchronic oral toxicity and analytical studies on nickel rutile yellow and chrome rutile yellow with rats. Toxicology Letters, 14, 189–194.

Test Substance	Chrome rutile yellow*		
Species	Rats (SPF-derived Wistar TNO W74)	Frequency	daily ad libitum
Gender	Male/Female	Duration	90 days
Age	4-5 weeks old	#/Group	15/sex/dose
Route	Oral (feed)	Protocol Guideline	See Notes
Dose(s)	0, 0.5, 5, 50, 500 mg/kg/day	Statistical Method(s)	None
Observed Responses	Toxicity: No changes in general condition, body weight gain or food consumption were detected in any of the groups. No adverse effect found for haematologica1 and measured serum/urinary biochemical parameters. No pathological anatomical changes were detected attributable to treatment. No effect on organ weights including thyroid, thymus, heart, lung, liver, spleen, kidneys, adrenals or gonads were reported. No change in macro- and micro-histopathological of the gonads (*i.e.*, testes, epididymis, prostate, seminal vesicle, ovary and uterus). Chemical analysis: (10 animals per sex per dose and 20 per sex for controls were used. After 1, 2, and 3 months, liver and kidneys from 5 animals per sex per dose were analyzed for chromium (Cr) and antimony (Sb) levels). In males and females, the Sb concentrations in liver and kidney were below the detection limit at doses up to 500 mg/kg/day. In the high dose groups the Sb		

	levels slightly increased with exposure duration and reached max. 27 ppb in the liver (3 mo) of males (range 15-40 ppb) and 17 ppb in females (kidney 14 ppb in males and 15 ppb in females). No measurable effect of Cr content on liver and kidney at any dose level and exposure duration. Neither the pigments nor the bioavailable traces of metals are considered to have toxicological significance even after extremely high oral exposure. C.I. Pigment Brown 24 did not induce reproductive toxicity. NOAEL = 500 mg/kg/day. Data quality: Reference is a peer-reviewed, health effect *in vivo* toxicity study performed under methods comparable to OECD guidelines 408. ECHA has assigned a reliability (Klimisch) score of 2 (with restrictions) to this study.
Notes:	Comparable to OECD guideline 408. Doses were reported as 1, 10, 100, 1,000 and 10,000 ppm and was converted into a dose from a feed concentration by a conversion factor of 0.05 (EFSA 2011)

C.I. Pigment Red 101

CAS # 1309-37-1

Toxicological Profile Summary

C.I. Pigment Red 101 (a.k.a., Iron oxide or Ferric oxide, CAS # 1309-37-1) is a red to brown solid with no distinct odor. It is an inorganic compound found naturally as hematite ore. The main use of C.I. Pigment Red 101 is a pigment, though it is also used in metallurgy, gas purification, magnetic tapes, dental abrasives, and polishing agents. Iron absorption from this water insoluble iron oxide is low. Reported hazard information includes an adverse health effect study, supporting studies, and secondary sources. C.I. Pigment Red 101, administered at high oral doses (up to 1000 mg/kg) to rodents, is reported to not elicit systemic toxicity. The calculated provisional tolerable intake (pTI) value for parenteral systemic toxicity is 1 mg/kg/day based on the lowest reported point-of-departure (1000 mg/kg) with a modifying factor of 1,000 to address the following sources of uncertainty: variation among humans (10), variation between species (10), and data quality/route-to-route extrapolation (10).

NOTE: This pTI is protective for the color additive including any impurities listed in Section I.1 "Substance Identity," as the impurities likely will be present at a very low percentage of the color additive, and the quantity of the impurity will be at an even lower percentage in the final device (see Section III, Table III.1 footnote). Unknown impurities are addressed by the CHRIS calculator.

Section I. Substance Information

I.1 Substance Identity

	Descriptor
Synonyms[47]	C.I. Pigment Red 101 (Iron oxide, Ferric oxide)
Formula[47]	Fe_2O_3
Molecular Weight[47]	159.69 g/mol
Physical Form[47]	Solid
Type[47]	An inorganic pigment that is the reaction product of high temperature calcination in which titanium (IV) oxide, chromium (III) oxide and antimony oxide in varying amounts are homogeneously and ionically interdiffused to form a crystalline matrix of rutile.
Physical/Chemical Characteristics[47]	C.I. Pigment Red 101 (Iron oxide, Ferric oxide) is a red to brown solid with no distinct odor. It is soluble in acids and insoluble in water, alcohol, and ether. C.I. Pigment Red 101 has a melting point of 1539°C, and no boiling point has been reported. It has a density of 5.25 g/cm^3, and is 6.5 on the Mohs' hardness scale.
Other[47]	Mixed phase dye based on titanium dioxide (rutile) where chromium & antimony ions partially replace titanium ions in the lattice. Its composition may include any one or a combination of the modifiers Al2O3, MnO, NiO, WO3, or ZnO. This substance is identified in the Colour Index™ by Colour Index™ Constitution Number, C.I. 77310.

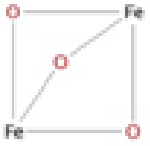


Figure I.1. Schematic of C.I. Pigment Red 101 molecule

Section II. Hazard Identification

Health effect data, supporting studies, and secondary sources were reviewed, see tables in sections II.1, and II.2, for details.

Acute exposure to C.I. Pigment Red 101 (Iron oxide, Ferric oxide) orally resulted in no treatment-related mortality or no clinical signs of toxicity in rats.

Inhalation exposure of rats to 30 mg/m³ on 5 consecutive days did not cause any observable adverse effect in the respiratory tract up to the 28-day observation period, which was examined by broncho-alveolar lavage and histopathology[48]. Human lungs repeatedly exposed to particles developed transient acute inflammation of the lungs which resolved after day 4 post-exposure[49]. An inhalation study in rodent reported similar results, which included pulmonary inflammation in rats administered a dose of 1 mg/m² of lung surface[49].

47 ChemID [WWW Document] https://chem.nlm.nih.gov/chemidplus/rn/1309-37-1

[48] Hofmann, Thomas, Lan Ma-Hock, Volker Strauss, Silke Treumann, Maria Rey Moreno, Nicole Neubauer, Wendel Wohlleben, et al. "Comparative Short-Term Inhalation Toxicity of Five Organic Diketopyrrolopyrrole Pigments and Two Inorganic Iron-Oxide-Based Pigments." Inhalation Toxicology 28, no. 10 (August 23, 2016): 463–79. https://doi.org/10.1080/08958378.2016.1200698

[49] Lay, J., Bennett, W., Ghio, A., Bromberg, P., Costa, D., Kim, C., Koren, H., & Delvin, R. (1999). "Cellular and biomedical response of the human lung after intrapulmonary instillation of ferric oxide particles." Am. J. Respir. Cell Mol. Biol. 20(4): 631-642.

In a comet assay, when A549 cells were exposed to 40 μg/cm² micrometer particles for 4 h, a small increase in DNA damage is reported. However, due to the low response level and absence of dose-dependency data, this reported result is inconclusive.[50].

Carcinogenetic potential was examined in a human cohort study, in which no observable relationship between occupational exposure to the color addtive and lung cancer mortality is reported.[51]. Similarly, in a 798-day rodent cancer study, doses up to 40 mg/kg/day resulted in no signs of carcinogenicity in rats.[52].

II.1 Systemic, Genotoxicity, Carcinogenicity, Reproductive/Developmental Toxicity

ADVERSE HEALTH EFFECT STUDIES

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
Systemic Toxicity (acute, subacute)							
Lay 1999	ND	Human	Inhalation	3.2 particles per AM (**LOAEL**)	Two exposures	Acute inflammation	NR
Test article: Ferric oxide (particles). ***Dose:*** A total of 10 ml SPSS containing 3 X 10^8 Fe_2O_3 microspheres was slowly instilled through the catheter coincident with inspirations to maximize aspiration of particles into the alveolar region. The authors calculated that instillation of 3X10^8 particles into a lingular subsegment should result in a concentration of about 3.2 particles per AM within that region. This was followed by an additional 10 ml SPSS from a different syringe (for a total of 20 ml) with the intent of washing particles remaining in airways into the alveoli. A total of 20 ml SPSS (without particles) was instilled, as described, into the medial segment of the right middle lung lobe to serve as a control. ***Treatment Duration:*** Two installations followed by a specified time 1, 2, 4, 28, or 91 d BAL recovery from the instillation sites. ***Species (strain):*** Human ***Gender:*** Male/female ***Number per Group:*** 34 healthy volunteers (27 male, 7 female) (19.6 to 35.5 yr of age). ***Methods:*** Inhalation (bronchopulmonary installation). 34 healthy non-smoking subjects were randomly assigned to one of five groups of six subjects each (except that the group studied 1 d after exposure included 10 subjects). Each subject underwent two bronchoscopy procedures. During the first bronchoscopy, Fe2O3 microspheres suspended in nonpyrogenic, sterile physiologic saline solution (SPSS) was instilled into an identified subsegment of the lingula that could readily be wedged by advancing the bronchoscope. As a control, SPSS (without particles) was instilled into a segment (medial or lateral) of the right middle lobe. Subsequently, during the second bronchoscopy procedure, particles, cells, and soluble materials were recovered from the instillation sites by BAL at a specified time PI (1, 2, 4, 28, or 91 d). Each subject was thus lavaged only once, and none underwent serial lavages. ***Adverse Effect(s) (brief):*** **LOAEL**=3.2 particles per AM. (3 X 10^8 Fe_2O_3 microspheres)**.** Human subjects were exposed to particles of ferric oxide via bronchopulmonary installation. The Fe_2O_3 particles resulted in significantly greater oxidant-generating capacity than that of saline or the commercial Fe_2O_3 samples examined. Lung cells and lung airspaces were examined for 91 days after exposure during which time acute inflammation was observed from days 1 through 4. The inflammation characterized by increased numbers of neutrophils and alveolar macrophages as well as increased amounts of protein, lactate dehydrogenase, and interleukin-8 in BAL fluids. This inflammation was transient, resolving after day 4 post-exposure. ***Data Quality:*** *NR* ***Reference:*** *Lay, J., Bennett, W., Ghio, A., Bromberg, P., Costa, D., Kim, C., Koren, H., & Delvin, R. (1999). "Cellular and biomedical response of the human lung after intrapulmonary instillation of ferric oxide particles." Am. J. Respir. Cell Mol. Biol. 20(4): 631-642.*							
Lay 1999	ND	Animal	Inhalation	1 mg/m² lung surface (**LOAEL**)	24hrs	Acute inflammation	NR
Test article: Ferric oxide (particles). ***Dose:*** Experiment one: 0-0.16-1.61-4.83 mg/animal (0-0.188-1.88-5.63 mg/m² lung surface). Experiment two: 1 mg; Controls were treated with saline. ***Treatment Duration: Species (strain):*** Rats (fischer 344) ***Gender:*** Male ***Number per Group:*** 4/ per group. ***Methods:*** First experiment: dose finding study spherical particles were instilled intratracheally Animals: 5 male fischer 344 rats per group 24 h after instillation rats were killed and lungs lavaged. Cells were isolated and differentially counted. Protein and LDH activity were measured. Second experiment: Comparison of several preparations two commercial preparations were tested in comparison to the spherical particles used in the first experiment. Particle sizes: 0.2 μm (1-5μm) Animals: 4 groups of 4 male Fischer 344 rats Dose: 1 mg in 0.5 ml sterile saline 24 h after instillation rats were killed and lungs lavaged. Cells were isolated and differentially (neutrophils) counted. Protein was measured in the liquid. ***Adverse Effect(s) (brief):*** First experiment: The total number of cells was markedly increased in mid and high dose animals, but not in the low dose. The increase was due almost exclusively to the influx of neutrophils. This influx was accompanied by an increase of protein and LDH. Second							

[50] Karlsson, Hanna L., Johanna Gustafsson, Pontus Cronholm, and Lennart Möller. "Size-Dependent Toxicity of Metal Oxide Particles—A Comparison between Nano- and Micrometer Size." Toxicology Letters 188, no. 2 (July 24, 2009): 112–18. https://doi.org/10.1016/j.toxlet.2009.03.014.

[51] Bourgkard E, Wild P, Courcot B, Diss M, Ettlinger J, Goutet P, Hémon D, Marquis N, Mur JM, Rigal C, Rohn-Janssens MP, Moulin JJ. Lung cancer mortality and iron oxide exposure in a French steel-producing factory. Occup Environ Med. 2009 Mar;66(3):175-81. doi: 10.1136/oem.2007.038299. Epub 2008 Sep 19. PMID: 18805881.

[52] Steinhoff, D., U. Mohr, and S. Hahnemann. "Carcinogenesis Studies with Iron Oxides." Exp. Pathol 43 (1991): 189–94.

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
experiment: The spherical particles used in the other experiment and in humans induced significantly greater effects than both commercial preparations: Protein: commercial preparations produced only minimal increases, while the spherical particles increased the concentration by a factor of ~1.7. Neutrophil influx: commercial preparations produced only minimal changes ~2% of cells, similar to control, while the spherical particles increased the concentration by a factor of ~20 (~40% of cells) compared to controls. ***Data Quality:*** *NR* ***Note:*** Alveolar surface area estimated to be 0.41 m2 for a 250-g rat based ***Reference*****:** *Lay, J., Bennett, W., Ghio, A., Bromberg, P., Costa, D., Kim, C., Koren, H., & Delvin, R. (1999). "Cellular and biomedical response of the human lung after intrapulmonary instillation of ferric oxide particles." Am. J. Respir. Cell Mol. Biol. 20(4): 631-642.*							
Systemic Toxicity (subacute, subchronic)							
Kumari, 2012	ND	Rat	Oral	1000 mg/kg/day (**NOAEL)**	28-days	No observable adverse effect reported	NR
Test article: Fe_2O_3-Bulk (<5000nm) ***Dose:*** 0, 30, 300 or 1000 mg/kg/day ***Treatment Duration:*** for 28 days. ***Species (strain):*** Rat. (Wistar) (8 weeks) ***Gender:*** female (120-150g) ***Number per Group:*** 10/ per dose. ***Control:*** untreated 10. ***Method:*** In a subacute repeated dose oral toxicity study complying with OECD TG 408 (OECD, 1998), groups of ten female Wistar rats received by gavage 0 (controls), 30, 300 or 1 000 mg/kg/day of micro sized red iron oxide (Fe_2O_3-Bulk). At the end of the treatment period, animals were sacrificed, serum and organs were collected. Biochemical enzymes were measured in erythrocytes, serum, brain, liver and kidney of rats. Histopathology was carried out in the liver, kidney, spleen, heart and brain of all animals. ***Adverse Effect(s) (brief):*** **NOAEL=1000 mg/kg/day.** No decrease in body weight, no change in feed intake, nor any adverse sign, symptoms or mortality were observed in rats exposed to any of the micro sized red iron oxide doses. Microsized red iron oxide did not induce any adverse effects in either biochemical parameters or histopathology in the treated rats, with the exception of a significant decrease (25 %) in lactate dehydrogenase activity in the kidneys of rats given the highest dose. However, the Panel noted that no significant change in plasma lactate dehydrogenase activity was observed in this group of animals and therefore did not consider the decreased lactate dehydrogenase activity in the kidney adverse. The results indicate that the no-observed-adverse effect (NOAEL) for microsized red iron oxide of 1 000 mg/kg/day, the highest dose tested. ***Data Quality:*** *NR* ***Reference:*** *Kumari, Monika. "Repeated Oral Dose Toxicity of Iron Oxide Nanoparticles: Biochemical and Histopathological Alterations in Different Tissues of Rats." Journal of Nanoscience and Nanotechnology 12 (2012): 2149–59.*							
Pauluhn, J. (2012)	ND	Animal	Inhalation	4.7 mg m^{-3} (**NOAEL)**and 4.4 mg m^{-3} **(BMCL)**	13-week	pulmonary inflammation; increased lung and lung-associated-lymph node (LALN) weights; Elevations of neutrophils	OECD 413
Test article: C.I. Pigment Red 101. ***Dose:*** actual concentrations of 0, 4.7, 16.6 and 52.1 mg m(-3) (mass median aerodynamic diameter ≈1.3 µm, geometric standard deviation = 2) in air. ***Treatment Duration:*** Animals were exposed 6 h per day, five days per week for 13 consecutive weeks ***Species (strain):*** Rats (Wistar) ***Gender:*** Male/ female. ***Number per Group:*** 10/ per sex/ per group. ***Methods:*** Inhalation (nose only). Rats were nose-only exposed to pigment-sized iron oxide dust (Fe_3O_4, magnetite) in a subchronic 13-week inhalation study according to the OECD testing guidelines TG#413 and GD#39. Groups of at least 10 male and 10 female rodents are exposed 6 hours per day for 90 days to a) the test chemical at three or more concentration levels, b) filtered air (negative control), and/or c) the vehicle (vehicle control). Animals are generally exposed 5 days per week. The results of the study include measurement and daily and detailed observations (haematology and clinical chemistry), as well as ophthalmology, gross pathology, organ weights, and histopathology. Animals were observed for mortality, body weights, food/water consumption, and clinical signs of toxicity. Animals also underwent bronchoalveolar lavage (BAL). ***Adverse Effect(s) (brief):*** **NOAEL=** 4.7 and BMCL=4.4 mg/m^3; OEL=2 mg m^{-3}. The exposure to iron oxide dust was tolerated without mortality, consistent changes in body weights, food and water consumption or systemic toxicity. Histopathology demonstrated responses to particle deposition in the upper respiratory tract (goblet cell hyper- and/or metaplasia, intraepithelial eosinophilic globules in the nasal passages) and the lower respiratory tract (inflammatory changes in the bronchiolo-alveolar region). Consistent changes suggestive of pulmonary inflammation were evidenced by BAL, histopathology, increased lung and lung-associated-lymph node (LALN) weights at 16.6 and 52.1 mg m^{-3}. Increased septal collagenous fibers were observed at 52.1 mg m^{-3}. Particle translocation into LALN occurred at exposure levels causing pulmonary inflammation. Elevations of neutrophils in bronchoalveolar lavage (BAL) appeared to be the most sensitive endpoint of study. The empirical no-observed-adverse-effect level (NOAEL) and the lower bound 95% confidence limit on the benchmark concentration (BMCL) obtained by benchmark analysis was 4.7 and 4.4 mg m^{-3}, respectively, and supports an OEL (time-adjusted chronic occupational exposure level) of 2 mg m^{-3} (alveolar fraction). ***Data Quality:*** *NR* ***Reference****: Pauluhn, J. (2012). "Subchornic inhalation toxicity of iron oxide (maganetite, Fe SUB 3 O SUB 4) in rats: pulmonary toxicity is determined by the particle kinetics typical of poorly soluble particles. ." J. Appl. Toxicol 32(7): 488-504.*							
Hofmann, 2016	ND	Animal	Inhalation	30 mg/m^3. (**NOAEC)**	Five exposures	Acute inflammation	NR

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
Test article: coarse and fine inorganic Pigment Red 101. ***Dose:*** 30 mg/m³**.** ***Treatment Duration:*** Wistar rats were exposed head-nose to atmospheres of the respective materials for 6 h/day on 5 consecutive days, followed by up to the 28-day observation period. ***Species (strain):*** Rat (Wistar) ***Gender:*** Male ***Number per Group:*** 5/dose/group (main group for blood sampling and BAL). ***Control*****:** up to the 28-day observation period ***Methods:*** Five-day dust inhalation study with investigations for local and systemic effects both after treatment and after a 3-week recovery period. ***Adverse Effect(s) (brief):*** **NOAEC=30 mg/m³.** Lung burden measurements reveal a mean 0.31 mg. Inhalation exposure of rats to 30 mg/m³ on 5 consecutive days did not cause any adverse effect in the respiratory tract up to the 28-day observation period, which was examined by broncho-alveolar lavage and histopathology. There were no changes of hematology and clinical chemistry parameters. A slightly decreased mean body weight intermittently reduced body weight change were observed during exposure and postexposure period. However, the deviation to the control were with -3 to -8 % not biologically relevant. Thus, under current study conditions, the no observed adverse effect concentration (NOAEC) was 30 mg/m³. ***Data Quality:*** *NR* ***Reference:*** *Hofmann, Thomas, Lan Ma-Hock, Volker Strauss, Silke Treumann, Maria Rey Moreno, Nicole Neubauer, Wendel Wohlleben, et al. "Comparative Short-Term Inhalation Toxicity of Five Organic Diketopyrrolopyrrole Pigments and Two Inorganic Iron-Oxide-Based Pigments." Inhalation Toxicology 28, no. 10 (August 23, 2016): 463–79. https://doi.org/10.1080/08958378.2016.1200698*							
Genotoxicity							
Bhattacharya K et al., 2012	ND	Human cells	Cell culture	25 µg/plate	24hrs	Genotoxic based on particle size and media conditions	NR
Test article: Diiron trioxide. ***Dose:*** 0, 10, 25, 50, and 250 µg/ml. Untreated and positive controls were included. ***Treatment duration:*** The cells were exposed for a time period of 24 h. For negative control, cells were exposed to normal cell culture medium. ***Species (strain):*** IMR 90 and BEAS-2B cells ***Gender:*** NA. ***Number per Group:*** NR. ***Methods***. Comet Assay. Genotoxic effect of the alpha-Fe_2O_3 nano- and microscale particles was determined by analyzing DNA fragmentation using the alkaline comet assay technique (pH 12.7) ***Adverse Effect(s) (brief):*** Both the nano- and microscale alpha-Fe2O3 particles were actively taken up by human lung cells in vitro, although they were not found in the nuclei and mitochondria. Significant genotoxic effects were only found at very high particle concentrations (> 50 mg/ml). The nanoscale particles were slightly more potent in causing cyto- and genotoxicity as compared with their microscale counterparts. Both types of particles induced intracellular generation of reactive oxygen species. This study underlines that a-Fe_2O_3 nanoscale particles trigger different toxicological reaction pathways than microscale particles. However, the immediate environment of the particles (biomolecules, physiological properties of medium) modulates their toxicity on the basis of agglomeration rather than their actual size. The study demonstrates that the surface reactivity of nanoscale alpha-Fe_2O_3 differs from that of microscale particles with respect to the state of agglomeration, radical formation potential, and cellular toxicity. Proteins were found to play an important role in agglomeration activity of both particle types. Particles suspended in native cellular protein fractions showed the highest agglomeration capacity compared with cell culture medium (DKSFM þ 0.1% EGF) and water. A modification of the composition or the chemical properties (e.g., the pH) of the surrounding medium resulted in changed agglomeration capacities of the particles. The study indicates that the surface charge and area of a-Fe_2O_3 nano- and microparticles are important for their toxicity to human lung cells. At relevant particle concentrations of < 10 lg/ml, no DNA breakage can be observed. ***Data Quality:*** ECHA Reliability 2 *(reliable without restriction) Reference Bhattacharya. "Comparison of Micro- and Nanoscale Fe+³–Containing (Hematite) Particles for Their Toxicological Properties in Human Lung Cells in Vitro." Toxicological Sciences 126, no. 1 (2012): 173–82.:*							
Karlsson et al., 2009	ND	Human cells	Cell culture	40 µg/ml.	24hrs	DNA damage	NR
Test article: Fe_2O_3, Fe_3O_4, $TiO_2$and CuO micrometer particles (<5µm) were tested in this study. ***Dose:*** 0, 20 or 40 µg/cm² (40 or 80 µg/ml) suspended in supplemented DMEM. Control cells were only exposed to DMEM medium. ***Gender:*** NR. ***Number per Group:*** three to four independent experiments. ***Treatment duration:*** 24hrs ***Species (strain):*** A549 cells. ***Methods***. Comet assay. For the mitochondrial assay: 0.4 million cells were seeded in each well of 6-well plates and grown for 24h before exposure to 40mg/cm² (80mg/ml) of each particle type for 16h. Cells were also exposed to 5, 10, 15, and 20mg/cm² (10, 20,30 and 40mg/ml) of CuO particles. To analyze mitochondrial depolarization, cells were treated with 50nM of the fluorescent probe tetramethylrhodamine ethyl ester (TMRE). In the Comet assay, 0.16 million cells were grown in each well of 24-well plates for 24h to get a confluent layer of 90–100% before exposure to 20 and 40mg/cm2(40 and 80 mg/ml) of particles for 4h. To study DNA damage, in forms of single strand breaks and alkali labile sites, the alkaline version of the Comet assay. For the cytotoxicity assay 0.08 million cells were seeded in each well of 24-well plates and grown for 24h to obtain a 50% covering layer. The cells were then exposed to 20 and 40mg/cm2(40 and 80mg/ml) of particles in 37◦C sterile DMEM medium for 18h. ***Adverse Effect(s) (brief):*** Micrometer particles did not cause any cytotoxic effects. When A549 cells were exposed to 40 µg/cm² micrometer particles for 4 h there was a small increase in DNA damage for Fe3O4 (11% tail, $p < 0.05$) and Fe2O3 (12% tail, $p < 0.05$). ***Note***: Cause-effect is unclear due to low response level and absence of dose-dependency. ***Data Quality:*** ECHA Reliability 2 *(reliable without restriction)* ***Reference****: Karlsson, Hanna L., Johanna Gustafsson, Pontus Cronholm, and Lennart Möller. "Size-Dependent Toxicity of Metal Oxide Particles—A Comparison between Nano- and Micrometer Size." Toxicology Letters 188, no. 2 (July 24, 2009): 112–18. https://doi.org/10.1016/j.toxlet.2009.03.014.*							

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
Guichard Y et al.,2012	ND	Animal cells	Cell culture	50 µg/cm²	24hrs	No observable adverse effect reported	NR
Test article: nanosized and microsized Fe_3O_4, and Fe_2O_3. ***Dose:*** 0, 1, 5, 10, and 50 µg/cm² ***Treatment duration:*** 24hrs***. Species (strain):*** Syrian hamster embryo (SHE) cells ***Gender:*** NR. ***Number per Group:*** three times and experimental data. ***Methods***. In the present study, the in vitro cytotoxicity and genotoxicity of commercially available nano-sized and micro-sized Fe3O4, and Fe2O3 particles were compared in Syrian hamster embryo (SHE) cells. Samples were characterized for chemical composition, primary particle size, crystal phase, shape, and specific surface area. ***Adverse Effect(s) (brief):*** No significant increase in DNA damage was detected from nanosized and microsized iron oxides. None of the samples tested showed significant induction of micronuclei formation after 24 h of exposure. Measurement of particle size in the culture medium showed that primary nano- and micro-materials are present in the form of micrometric agglomerates of high poly-dispersed size. Among the iron oxide samples, the particle population of the nano-form Fe3O4 was finer than the micron-form, while the opposite was observed for Fe2O3 samples. ***Data Quality:*** NR. Reference: *Guichard, Yves, Julien Schmit, Christian Darne, Laurent Gaté, Michèle Goutet, Davy Rousset, Olivier Rastoix, et al. "Cytotoxicity and Genotoxicity of Nanosized and Microsized Titanium Dioxide and Iron Oxide Particles in Syrian Hamster Embryo Cells." The Annals of Occupational Hygiene 56, no. 5 (July 1, 2012): 631–44. https://doi.org/10.1093/annhyg/mes006.*							
Carcinogenicity							
Saffiotti 1972	ND	Animal	Intratracheal instillation	50 mg **(LOAEL)**	Single administration; 15 doses; 10 doses; 5 doses. Up to 140 weeks	No observable adverse effect reported (ferric oxide alone); Respiratory tract tumors (ferric oxide +BP)	NR
Test article: Benzo(a)pyrene (BP), prepared as a suspension of fine crystalline particles attached to particles of ferric oxide. ***Dose:*** *Single administration:* ***3***7.5 mg BP with 12.5 mg ferric oxide; 5 mg BP with 45 mg ferric oxide, 50 mg ferric oxide in 0.5 ml 0.9% saline. Multiple *administrations:* 3 mg BP with 3 mg ferric oxide in 0.20 ml 0.9% NaCl solution. ***Treatment Duration:*** Single administration; 15 doses; 10 doses; 5 doses. Up to 140 weeks. ***Methods:*** Benzo(a)pyrene (BP), prepared as a suspension of fine crystalline particles attached to particles of ferric oxide, or ferric oxide only in solution were administered by intratracheal instillation to animals. Four groups of animals received doses in single administrations, or six groups received multiple administration 5,10, or 15 times, two experimental groups were used at each dose level. Each treatment consisted of 3 mg BP and 3 mg ferric oxide suspended in 0.2 ml of 0.9% NaCl solution; intratracheal instillations were performed weekly, except in Group 10, to which the 5 intratracheal instillations were given at 25-day intervals. Animal were observed up to 140 weeks, survival rats were determined weekly, and animals were weighed once weekly. Animals were then sacrificed for autopsy and for fixation of the lungs, the respiratory organs, and other pertinent tissues were examined histologically. ***Species (strain):*** hamsters (Syrian golden) ***Gender:*** Male/ Female***. Number per Group:*** Single administration; 39-110/ Multiple 30-36. ***Adverse Effect(s) (brief):*** **LOAEL= 50 mg ferric oxide**. Rat were given a single administration of 50 mg ferric oxide in 0.5 ml 0.9% saline resulted There was no differences in formation of any respiratory tumors when compared to control group. Other respiratory lesions were seen among this group, 1 animal was found with a patch of bronchiolar squamous metaplasia and 3 were found with small bronchiolar adenomatoid lesions. Papillomas of the forestomach appear spontaneously in hamsters in the study. 50 mg ferric oxide groups and control group, showed, respectively, 4 and 9% incidences of forestomach papillomas (or a total of 14 animals with 16 forestomach papillomas out of the 277 controls of both groups). No lesions of the respiratory tract were seen in 0.9% NaCl solution controls. A single administration of 37.5 mg BP with 12.5 mg ferric oxide in 0.5 ml 0.9% NaCl solution induced five bronchogenic carcinomas and five histologically benign respiratory tumors in a total of 61 hamsters. Four were of squamous cell type and one was of large cell anaplastic type), one bronchial papilloma, two bronchial adenomas, and two alveolar adenomas. The earliest tumor to be observed was a bronchogenic squamous cell carcinoma in a female hamster that died at 28 weeks; the other tumors were found throughout the experiment, the last in a hamster that died at 101 weeks. A single administration of 5 mg BP with 45 mg ferric oxide in 0.5 ml 0.9% NaCl solution induced one peripheral adenocarcinoma and six histologically benign respiratory tumors in a total of 189 hamsters (Bronchial adenoma, an alveolar adenoma, 2 tracheal polyps, and 2 trachea papillomas were observed). In Group 1, 15% of the effective number of animals developed respiratory tumors. The smaller single dose of 5 mg BP in Group 2 induced a respiratory tumor incidence of only 4% of the effective number of animals. The results in Group 2 are relatively scanty but appear attributable to the treatment, in comparison to negative findings in large control groups (6) and considering the fact that, qualitatively, the same tumor types were obtained as in Group 1 or following repeated BP administrations. Repeated instillations, each of 3 mg BP with 3 mg ferric oxide in 0.20 ml 0.9% NaCl solution resulted in respiratory tract tumors, mostly bronchogenic carcinomas, which were induced in all groups, and a positive dose-response relationship was demonstrated. The different frequency of administration of 5 doses in Groups 9 and 10 over a period of 4 and 14 weeks, respectively, did not lead to significant differences in the results. The highest incidences of respiratory tumors were obtained with a total dose of 45 mg BP. The lower dose of 15 mg BP still induced a 15% tumor incidence. The present results show that single respiratory exposures to carcinogenic polynuclear hydrocarbons are sufficient to induce bronchogenic carcinomas. However, ferric oxide alone did not result in any differences in formation of any respiratory tumors compared to control group. ***Data Quality:*** *NR* ***Reference:*** *Saffiotti, Umberto, Ruggero Montesano, Arthur R. Sellakumar, Francesco Cefis, and David G. Kaufman. "Respiratory Tract Carcinogenesis in Hamsters*								

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
Induced by Different Numbers of Administrations of Benzo(a)Pyrene and Ferric Oxide." Cancer Research 32, no. 5 (May 1, 1972): 1073–81.							
Wright et al., 1988	ND	Animal	intratracheal injection	10 mg **(NOAEL)**	30-day	No observable adverse effect reported	NR
Test article: Ferric oxide (particles). ***Dose:*** 0.5ml saline (control), 10 mg iron oxide in 0.5 ml saline, 10mg quartz, or 30 mg quartz in 0.5 ml saline was administered via intratracheal injection. ***Treatment Duration:*** 30-day ***Species (strain):*** Rats (Sprague -Dawley) (200g) ***Gender:*** Female. ***Number per Group:*** 5 per group. ***Control***: 7/0.5ml saline ***Methods:*** Intratracheal injection. Rats were divided to four groups and administered the test substance via intratracheal injection. One month after exposure, the animals were anesthetized, and a series of pulmonary function test were performed. Animals were then sacrificed on day 30 and the lungs were removed, and morphologic changes were examined. ***Adverse Effect(s) (brief):*** **NOEAL=10 mg iron oxide**. 10 mg iron oxide exposure resulted in no notable or significant changes. 10mg Iron oxide exposure resulted in no air-flow obstruction, functional changes, or morphometric changes compared to the control group. However, animals exposed to quartz showed evidence of air-flow obstruction, with sever abnormalities in the high dose group. The findings correlate with morphometric observation. Emphysem and thicken airway walls. Early silioctic nodule were observed. ***Data Quality:*** *NR* ***Reference:*** *Wright, J. L., Nigel Harrison, Barry Wiggs, and Andrew Churg. "Quartz but Not Iron Oxide Causes Air-Flow Obstruction, Emphysema, and Small Airways Lesions in the Rat." American Journal of Respiratory and Critical Care Medicine 138, no. 1 (1988): 129–35.*							
Steinhoff D et al., 1991	ND	Animal	Intratracheal instillation; Intraperitoneal	1530 mg/kg. **(NOAEL)**	798 days	No observable adverse effect reported	NR
Test article: Bayferrox 1352, Bayferrox 920, Bayferrox 130, Brazilian iron ore AC 5031 ***Dose:*** 10 mg/kg b.w. every 14 days; subsequent doses could be doubled after 13 treatments and doubled again following 6 further treatments. Total dose 1530 mg/kg (Intratracheal instillation) 600 mg/kg (Intraperitoneal Administration) ***Treatment duration:*** up to 798 days ***Species (strain):*** Rats (Sprague-Dawley). ***Gender:*** Female. ***Number per Group:*** 50 per sex/per group; including control group. ***Control:*** Rats appropriately treated with physiological saline solution, and completely untreated animals were used as controls. 10 male and 10 female ***Methods:*** iron oxides (in physiological saline solution) were also tested by intraperitoneal injection. This involved injection of three 200 mglkg doses at intervals of 8 weeks (total dose 600 mg/kg b.w.). The surviving rats were sacrificed after 790/914 study days. rats were intraperitoneally treated with AC 5100 M, 60 males and 60 females with RBW 07105/SV2, and 50 male and 50 female rats with the other iron oxides. Control groups of identical size (treated with physiological saline solution and untreated) were used for all test groups. Only the abdominal cavity tumors were histologically examined in these rats. Pilot tests had shown that in the case of single intratracheal instillation in Sprague-Dawley rats, the administrability rather than the toxicity limited the size of the doses to be used in the chronic study. A dose of 1 X 10 mg/ kg b.w. every 14 days was initially established for intratracheal instillation. ***Adverse Effect(s) (brief):*** **NOAEL= 1530 mg/kg.** Following exposure via intratracheal instillation, C.I. Pigment Red 101 exhibited no signs of carcinogenicity in rats. Rats were initially exposed to 10 mg/kg b.w. every 14 days; subsequent doses could be doubled after 13 treatments and doubled again following 6 further treatments. Surviving rats were sacrificed after 798 study days. Following exposure, there were no relevant effects on survival or body weight gain in any rat. Substance related effects were seen in the lungs in the form of metaplasia due to foreign body deposits. Weak increase in tumors were observed in the test groups; however, the investigator attributed this response to stress and therefore the iron oxides were considered negative for carcinogenicity. ***Data Quality:*** *ECHA Reliability (reliable with restrictions) 2* ***Reference****: Steinhoff, D., U. Mohr, and S. Hahnemann. "Carcinogenesis Studies with Iron Oxides." Exp. Pathol 43 (1991): 189–94.*							

SUPPORTING STUDIES

Epidemiology							
Bourgkard 2009	ND	Epidemiology (Human)	Cohort	ND	NR	No observable adverse effect reported	NR
Test article: C.I. Pigment Red 101 ***Dose: Treatment Duration: Gender:*** Male and female. ***Number per Group:*** 16 742 males and 959 females. ***Control:*** Not defined. ***Methods:*** Epidemiology. Males and females ever employed for at least 1 year between 1959 and 1997 were followed up for mortality from January 1968 to December 1998. Causes of death were ascertained from death certificates. Job histories and smoking habits were available for 99.7% and 72.3% of subjects, respectively. Occupational exposures were assessed by a factory-specific job-exposure matrix (JEM) validated with atmospheric measurements. Standardized mortality ratios (SMRs) were computed using local death rates (external references). Poisson regressions were used to estimate the relative risks (RRs) for occupational exposures (internal references), adjusted on potential confounding factors. ***Results (brief):*** A cohort study of male and female workers employed at a carbon steel-producing factory in France failed to find any evidence to support a relationship between occupational exposure to C.I. Pigment Red 101 and lung cancer mortality. ***Data Quality:*** NR ***Reference:*** *Bourgkard E, Wild P, Courcot B, Diss M, Ettlinger J, Goutet P, Hémon D, Marquis N, Mur JM, Rigal C, Rohn-Janssens MP, Moulin JJ. Lung cancer mortality and iron oxide exposure in a French steel-producing factory. Occup Environ Med. 2009 Mar;66(3):175-81. doi: 10.1136/oem.2007.038299. Epub 2008 Sep 19. PMID: 18805881.*							

SECONDARY SOURCES

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
Systemic Toxicity (acute)							
ECHA, 1986	ND	Animal	Oral (water)	> 5000 mg/kg bw **(LD_{50})**	single administration	No observable adverse effect reported	EU Method B.1 (Acute Toxicity (Oral))
Test article: C.I. Pigment Red 101 ***Dose:*** single dose of 5000 mg/kg b.w. in water. ***Treatment duration:*** single administration followed by a 14-day observation period. ***Species (strain):*** Wistar rats. ***Gender:*** Male and Female. ***Number per Group:*** 5 animals per sex per dose. ***Control:*** No controls were used. ***Methods:*** Oral (water). Using a standard acute method, rats were exposed to a single dose of. C.I. Pigment Red 101 in water via oral gavage. After 14-day observation period animals were sacrificed. ***Adverse Effect(s) (brief):*** **LD_{50} > 5,000 mg/kg bw.**, C.I. Pigment Red 101 demonstrated no toxicity potential. There was no mortality and no clinical signs of toxicity noted. A LD_{50} of >5000 mg/kg b.w. was determined for both sexes. ***Data Quality:*** *ECHA Reliability (reliable with restrictions) 2*							
ECHA, 1977	ND	Animal	Oral (gavage)	>10,000 mg/kg bw **(LD_{50})**	single administration	No observable adverse effect reported	NR
Test article: C.I. Pigment Red 101. ***Dose:*** A single application of 10,000 mg/kg b.w. was administered in water via oral gavage. ***Treatment duration:*** single administration followed by a 14-day observation period. ***Species (strain):*** Rat (Wistar). ***Gender:*** Male. ***Number per Group:***10 animals per sex per dose. ***Control:*** No controls were used ***Methods:*** Oral (gavage). Using a standard acute method, C.I. Pigment Red 101 was administered in a single application to rats in water via oral gavage. Exposure was followed by a 14-day observation period, animals were observed for clinical signs of toxicity. ***Adverse Effect(s) (brief):*** **LD_{50} = 10,000 mg/kg bw.** A single application of 10,000 mg/kg b.w. was considered not acutely toxic. During the 14-day observation period, there was no mortality and no signs of toxicity. ***Data Quality:*** *ECHA Reliability (reliable with restrictions) 2*							
ECHA 2015	ND	Animal	Inhalation (snout only exposure)	>5 mg/L. **(MLC)**	4hrs	No observable adverse effect reported	OECD 403; GLP
Test article: Diiron trioxide. ***Dose:*** single to 5 mg/l CERAC-Pigment (average particle size = 35 nm). ***Treatment duration:*** For 4 hours in air. followed by a 14-day observation period. ***Species (strain):*** Rats (Sprague-Dawley) ***Gender:*** Male/female ***Number per Group:*** 5 per group. ***Control:*** No controls were used ***Methods:*** Inhalation (snout only exposure). The animals were observed for mortality, clinical signs and body weight during a post-observation period of 14 days. A pathological examination was performed on all animals which died during the observation period or were sacrificed at the end of the study period. ***Adverse Effect(s) (brief):*** No morality occurred and no significant changes in body weight were observed. Brown staining around the head, rolling gait, and were observed. 3 of 5 females had uterine dilatation with one of these having a scab on the tail. The Median Lethal Concentration (MLC) was therefore considered to be in excess of 5 mg/L. **Data *Quality:*** *ECHA Reliability (reliable with restrictions) 1*							
Genotoxicity							
ECHA, 2003	ND	Animal cells (in vivo)	endotracheal instillation	3.75 mg/kg bw	single dose/ 24hr	No observable adverse effect reported	NR
Test article: C.I. Pigment Red 101. ***Dose:*** Iron oxide 0 or ~3.75 mg/kg ***Treatment duration:*** 24 hrs. ***Species (strain):*** Sprague-Dawley rats. ***Gender:*** Male. ***Number per Group:*** 3/ per group (4 groups). ***Control:*** Saline controls were used ***Methods:*** *Comet assay:* Animals were anesthetized by i.p, injection Treatment was performed by intratracheal instillation of a suspension of iron oxide, benzopyrene or a mixture of both. The control group was treated with the vehicle (saline) only. or the sum of these 24 hours after treatment animals were killed and cells isolated (alveolar macrophages, lung cells, peripheral lymphocytes, hepatocytes) Parameters: alkaline single cell electrophoresis (Comet Assay; 100 cells per slide). ***Adverse Effect(s) (brief):*** **NOAEL**=3.75 mg/kg bw. No damage was observed in cells from the four investigated organs in rats treated with iron oxide alone compared with control animals in all tested cell types. ***Data Quality:*** ECHA Reliability *2 (reliable with restrictions)*							

II.2 Other Health Effects

Pigment	Endpoint	Outcome	Reference
Dermal irritation			
C.I. Pigment Red 101	Dermal irritation	No observable adverse effect reported	ECHA, 1985
Test article: C.I. Pigment Red 101 ***Dose:*** 0.5 g of the test substance were dissolved in distilled water at a concentration of 20%, and 0.5 mL. ***Treatment duration:*** 24 hrs, followed by a 14-day observation period. ***Species (strain):*** Rabbit (New Zealand White). ***Gender:*** male/ female; 2.3 and 2.8 kg. ***Number per Group:*** 6/ per sex. ***Control:*** not required ***Method:*** 0.5 g of the test substance were dissolved in distilled water at a concentration of 20%, and 0.5 mL of this solution was applied to the intact and scarified back skin of three rabbits, respectively. The skin was evaluated for edema and erythema at removal of the test substance, and again at daily intervals until study end at 14 days after exposure. The skin was evaluated by two independent investigators. In addition, clinical signs, behavior, general condition, and food consumption was recorded. Body weights were determined daily. ***Adverse Effect(s) (brief):*** None of the animals exhibited any signs of skin irritation (no edema, no erythema at any of the observations). No signs of systemic toxicity were noted. Behavior, general condition, and body weight gain were not affected by treatment. ***Data Quality:*** ECHA Reliability 1 (reliable without restriction).			
C.I. Pigment Red 101	Dermal irritation	No observable adverse effect reported	ECHA, 1982
Test article: C.I. Pigment Red 101. ***Dose:*** NR ***Treatment duration:*** for 4 hours and were observed for 8 days. No controls. ***Species (strain):*** Rabbits (albino) ***Gender:*** NR. ***Number per Group:*** 3 rabbits. ***Control***: Not required. ***Methods:*** Acute Dermal Irritation: The test material with oil vehicle was applied to the shaved, semiocclusive skin of New Zealand White rabbits (3 rabbits). Rabbits were exposed to test material for 4 hours and were observed for 8 days. ***Adverse Effect(s) (brief): No NOAEL*** Following observations, there were no skin reactions (scores for edema and erythema were 0). C.I. Pigment Red 101 was not irritating in rabbits. ***Data Quality:*** *ECHA Reliability 2 (reliable with restrictions) 2*			
Toxicokinetic			
C.I. Pigment Red 101	Dermal irritation	No observable adverse effect reported	ECHA, 2003
Test article: Iron oxide aerosol. ***Dose:*** at iron concentrations up to 700 mg/m^3 ***Treatment duration:*** for continuous periods up to 235 minutes, and for immediate periods of 30 minutes over a 10–100-day period. ***Species (strain):*** Rat (Species not specified). ***Gender:*** male ***Number per Group:*** NR. ***Control:*** Controls not defined ***Methods:*** Rats have been exposed to iron oxide aerosol (MAD 0.3 µm) at iron concentrations up to 700 mg/m³ for continuous periods up to 235 min, and for immediate periods of 30 min over a 10 day period. Rats were killed one day post each exposure and also at several predetermined periods up to 100 d post exposure in order to follow the elimination of deposited iron from the lung. The total lung being removed, and iron was determined by instrumental neutron activation analysis. ***Adverse Effect(s) (brief):*** **No NOAEL.** Rats exposed to iron oxide aerosol at iron concentrations up to 100 mg/m^3 for continuous periods up to 235 minutes, and for immediate periods of 30 minutes over a 10-day period showed that inhaled iron oxide is translocated form the lungs to the gastrointestinal tract. The contribution of inhaled iron oxide to blood and organs other than the lungs is insignificant; distribution throughout the organism is dependent upon particle size. Iron oxide behaves as an insoluble material and is efficiently eliminated from the gastrointestinal tract in the feces ***Data Quality:*** *ECHA Reliability (reliable with restrictions) 2*			

Section III. Dose-Response Assessment

III.1 Derivation of Provisional Tolerable Intake

Contact Duration	Exposure Route	Patient	POD	MF	pTI
Permanent	Parenteral	All	1000 mg/kg/d	1,000	1 mg/kg/d

pTI = POD ÷ MF

Note: When a supplier-specific color additive safety data sheet (SDS) does not report the presence of a carcinogen or non-carcinogen impurity, and SDS complies with Globally Harmonized System (GHS) hazard communication limits (i.e., <0.1% and <1%, respectively), then this pTI is low enough to be protective for naturally occurring impurities which could be present in the color additive. Processes used to color a polymer system involve diluting the pigment and its impurities by at least 50x (i.e., the CHRIS calculator is valid when the maximum concentration of pigment in a colored polymer is 2%, i.e., 1 ÷ 2% = 50); therefore, the concentration of naturally occurring impurities in the finished colored polymer will be significantly lower compared to the concentration of these substances in the pigment (i.e., 0.002% (i.e., 0.1% ÷ 50) for carcinogens and 0.02% (i.e., 1% ÷ 50) for non-carcinogens). Unknown impurities are addressed by the CHRIS calculator.

III.2 Derivation of a Modifying Factor

Critical Study	Biological Endpoint	Exposure Route	POD Type	POD Value	UF_1	UF_2	UF_3	MF
Kumari, 2012	Food consumption, body weight, organ histopathology	Oral	NOAEL	1000 mg/kg/d	10	10	10	1,000

MF = modifying factor (UF_1 x UF_2 x UF_3)

III.3 Toxicological Uncertainties Applied to POD

Uncertainty	Justification
UF_1 Error! Bookmark not defined.	(10) Default value to account for susceptible individuals.
UF_2 Error! Bookmark not defined.	(10) Default value to account for differences between species.
UF_3 Error! Bookmark not defined.	(10) Default value to account for differences in exposure route and data quality.

UF_1, UF_2, and UF_3, are used to extrapolate the POD to the general human population (including pediatric) exposed parenterally for a lifetime.

III.4 Critical Health Study Design and Outcome

Kumari, M. (2012) Repeated Oral Dose Toxicity of Iron Oxide Nanoparticles: Biochemical and Histopathological Alterations in Different Tissues of Rats. Journal of Nanoscience and Nanotechnology 12, 2149–2159.

Test Substance	Fe_2O_3-Bulk (<5000nm)		
Species	Rats (Wistar)	Frequency	daily ad libitum
Gender	Female	Duration	28 days
Age	8 weeks old	#/Group	10/dose
Route	Oral (feed)	Protocol Guideline	OECD
Dose(s)	0, 30, 300 or 1000 mg/kg/day	Statistical Method(s)	None
Observed Responses	NOAEL=1000 mg/kg/day for microsized particles (<5000nm). No decrease in body weight, no change in feed intake, nor any adverse sign, symptoms or mortality were observed in rats exposed to any of the micro sized red iron oxide doses. Micro-sized red iron oxide did not induce any adverse effects at any of the treatment doses in either biochemical parameters or histopathology, with the exception of a significant decrease (25 %) in lactate dehydrogenase activity in the kidneys of rats given the highest dose. However, the Panel noted that no significant change in plasma lactate dehydrogenase activity was observed in this group of animals and therefore did not consider the decreased lactate dehydrogenase activity in the kidney adverse.		
Notes:	None		

C.I. Pigment Yellow 138

CAS # 30125-47-4

Toxicological Profile Summary

C.I. Pigment Yellow 138 (CAS # 30125-47-4) is an insoluble organic pigment. Reported hazard information includes an adverse health effect study, supporting studies, and secondary sources. C.I. Pigment Yellow 138, administered at high oral doses (up to 1000 mg/kg bw) to rodents, is reported to not elicit systemic toxicity. For assessing C.I. Pigment Yellow 138 exposure that will be without appreciable harm to health, the calculated provisional tolerable intake (pTI) value for parenteral systemic toxicity is 1 mg/kg/day based on the lowest reported point-of-departure (1000 mg/kg/day, oral, 28-day, rodent) with a modifying factor of 1000 to address the following sources of uncertainty: variation among humans (10), variation between species (10), and data quality/route-to-route extrapolation (10).

NOTE: This pTI is protective for the color additive including any impurities listed in Section I.1 "Substance Identity," as the impurities likely will be present at a very low percentage of the color additive, and the quantity of the impurity will be at an even lower percentage in the final device (see Section III, Table III.1 footnote). Unknown impurities are addressed by the CHRIS calculator.

Section I. Substance Information

I.1 Substance Identity

	Descriptor
Synonyms[53]	C.I. Pigment Yellow 138 (C.I. 56300)
Formula[53]	$C_{26}H_6C_{l8}N_2O_4$
Molecular Weight[53]	Not available (no data)
Physical Form[53]	Solid
Type[53]	quinophthalone pigment.
Physical/Chemical Characteristics[54], [55],[53]	Melting point of 460°C, a vapor pressure of $1.0x10^{-6}$ hPa at 20°C Density of 1.852 g/cm^3 at 20°C Sparingly soluble in water (less than 0.05 mg/L at 20°C) and in organic solvents
Production	Data not found in literature
Impurities	Data not found in literature
Other[56]	It is an FDA approved food contact grade colorant per 21CFR 178.3297 for use not to exceed 1% by weight of polymers
Surrogates	Data not found in literature

[53] ECHA (2013). 3,4,5,6-tetrachloro-N-[2-(4,5,6,7-tetrachloro-2,3-dihydro-1,3-dioxo-1H-inden-2-yl)-8-quinolyl]phthalimide. Registered Substances Database. European Chemical Agency (ECHA).
[54] Eurocolour, ETAD, VdMi (2002). Colourants for Food Contact Plastics, Aspects of Product Safety (Heidelberg, Germany: Neumann Druck).
[55] European Commission -European Chemicals Bureau (2014). 3,4,5,6-tetrachloro-N-[2-(4,5,6,7-tetrachloro-2,3-dihydro-1,3-dioxo-1H-inden-2-yl)-8-quinolyl]phthalimide, C.I. Pigment Yellow 138.
[56] Clariant (2013). Product Composition Mevopur-Yellow NC1M415072-ZN.

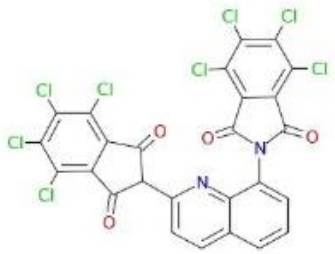


Figure I.1. Schematic of C.I. Pigment Yellow 138 molecule

Section II. Hazard Identification

Health effect data, supporting studies and secondary sources were reviewed, see tables in sections II.1 and II.2 for details.

Acute oral lethal dose (50) values were found to exceed 2,000 mg/kg bw in test animals. Rats doses up to 10,000 mg/kg bw experienced no observable adverse effects[57].

In a 28-day repeat dose study in rodents dosed up to 1000 mg/kg bw (NOAEL), no observable signs of treatment-related systemic toxicity or reproductive/ in both sexes compared to controls are reported[58]. C.I. Pigment Yellow 138 was non-mutagenic in 5 strains of *S. typhimurium* with or without metabolic activation up to 5000 mg/plate[59].

II.1 Systemic, Genotoxicity, Carcinogenicity, Reproductive/Developmental Toxicity

ADVERSE HEALTH EFFECT STUDIES

None reported, see Section III.4 for the critical adverse health effect study.

SUPPORTING STUDIES

None reported.

SECONDARY SOURCES

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Guideline
Systemic Toxicity (acute)							
ECHA, 1975b	ND	Animal	Oral	> 10,000 mg/kg bw (**LD_{50}**)	Once	No observable adverse effect reported	Similar to OECD 401; non-GLP
Test article:3,4,5,6-tetrachloro-N-[2-(4,5,6,7-tetrachloro-2,3-dihydro-1,3-dioxo-1H-inden-2-yl)-8 quinolyl]phthalimide. ***Dose:*** Rat were treated with 10000 mg/kg bw (28.5 mL/kg bw) suspension in 0.5 % aqueous CMC solution as vehicle. ***Treatment duration:*** Single administration, followed by 7-day observation period**.** *No controls were used.* ***Species (strain):*** Rat (Sprague-Dawley). ***Gender:*** males: 229 g (208-246 g); females: 170 g (165-171 g) ***Number per Group:*** (5/per sex/ per dose). ***Control:*** No control groups. **Method:** oral (gavage). 7 days observation period following administration. Animals were weighted on day 1, 5 and 8. Observation of clinical signs were observed on the day of administration and once daily afterwards. Necropsy of survivors performed. ***Adverse Effect(s) (brief)****:* **LD_{50} >10,000 mg/kg**. No mortality was observed, abnormalities in gross pathology were observed, and no adverse effects were observed in the treated animals. Yellow feces were observed in the study. ***Data Quality:*** ECHA Reliability, 2 (reliable with restrictions)							
ECHA, 1975c	ND	Animal	Inhalation	0.046 mg/L air (**LC_0**)	Once	No observable adverse effect reported	Similar to OECD 403; non-GLP

[57] ECHA, 1975b
[58] ECHA 2012b
[59] ECHA, 1983

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Guideline
Test article: 3,4,5,6-tetrachloro-N-[2-(4,5,6,7-tetrachloro-2,3-dihydro-1,3-dioxo-1H-inden-2-yl)-8-quinolyl]phthalimide. ***Dose:*** Rat were exposed to whole body concentrations of 0.046 mg/L air as vehicle. ***Treatment duration:*** Single administration, the duration of exposure was 8hrs; followed by 7-day observation period***. Species (strain):*** Rat (NR). ***Gender:*** male (250 g)/female (193 g) ***Number per Group:*** (6 / per sex/ per dose) (3 control). ***Control:*** No controls were used. **Method:** Inhalation. whole body exposure for 8 h, no analytics to determine concentrations or particle size, 7-day observation period. Clinical observations were conducted duration the observation period of 7 days. The weighing was done after 7 days, and sacrifice. Necropsy of survivors was performed. ***Adverse Effect(s) (brief):*** **LC_0 >0.046 mg/L air.** No clinical signs of toxicity were observed during the observation period. No mortality occurred because of exposure. Gross pathology results found no abnormalities. ***Data Quality:*** ECHA Reliability 3 (not reliable)							
ECHA, 1975d	ND	Animal	Dermal (occlusive)	>2500 mg/kg bw **(LC_{50})**	24hrs	No observable adverse effect reported	Similar to OECD 402; non-GLP
Test article: 3,4,5,6-tetrachloro-N-[2-(4,5,6,7-tetrachloro-2,3-dihydro-1,3-dioxo-1H-inden-2-yl)-8-quinolyl]phthalimide. ***Dose:*** Rat were treated with 2500 mg/kg bw 50 % suspension solution (in water 0.5% CMC). No controls were used. ***Treatment duration:*** Single administration; followed by 14- day observation period. ***Species (strain):*** Rat (Sprague-Dawley). ***Gender:*** male (136 g)/female (119 g) ***Number per Group:*** (5 / per dose). ***Control:*** None ***Method:*** Dermal (Occlusive). The dorsal area was the site of exposure an area of 50 cm^2 was treated. Clinical observations were conducted duration the observation period of 14 days. The weighing was done before application. Necropsy of survivors was performed. ***Adverse Effect(s) (brief):*** **LD_{50} > 2 5,00 mg/kg bw.** No clinical signs of toxicity were observed during the observation period. No mortality occurred because of exposure. Gross pathology results found no abnormalities. After 24 h and 8 days: local yellow substance residues. ***Data Quality:*** ECHA Reliability, 2 (not reliable)							
ECHA, 1975e	ND	Animal	intraperitoneal injection	> 10 000 mg/kg bw **(LD_{50})**	ND	slight adhesions and spleen enlargement and intra-abdominal substance	Non-guideline study; Non-GLP
Test article: 3,4,5,6-tetrachloro-N-[2-(4,5,6,7-tetrachloro-2,3-dihydro-1,3-dioxo-1H-inden-2-yl)-8-quinolyl]phthalimide. ***Dose:*** Rat were treated with 6810 and 10000 mg/kg bw (35% solution of test substance in 0.5% CMC-solution). No controls were used. ***Treatment duration:*** Single administration; followed by 7-day observation period. ***Species (strain):*** Mouse (NMRI). ***Gender:*** 27 g (males), 23 g (females)***Number per Group:*** (5/per sex/per dose). ***Control:*** None ***Method:*** intraperitoneal injection. 5 mice per sex per dose were administered a single dose of the test substance via intraperitoneal injection. Clinical signs and body weight were monitored, and necropsy was carried out at termination (or after death, if it occurred) on all animals. The animals were sacrificed on day 7 after application. ***Adverse Effect(s) (brief):*** **LD_{50} > 10 000 mg/kg bw.** 1 animal died within 48 h after application of the test substance (10000 mg/kg bw). The clinical signs observed included dyspnoea, apathy, and tremor. In gross pathology, intra-abdominal substance residues (10/10 animals); slight adhesions and spleen enlargement (9/10 animals) were observed at 10000 mg/kg bw. At 6810 mg/kg bw, intra-abdominal substance residues; slight adhesions and megaly of the spleen were observed. ***Quality:*** ECHA Reliability, 2 (not reliable)							
Systemic Toxicity (subchronic)							
ECHA 2012b	ND	Animal	Oral (gavage)	1000 mg/kg bw **(NOAEL)**	~60 days (females)	No observable adverse effect reported	OECD 422; GLP
Test article: C.I. Pigment Yellow 138. ***Dose:*** Administrated by gavage daily at 0,100, 300 and 1000 mg/kg bw in water. Controls were treated with water. ***Treatment duration:*** The duration of treatment covered a 2-week premating and a mating period in both sexes, approximately 1-week post-mating in males, and the entire gestation period as well as 4 days of lactation and 2 weeks thereafter in females. ***Species (strain):*** Rat (Sprague-Dawley). 10-11 weeks ***Gender:*** Male and female. ***Number per Group:*** (10/per sex/ per dose). ***Control:*** Untreated group***.*** ***Method*****:** Body weights were examined determined on study day 0 (start of the administration period) and thereafter once a week at the same time of the day. Other clinical examinations include: heamatology, clinical chemistry, urinalysis, neurobehavioral, gross pathology (all tissues), and histopathology (all tissues). The NOAEL was defined based on metabolome analysis. In this method, K-EDTA samples were taken from the retroorbital sinus in all rats under isoflurane anesthesia on study day 7, 14 and 28 for mass spectrometry-based metabolite profiling analysis. For analysis GC–MS (gas chromatography–mass spectrometry) and LC–MS/MS (liquid chromatography–MS/MS) were used for broad profiling and hormone measurement and SP-LC–MS/MS (solid phase extraction-LC–MS/MS) was applied for the determination of catecholamine and steroid hormone levels. ***Adverse Effect(s) (brief):*** **NOAEL= 1000 mg/kg bw.** All male and female animals of test group 3 (1000 mg/kg/d) showed yellowish discolored feces at the end of the study. 7 females of test group 3 (1000 mg/kg/d) revealed a yellow discoloration of the glandular stomach contents. 3 females of test group 3 (1000 mg/kg/d) showed the same discoloration of the contents of the jejunum. 2 females of test group 3 (1000 mg/kg/d) revealed histopathological yellow crystalline particles within the alveoli, often close to or within macrophages, rarely inside multinucleated giant cells. These discolorations were caused by the test substance but were not regarded to be a treatment-related adverse finding**.** No other adverse effects were observed up to and including the limit dose. ***Data Quality:*** ECHA Reliability 1 (reliable without restriction)							
Genotoxicity							

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Guideline
ECHA, 1983	ND	Bacteria (in vitro)	NR	5000 mg/ml	48hrs	No observable adverse effect reported	Similar to OECD 471; non-GLP
Test article: C.I. Pigment Yellow 138 ***Dose:*** 0, 20, 100, 500, 2500 and 5000 mg/plate in DMSO (vehicle / solvent). Positive and negative controls were used. ***Treatment duration:*** exposure duration was 48 hrs ***Species (strain):*** S. typhimurium. TA 1535, TA 100, TA 1537, TA 1538, TA 98 ***Number per Group:*** 3 dishes per dose per group (2 experiments) ***Control:*** Positive (+S9): 2-Aminoanthracen (all strains); (-S9): MNNG (TA 100, TA 1535) , 4-nitro-o-phenylendiamine (TA 1538, TA 98), 9-aminoacridine chloride monohydrate (TA 1537)/ untreated group. **Method:** Bacterial reverse mutation assay. Cells were preincubated for 20 min and exposure duration was 48 hrs. ***Adverse Effect(s) (brief):*** No cytotoxicity occurred but tested up to precipitating concentrations. C.I. Pigment Yellow 138 was non-mutagenic in 5 strains of S. typhimurium with or without metabolic activation up to 5000 mg/plate. ***Data Quality:*** ECHA Reliability 2 (reliable with restrictions)							
ECHA, 1985	ND	in vivo micronucleus assay	Oral (gavage)	4000 mg/kg bw	single oral administration	No observable adverse effect reported	Similar to OECD 474; non-GLP
Test article: 3,4,5,6-tetrachloro-N-[2-(4,5,6,7-tetrachloro-2,3-dihydro-1,3-dioxo-1H-inden-2-yl)-8-quinolyl]phthalimide (Purity > 98%). ***Dose:*** The exposure duration and concentration are listed: 16 h (4000 mg/kg bw), 24 h (0, 1000, 2000, 4000 mg/kg bw and positive control), 48 h (4000 mg/kg bw). The vehicle was 0.5 % CMC (carboxymethyl cellulose). ***Treatment duration:*** 16, 24 and 48 hours after the treatment ***Species (strain):*** Mouse (NMRI) ***Gender:*** male/female. ***Number per Group:*** 5/per sex/ per dose. ***Control:*** Untreated group/ cyclophosphamide. ***Method:*** micronucleus assay. The animals were sacrificed 16, 24 and 48 hours after the treatment, respectively. At the end of the experiment, the animals were sacrificed and, after removal of the two femora for bone marrow preparation, necropsied and examined for gross-pahtological changes of the internal organs. ***Adverse Effect(s) (brief):*** **NOAEL=4000 mg/kg bw.** Clinical signs of toxicity in test animals were observed 15 minutes post exposure. Administration doses of 4000 mg/kg bw led to irregular respiration which lasted for 3 - 5 hours. No other signs of toxicity were observed during the study. The number of normochromatic erythrocytes containing micronuclei did not differ to any appreciable extent in the negative control (solvent control). The test substance at 4000 mg/kg bw (highest dose tested) was found to be negative for mutagenic activity in mice. ***Data Quality:*** ECHA Reliability 2 (reliable with restrictions)							
Reproductive/Developmental Toxicity							
ECHA 2012b	ND	Animal	Oral (gavage)	1000 mg/kg bw (**NOAEL**)	28 days	No observable adverse effect reported	OECD 422; GLP
Test article: C.I. Pigment Yellow 138. ***Dose:*** Administrated by gavage at 0,100, 300 and 1000 mg/kg bw in water. Controls were treated with water. ***Treatment duration:*** The duration of treatment covered a 2-week premating and a mating period in both sexes, approximately 1-week post-mating in males, and the entire gestation period as well as 4 days of lactation and 2 weeks thereafter in females. ***Species (strain):*** Rat (Sprague-Dawley). 10-11 weeks ***Gender:*** Male and female. ***Number per Group:*** (10/per sex/ per dose). ***Control:*** Untreated group**. Method:** Treatment occurred daily. The key examinations included observation of body weight, haematology, clinical chemistry, urinalysis, gross pathology, and histopathology (all tissues were examined excluding skeletal muscle). The reproductive parameter examined include observation of the number of implantations, gravid uterus weight, and external examinations of fetus. ***Adverse Effect(s) (brief):*** Maternal **NOAEL= 1000 mg/kg bw; fetuses NOAEL= 1000 mg/kg bw.** The mean post implantation loss was highest in test group 2 (300 mg/kg/d), i.e. 28.6% compared to the control group (13.8%). As no dose-response relationship was observed, the finding was assessed as not being related to treatment. All female animals of test group 3 (1000 mg/kg/d) showed yellowish discolored feces at the end of the study. 7 females of test group 3 (1000 mg/kg/d) revealed a yellow discoloration of the glandular stomach contents. 3 females of test group 3 (1000 mg/kg/d) showed the same discoloration of the contents of the jejunum. 2 females of test group 3 (1000 mg/kg/d) revealed histopathological yellow crystalline particles within the alveoli, often close to or within macrophages, rarely inside multinucleated giant cells. These discolorations were caused by the test substance but were not regarded to be a treatment-related adverse finding Mean pup body weights were significantly lower on PND1 in test groups 2 (300 mg/kg/d) and 3 (1000 mg/kg/d). One female runt was seen in test group 2 (300 mg/kg/d) and 2 male and 3 female runts in test group 3 (1000 mg/kg/d). These values were within the range of the biological variation inherent in the strain of rats used for this study. The viability index as indicator for pup mortality between PND 0 and 4 was 99% for test groups 1 and 3 (100 and 1000 mg/kg/d; 1 pup of 1 female in group 1 and 3 was found dead). These findings were assessed to be incidental and not related to treatment. No dose-response relationship was observed in the mean post implantation loss, fetal body weight, number of live offspring, and postnatal survival**.** No adverse effects were observed up to and including the limit dose. ***Data Quality:*** ECHA Reliability 1 (reliable without restriction)							

II.2 Other Health Effects

Pigment	Endpoint	Outcome	Reference
C.I. Pigment Yellow 138	Sensitization	No observable adverse effect reported	ECHA, 2003

Pigment	Endpoint	Outcome	Reference
Test article: 3,4,5,6-tetrachloro-N-[2-(4,5,6,7-tetrachloro-2,3-dihydro-1,3-dioxo-1H-inden-2-yl)-8-quinolyl] phthalimide. ***Dose:*** Mice were treated with concentrations of 0, 3, 10 and 30 % in vehicle DAE 4:3:3 (Dimethylacetamid 99+%, acetone and ethanol in a ratio of 4:3:3 (volume parts)). 25 µl of the test substance per ear was applied on the dorsal part of both ears. ***Treatment duration:*** for 3 consecutive days. The animals were sacrificed on study day 5 by cervical dislocation. ***Species (strain):*** Mouse (CBA). 8 weeks ***Gender:*** Female (17.1 g - 21.5 g) ***Number per Group:*** 6 per dose ***Control:*** untreated site of the same animal ***Methods:*** Dermal. mouse local lymph node assay (LLNA). Groups of 6 female CBA/Ca mice each were treated with 3%, 10% and 30% w/w preparations of the test substance in DAE 4:3:3 (= NN-Dimethylacetamid 99+%, acetone and ethanol in a ratio ot 4:3:3) or with the vehicle alone. The study used 3 test groups and 2 control groups. Each test animal was applied with 25 µL per ear of the respective test substance preparation to the dorsum of both ears for three consecutive days. Three days after the last application the mice were sacrificed, and the auricular lymph nodes were removed. Lymph node response was evaluated by measuring the cellular content (indicator of cell proliferation) and weight of each animal's pooled lymph nodes. ***Adverse Effect(s) (brief):*** No signs of systemic toxicity were noticed. The test substance did not induce a statistically significant and biologically relevant increase in lymph node weights when applied as 3%, 10% or 30% preparations in DAE 4:3:3. Treatment of the mice with 30% test substance preparations induced a statistically significant increase in ear weights as compared to the vehicle control group. Based on the results it was determined that the test substance is negative for skin sensitization. ***Data Quality:*** *ECHA Reliability 2 (reliable with restrictions)*			
Dermal Irritation			
C.I. Pigment Yellow 138	Dermal irritation	No observable adverse effect reported	ECHA, 1975f
Test article: 3,4,5,6-tetrachloro-N-[2-(4,5,6,7-tetrachloro-2,3-dihydro-1,3-dioxo-1H-inden-2-yl)-8-quinolyl] phthalimide. ***Dose:*** Rabbits were treated using single exposure without washing of the eyes. 50 mg of test substance (100%) was applied. ***Treatment duration:*** 48hr, Observations were taken for 8-day post exposure. ***Species (strain):*** Rabbits (Vienna White). ***Gender:*** Female 3.17 kg, male: 3.68 kg. ***Number per Group:*** 2 ***Control:*** Control was talcum (amorph) into the other eye. ***Methods:*** Eye: Single exposure without washing of the eyes. The evaluation was carried out according to the Draize scoring system. ***Adverse Effect(s) (brief):*** After 8 days, only yellow substance residues in both animals was observed. No eye irritation occurred in rabbits following exposure or during the observation period. ***Data Quality:*** *ECHA Reliability 2 (reliable with restrictions)*			
C.I. Pigment Yellow 138	Dermal irritation	No observable adverse effect reported	ECHA, 1975a
Test article: 3,4,5,6-tetrachloro-N-[2-(4,5,6,7-tetrachloro-2,3-dihydro-1,3-dioxo-1H-inden-2-yl)-8-quinolyl] phthalimide. ***Dose:*** Rabbits were treated with 50 % aqueous solution in water. ***Treatment duration:*** for 20hr. Observations were taken for 8-day post exposure. ***Species (strain):*** Rabbits (Vienna White). ***Gender:*** Female 3.17 kg, male: 3.68 kg. ***Number per Group:*** 2 ***Control:*** untreated site of the same animal ***Methods:*** Dermal. occlusive treatment, 20 h treatment duration. A 2.5 x 2.5 cm area was used for exposure. Untreated site of the same animal was used as control. Descriptive scores of the raw data were converted to Draize numerical scores for final assessment. ***Adverse Effect(s) (brief):*** After 24 h and 8 days, only yellow substance residues in both animals, and slight scaling was observed. No skin irritation occurred in rabbits following exposure or during the observation period. ***Data Quality:*** *ECHA Reliability 2 (reliable with restrictions)*			

Section III. Dose-Response Assessment

III.1 Derivation of Provisional Tolerable Intake

Contact Duration	Exposure Route	Patient	POD	MF	pTI
Permanent	Parenteral	All	1,000 mg/kg/d	1,000	1 mg/kg/d

pTI = POD ÷ MF

Note: When a supplier-specific color additive safety data sheet (SDS) does not report the presence of a carcinogen or non-carcinogen impurity, and SDS complies with Globally Harmonized System (GHS) hazard communication limits (i.e., <0.1% and <1%, respectively), then this pTI is low enough to be protective for naturally occurring impurities which could be present. Processes used to color a polymer system involve diluting the pigment and its impurities by at least 50x (i.e., the CHRIS calculator is valid when the maximum concentration of pigment in a colored polymer is 2%, i.e., 1 ÷ 2% = 50); therefore, the concentration of naturally occurring impurities in the finished colored polymer will be significantly lower compared to the concentration of these substances in the pigment (i.e., 0.002% (i.e., 0.1% ÷ 50) for carcinogens and 0.02% (i.e., 1% ÷ 50) for non-carcinogens). Unknown impurities are addressed by the CHRIS calculator.

III.2 Derivation of a Modifying Factor

Critical Study	Critical Health Effect	Exposure Route	POD Type	POD Value	UF_1	UF_2	UF_3	MF
ECHA 2012b	discoloration of the glandular stomach contents	Oral	NOAEL	1000 mg/kg/d	10	10	10	1,000

MF = modifying factor (UF_1 x UF_2 x UF_3)

III.3 Toxicological Uncertainties Applied to POD

Uncertainty	Justification
UF_1 Error! Bookmark not defined.	(10) Default value to account for susceptible individuals.
UF_2 Error! Bookmark not defined.	(10) Default value to account for differences between species.
UF_3 Error! Bookmark not defined.	(10) Default value to account for differences in exposure route and data quality.

UF_1, UF_2, and UF_3, are used to extrapolate the POD to the general human population (including pediatric) exposed parenterally for a lifetime.

III. 4 Critical Health Study Design and Outcome

Reference: ECHA 2012b.			
Test Substance	C.I. Pigment Yellow 138		
Species	Rat/Crl:CD(SD)	Frequency	Daily
Gender	Male and female	Duration	28 days
Age	No age reported	#/Group	Not reported
Route	Oral gavage	Protocol Guideline	OECD Test Guideline 422; GLP compliant
Dose(s)	100, 300 and 1,000 mg/kg/day	Statistical Method(s)	Not reported
Observed Responses	No dose-response relationship was observed in the mean post implantation loss, fetal body weight, number of live offspring, and postnatal survival. No adverse effects were observed up to and including the limit dose.		
Notes:	No Adverse effects at the highest dose tested.		

METALLIC PHTHALOCHYANINES

Copper(II) Phthalocyanine (CuPc) – CAS # 147-14-8.[60]

Manganese(II) Phthalocyanine (MnPc) – CAS # 14325-24-7

Phthalocyanine Green (PcGn) – CAS # 1328-53-6.[61]

Toxicological Profile Summary

Copper phthalocyanine, manganese phthalocyannine, and phthalocyanine green are metallo-organic phthalocyanine (MPc) pigments used to impart color in plastic medical device components. When used for coloring plastics, optimal aggregate particle size of MPc is between 100 and 400 nanometers. MPc pigments are manufactured by chemical reaction of phthalonitrile or phthalic anhydride with metal salts. Some MPc require crystal stabilization and/or surface treatment to achieve desired stability, dispersibility and fastness characteristics in a polymer system. MPc size, shape, surface characteristics indicate low reactivity potential. Reported hazard information includes adverse health effect studies, supporting studies, and secondary sources. MPc doses above 40 mg/kg/day is reported to result in hemotological changes.[62]. The calculated provisional tolerable intake (pTI) value for systemic toxicity is 0.15 mg/kg/day based on (a) the lowest reported point-of-departure (40 mg/kg/day, oral, 28-day, rodent) and modifying factor of 270 to address the following sources of uncertainty: variation among humans (3), variation between species (3), and data quality/route-to-route extrapolation (30).

NOTE: This pTI is protective for the color additive including any impurities listed in Section I.1 "Substance Identity," as the impurities likely will be present at a very low percentage of the color additive, and the quantity of the impurity will be at an even lower percentage in the final device (see Section III, Table III.1 footnote). Unknown impurities are addressed by the CHRIS calculator.

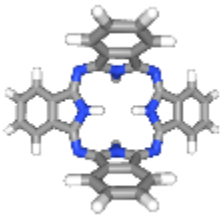
Note: This toxicity profile does not apply to non-metallic or other metallic phthalocyanines.

Section I. Substance Information

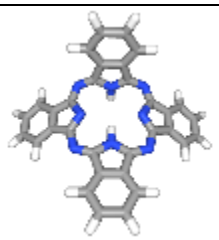
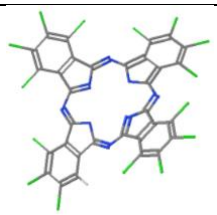
I.1 Substance Identity

Physical Form = gas, liquid, solid (at room temperature); Type = gas, liquid, or solid (include temp & pressure if available); RT = room temperature

I.2 Molecular Descriptors

Name	Short Name	CAS #	Formula	Mass	Structure
Copper(II) phthalocyanine	CuPc	147-14-8	$C_{32}H_{16}CuN_8$	578.0962	
3D Image.[63]					

[60] Includes Direct Blue 86 (CAS # 1330-38-7) and Acid Blue 185 (CAS # 12234-64-9/1330-39-8), which are same as CuPc plus sulfate groups (i.e., more water soluble).
[61] Includes Reactive Blue 21-CAS # 12236-86-1, which is similar to PcGn plus covalently bound sulfate groups (i.e., more water soluble).
[62] JECDB (date not reported) Phthalocyanine Blue. Japan Existing Chemical Data Base (JECDB). http://dra4.nihs.go.jp/mhlw_data/home/file/file147-14-8.html. Webpage accessed: January 21, 2017
[63] U.S. NLM (2020) Pigment Blue 15:3. PubChem. U.S. National Library of Medicine.

Name	Short Name	CAS #	Formula	Mass	Structure
Manganese(II) Phthalocyanine	MnPc	14325-24-7	$C_{32}H_{16}MnN_8$	567.4720	
3D Image[64]					
Phthalocyanine Green	PcGn	1328-53-6	$C_{32}Cl_{16}CuN_8$	1092.7569	
3D Image [65]					

CuPc and MnPc molecular formulas differ in the metal ion only. Unlike CuPc and MnPc, PcGn molecule contains chlorine atoms in place of hydrogen atoms, resulting in a molecular weight approximately 2x greater than CuPc and MnPc. CuPc, MnPc, and PcGn molecules contain an eight (8) ring structure. 3D images indicate CuPc molecule is less planar as compared to MnPc and PcGn molecules. Molecular structure is a determinant of particle crystal structure and stability.[66]

I.3 Particle Characteristics

Aggregates smaller than 100 nm absorb, rather than scatter, light.[67] In general, phthalocyanine pigments are more difficult to disperse, as compared to inorganic pigments, in a polymer/resin.[68]

I.4 Metallic Phthalocyanine Pigment Types, Stability, Incompatibilities, and Uses

Pc	Type	Color	Stability	Incompatibility	General Use
PB 15	α/non-stabilized	Reddish blue	Temperature: <200°C Chemical: Acids, alkali, soap, butter, and sterilization	Solvents: Aromatic Polymers: High temp processing	Printing inks (water based) Low temp polymers (e.g., PE and PVC) Rubbers Cosmetics, Soaps, Crayons Paper, Wax
PB 15:1	α/stabilized*	Reddish blue	Temperature: <300°C Chemical: Most acids and organic solvents Excellent migration fastness	Solvents: None reported Polymers: Low chlorinated Pc and partially crystalline thermoplastics (PE/PP) can result in polymer shrinkage	Coatings, paints, printing inks Plastics and Rubber

[64] U.S. NLM (2020a) Manganese phthalocyanine. PubChem. U.S. National Library of Medicine.
[65] U.S. NLM (2020b) Pigment Green 7 (CAS # 1328-53-6). ChemID Plus. U.S. National Library of Medicine (NLM)
[66] U.S. NLM (2020c) PubChem. U.S. National Library of Medicine (NLM). National Center for Biotechnology Information. PubChem.
[67] Schröder, J. (1984) Morphology of organic pigments with special reference to copper phthalocyanine. Prog. Org. Coat., 12, 181–210.
[68] Müller A. (2003) Coloring of Plastics: Fundamentals - Colorants – Preparations. Hanser Publications. https://doi.org/10.3139/9783446434158.fm

			Lightfast		
PB 15:2	α/stabilized, surface treated	Reddish blue	Temperature: <300°C Chemical: Most acids and organic solvents Excellent migration fastness Lightfast and weatherfast	Solvents: None reported Polymers: Low chlorinated Pc and partially crystalline thermoplastics (PE/PP) can result in polymer shrinkage	Same as PB 15:1, as well as, applications PB 15:1 does not perform.
PB 15:3	β	Greenish blue	Temperature: <300°C Chemical: Most acids and organic solvents Lightfast and weatherfast	Solvents: None reported Polymers: Low chlorinated Pc and partially crystalline thermoplastics (PE/PP) can result in polymer shrinkage	Coatings, paints, printing inks Plastics Rubbers Paper
PB 15:4	β/surface treated	Greenish blue	Temperature: <300°C Chemical: Most acids and organic solvents		Printing ink (toluene-based)
PB 15:6	ε	Strongly reddish blue			Color filters for LCD and TFT displays
Green 7	Not applicable	Bluish green	Temperature: <300°C Chemical: Most acids and organic solvents Lightfast and weatherfast		Coatings, paints, inks Plastics Textiles

Sources:[69] , [70] *Chlorination

Section II. Hazard Identification

Health effect data, supporting studies and secondary sources were reviewed, see tables in sections II.1 and II.2 for details.

Acute oral single administrations at doses up to 5000 mg/kg had no effects on survival, body weight, food consumption, or clinical symptoms[71].

Repeated intraperitoneal exposure (up to 2 g/kg/d) for 30-days resulted in no occurrence of mortality. Transient increase in blood ceruloplasmin in rats is reported[72]. In a 90-day oral study, the highest dose administered (i.e., 16000 and 20000 mg/kg/day for Males and Females, respectively) did not result in an observable adverse effect[73]. Carcinogenicity studies were conducted using mice exposed animals to Copper phthalocyanine 0.5 mg subcutaneously. This dose in mice resulted no increase of tumors compared to controls[74]. There were no effects on reproductive/developmental in rats exposed up to 1,000 mg/kg/day[75].

[69] Erk, Peter, and Heidi Henglesberg. “Chapter 119. Phthalocyanine Dyes and Pigments.” In The Porphyrin Handbook, edited by K. M. Kadish and S. R. Guilard, 19 / Applications of Phthalocyanines:105–46. New York, NY: Academic Press, 2003.
[70] Lomax, Suzanne Quillen. “Phthalocyanine and Quinacridone Pigments: Their History, Properties and Use.” Studies in Conservation 50, no. sup1 (June 1, 2005): 19–29. https://doi.org/10.1179/sic.2005.50.Supplement-1.19.
[71] Marhold, J.P. 1986 "Prehled Prumyslove Toxikologie; Organicke Latky,", Czechoslovakia, Avicenum, Vol. -, pg. 1329.
[72] Kurliandskii, B.A. et al. (1985) [Toxicity of copper phthalocyanine]. Gig Sanit, 92–3.
[73] ECHA, 1979c (PcGn))
[74] Ohba, K. (1990) Repeated oral administration toxicity test for 28 days using rats of Pigment Green No. 7.
[75] JECDB Phthalocyanine Blue. Japan Existing Chemical Data Base (JECDB): https://dra4.nihs.go.jp/mhlw_data/jsp/FileListPageENG.jsp?parameter_csno=147-14-8 .

II.1 Systemic, Genotoxicity, Carcinogenicity, Reproductive/Developmental Toxicity

ADVERSE HEALTH EFFECT STUDIES

Source Citation	Source Type	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
Systemic Toxicity (acute)							
(Kurliandskii *et al.*, 1985 (CuPc))	Journal Article	Animal	Oral (gavage)	6 g/kg 15 g/kg	Single administration	No observable adverse effect reported	NR
Test article: Pigment Blue 15 (99.8%, 0.2% copper salt). ***Species (strain):*** Rat (strain not reported). **Doses:** 6,000 mg/kg; 15,000 mg/kg ***Treatment duration:*** Single administration. ***Gender:*** Female. ***Number per Group:*** Not reported. ***Control***: NR***Adverse Effect(s) (brief):*** **NOAEL** adverse effect on survival, body weight, food consumption, or clinical symptoms. ***Data Quality:*** Not reported. ***Note:*** Not available in English ***Reference:*** Kurliandskii, B.A. et al. (1985) [Toxicity of copper phthalocyanine]. Gig Sanit, 92–3.							
(Kurliandskii *et al.*, 1985 (CuPc))	Journal Article	Animal	Intraperitoneal	3 g/kg	Single administration	No observable adverse effect reported	NR
Test article: Pigment Blue 15 (99.8%, 0.2% copper salt).***: Species (strain):*** Rat (strain not reported). **Doses:** 3,000 mg/kg ***Treatment duration:*** Single administration. ***Gender:*** Female. ***Number per Group:*** Not reported. ***Control***: ***Method: Adverse Effect(s) (brief):*** **NOAEL** No adverse effect on survival. ***Data Quality:*** Not reported. ***Note:*** Not available in English ***Reference:*** Kurliandskii, B.A. et al. (1985) [Toxicity of copper phthalocyanine]. Gig supSanit, 92–3.							
(Kurlandsky *et al.*, 1993 (DB86))	ND	Animal	Oral (gavage)	>5,000 mg/kg bw **(LD_{50})**	Single administration	No observable adverse effect reported	NR
Test article: Direct Blue 86 (CAS # 1330-38-7) (purity not reported). ***Dose:*** 5,000 mg/kg bw as suspension on the starch or dimethylsulphoxide. ***Treatment duration:*** Single administration. ***Species (strain):*** Rat (albino). ***Gender:*** NR. ***Number per Group:*** Not reported. ***Control***: Controls were used but no detail was provided. ***Method: Adverse Effect(s) (brief):*** **LD_{50} =** >5,000 mg/kg bw. No mortality occurred at 5,000 mg/kg bw. It was found that the test substance is adsorbed on the tissue surface and are linked with the proteins of blood serum, preferably albumines. Oral administration of 150 mg/kg bw does not "penetrate" blood. Other adverse effect(s) was/were not reported. ***Data Quality:*** NR. ***Reference****: Kurlandsky, B.A. et al. (1993) Toxic effect of copper phthalocyanine-based dyes. Curr. Toxicol., 1, 243–246.*							
(Kurlandsky *et al.*, 1993 (AB185))	ND	Animal	Oral (gavage)	>7,800 mg/kg bw. **(LD_{50})**	Single administration	No observable adverse effect reported	NR
Test article: Acid Blue 185 (CAS # 12234-64-9/1330-39-8) (purity not reported). **Doses:** 7,800 mg/kg bw as suspension on the starch or dimethylsulphoxide. Controls were used but no detail was provided. ***Treatment duration:*** Single administration. ***Species (strain):*** Rat (albino). ***Gender:*** Not reported. ***Number per Group:*** Not reported. Controls were used but no detail was provided. ***Method:*** NR ***Adverse Effect(s) (brief):*** LD_{50} = >7,800 mg/kg bw. No mortality occurred at 7,800 mg/kg bw. It was found that the test substance is adsorbed on the tissue surface and are linked with the proteins of blood serum, preferably albumines. Oral administration of 150 mg/kg bw does not "penetrate" blood. Other adverse effect(s) was/were not reported. ***Data Quality:*** NR. ***Reference:*** *Kurlandsky, B.A. et al. (1993) Toxic effect of copper phthalocyanine-based dyes. Curr. Toxicol., 1, 243–246.*							
(Kurlandsky *et al.*, 1993 (RB21))	ND	Animal	Oral (gavage)	>10,000 mg/kg bw **(LD_{50})**	Single administration	No observable adverse effect reported	NR
Test article: Reactive Blue 21 (CAS # 12236-86-1) (purity not reported). **Doses:** 10,000 mg/kg as suspension on the starch or dimethylsulphoxide. Controls were used but no detail was provided. ***Treatment duration:*** Single administration. ***Species (strain):*** Rat (albino). ***Gender:*** Not reported. ***Number per Group:*** Not reported. **Control**: Controls were used but no detail was provided. ***Adverse Effect(s) (brief):*** LD_{50} = >10,000 mg/kg bw. Oral administration of 150 mg/kg bw does not "penetrate" blood. Other adverse effect(s) was/were not reported. ***Data Quality:*** NR. ***Reference:*** *Kurlandsky, B.A. et al. (1993) Toxic effect of copper phthalocyanine-based dyes. Curr. Toxicol., 1, 243–246.*							
(Kurlandsky *et al.*, 1993 (RB21))	ND	Animal	Oral (gavage)	>5,800 mg/kg bw **(LD_{50})**	Single administration	No observable adverse effect reported	NR
Test article: Reactive Blue 21 (CAS # 12236-86-1) (purity not reported). **Doses:** 5,800 mg/kg bw as suspension on the starch or dimethylsulphoxide. ***Treatment duration:*** Single administration. ***Species (strain):*** Mouse (albino). ***Gender:*** Not reported. ***Number per Group:*** Not reported***. Control:*** Controls were used but no detail was provided. ***Adverse Effect(s) (brief):*** **LD_{50} = >5,800 mg/kg bw.** Mortality occurred at 5,800 mg/kg bw. It was found that the test substance is adsorbed on the tissue surface and is linked with the proteins of blood serum, preferably albumines. Oral administration of 150 mg/kg bw does not "penetrate" blood. Other adverse effect(s) was/were not reported. ***Data Quality:*** NR. ***Reference:*** *Kurlandsky, B.A. et al. (1993) Toxic effect of copper phthalocyanine-based dyes. Curr. Toxicol., 1, 243–246.*							

(Kurlandsky *et al.*, 1993 (DB86))	ND	Animal	Intraperitoneal	800 mg/kg bw **(LD_{50})**	Single administration	No observable adverse effect reported	NR
Test article: Direct Blue 86 (CAS # 1330-38-7) (purity not reported). **Doses:** 800 mg/kg bw as suspension on the starch or dimethylsulphoxide. ***Treatment duration:*** Single administration. ***Species (strain):*** Rat (albino). ***Gender:*** Not reported. ***Number per Group:*** Not reported. ***Control:*** Controls were used but no detail was provided ***Method: Adverse Effect(s) (brief):*** LD_{50} = 800 mg/kg bw. No mortality occurred at 800 mg/kg bw. It was found that the test substance is adsorbed on the tissue surface and islinked with the proteins of blood serum, preferably albumines. Oral administration of 150 mg/kg bw does not "penetrate" blood. Other adverse effect(s) was/were not reported. Other adverse effect(s) was/were not reported. ***Data Quality:*** NR. ***Reference:*** *Kurlandsky, B.A. et al. (1993) Toxic effect of copper phthalocyanine-based dyes. Curr. Toxicol., 1, 243–246.*							
(Kurlandsky *et al.*, 1993 (AB185))	ND	Animal	Intraperitoneal	1,400 mg/kg bw **(LD_{50})**	Single administration	No observable adverse effect reported	NR
Test article: Acid Blue 185 (CAS # 12234-64-9/1330-39-8) (purity not reported). **Doses:** 1,400 mg/kg bw as suspension on the starch or dimethylsulphoxide. ***Treatment duration:*** Single administration. ***Species (strain):*** Rat (albino). ***Gender:*** Not reported. ***Number per Group:*** Not reported. ***Control:*** Controls were used but no detail was provided ***Method: Adverse Effect(s) (brief):*** **LD_{50} = 1,400 mg/kg bw.** No mortality occurred at 1,400 mg/kg bw. It was found that the test substance is adsorbed on the tissue surface and islinked with the proteins of blood serum, preferably albumines. Oral administration of 150 mg/kg bw does not "penetrate" blood. Other adverse effect(s) was/were not reported. ***Data Quality:*** NR. ***Reference:*** *Kurlandsky, B.A. et al. (1993) Toxic effect of copper phthalocyanine-based dyes. Curr. Toxicol., 1, 243–246.*							
(Kurlandsky *et al.*, 1993 (RB21))	ND	Animal	Intraperitoneal	620 mg/kg bw **(LD_{50})**	Single administration	No observable adverse effect reported	NR
Test article: Reactive Blue 21 (CAS # 12236-86-1) (purity not reported). **Doses:** 620 mg/kg bw as suspension on the starch or dimethylsulphoxide. ***Treatment duration:*** Single administration. ***Species (strain):*** Mouse (albino). ***Gender:*** Not reported. ***Number per Group:*** Not reported. ***Control:*** Controls were used but no detail was provided ***Method: Adverse Effect(s) (brief):*** LD_{50} = 620 mg/kg bw. Less than 6% of 150 mg/kg bw injected intraperitoneally is found in blood, and <8% of same dose is "*removed with urea*". Majority (70.5%) of DB86 in blood is bound to proteins (mostly albumin). Intraperitoneal toxicity is correlated with protein binding in blood. Other adverse effect(s) was/were not reported. ***Data Quality:*** NR. ***Reference:*** *Kurlandsky, B.A. et al. (1993) Toxic effect of copper phthalocyanine-based dyes. Curr. Toxicol., 1, 243–246.*							
Systemic Toxicity (subacute/subchronic/chronic)							
(Kurliandskii *et al.*, 1985 (CuPc))	Journal Article	Animal	Intraperitoneal	2 g/kg	Daily for 30 days	Increased ceruloplasmin (transient)	NR
Test article: Pigment Blue 15 (99.8%, 0.2% copper salt). ***Dose: Species (strain):*** Rat (strain not reported). **Doses:** 2,000 mg/kg ***Gender:*** Not reported. ***Number per Group:*** 50. ***Observations (brief):*** No treatment-related effect on: survival, body weight food consumption, clinical symptoms, clinical chemistry, or organ weight. Ceruloplasmin (a marker of copper toxicity) was increased after 10 days of treatment; but returned to normal by study termination. Only 0.01% of administered dose was absorbed and distributed to the circulation. Removal of 0.2% copper salt eliminated the increased ceruloplasmin of CuPc treated rats. ***Data Quality:*** Not reported. ***Reference:*** *Kurliandskii, B.A. et al. (1985) [Toxicity of copper phthalocyanine]. Gig Sanit, 92–3.*							

Systemic Toxicity (subacute/subchronic/chronic)							
Oba, 1990 (PcGn))	N	Animal	Oral (gavage)	1,000 mg/kg/day (NOAEL)	Daily for 28 days	No observable adverse effect reported	NR

Test article: Pigment green no. 7 (purity 99.1%) **Doses:** 0,100,300 and 1000 mg/kg in corn oil; 5 females and 5 females in the group, and a recovery group of 0 and 1000 mg/kg. ***Treatment duration:*** 28-days, followed by recovery group for 14 days. ***Species (strain):*** Rat (Sprague Dawley). 5 weeks. ***Gender:*** Male (138-156g) /Female (110-120g). ***Number per Group:*** 5/sex/dose. ***Control:*** untreated group ***Method:*** The test substance was suspended in corn oil and administered orally to the stomach using gastric sonde. The dosage volume was 0.5 mL per 100 g of body weight. Only media were administered to the control group. The administration period All animals were observed 3 times daily (before administration, 1 and 5 hours after administration) during the administration period, and the presence or absence of poisoning symptoms, behavioral abnormalities, animals approaching death and dead animals, etc. were recorded. Body weight was measured once a week from the start of administration to the end of the recovery period. Bodyweight was measured once a week from the start of administration to the end of the recovery period. The remaining amount of feed was measured once a week, and the feed intake (g/week) was calculated. Samples were collected for hematology test, blood biochemical testing, and urine test. The animals were anesthetized at the end of the administration period and the end of the recovery period, and the pathological autopsy was performed by hemolysesthesize the animal. Weight was measured for the brain, liver, kidneys, adrenal glands, thymus, heart, spleen, testes, epidesodic and ovaries, and the organ weight/weight ratio was calculated. The above weight measurement organ and spinal cord, pituitary gland, eyeball, salivary gland (submandibular gland, sublingus gland), thyroid gland, epithelial body, lung (injection fixation, including bronchi), trachea, pancreas, stomach, small intestine (mr. paiel board) including), large intestine, secrotum, prostate, uterus, vagina, bladder, peripheral nerve (sciatic nerve), lymph nodes (mandibular lymph nodes, mesometic lymph nodes), bone marrow (femur), aorta, skin, mammary glands, other organs and tissues that had changed in naked eye observation were fixed with a sufficient amount of 10% buffer formalin solution. however, the testes and epidesis were pre-fixed with buan solution and then fixed with 10% buffer formalin solution. Histopathological examinations were performed on the lungs (including bronchi) and liver and control groups and high dose groups of thymus, heart, spleen, kidneys, adrenal glands, stomach, small intestine, large intestine, testes, epididyms, uterus, ovaries and bone marrow (femur) of all animals dissected at the end of the administration period. paraffin embedding and thinning were stained with hematoxylin eoline and mirrored according to the normal method. ***Adverse Effect(s) (brief):*** **NOAEL = 1,000 mg/kg/day.** Following the observation period autopsy revealed no lesions in the test drug administration group compared to the control group, but green spots/areas of the lungs were observed in a female group of 1000 mg/kg. In addition, scarring of the kidneys was observed in both males and females. In the observation of the general condition, no abnormality was observed in both males and females, and there were no deaths. In addition, in weight, feeding quantity, hematology test, hematology test, urine test and pathology test, there was no change caused by the administration of the test substance in both males and females. ***Data Quality:*** Insufficient information is reported to evaluate study reliability. ***Reference:*** *Ohba, K. (1990) Repeated oral administration toxicity test for 28 days using rats of Pigment Green No. 7.*

Carcinogenicity							
Haddow and Horning, 1960 (CuPc))	Journal Article	Animal	Subcutaneous	0.5 mg	Weekly / 8 months	No tumors	NR

Test article (purity): Copper phthalocyanine (purity not reported), **Doses:** 0.5 mg vehicle not reported. ***Treatment duration:*** Weekly / 8 months. ***Species (strain):*** Stock mice. ***Number per Group:*** 20. ***Gender:*** NR. ***Control:*** It is not specified if controls were used. ***Method:*** All preparations were administered by repeated subcutaneous injection in the flank at weekly intervals on 34 occasions. Survival and tumor numbers were observed. ***Adverse Effect(s) (brief):*** The weekly subcutaneously dose of 0.5mg administered for 8 months resulted in no tumors. 17/20 mice survived the duration of the study. No other effects were reported. ***Data Quality:*** Not reported. ***Note*****:** Data quality cannot be assessed because the report contains insufficient information. Animal body weight was not reported. Organs removed for pathology was not reported. ***Reference:*** *Haddow, A, and E S Horning. "On the Carcinogenicity of an Iron-Dextran Complex." Journal of the National Cancer Institute 24 (January 1960): 109–47.*

SECONDARY STUDIES

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
Systemic Toxicity (acute)							
ECHA, 1971a (CuPc))	N	Animal	Oral (gavage)	NR	Single administration	No observable adverse effect reported	Similar to OECD 401/non-GLP
Test article: [29H,31H-phthalocyaninato(2-)-kappa~2~N~29~,N~31~]copper. ***Species (strain):*** Rat (Sprague Dawley). **Doses:** Rats received oral doses of 200, 1600, and 6400 mg/kg bw (purity 100%) in carboxymethyl cellulose (CMC). ***Treatment duration:*** Single administration, followed by 14 day observation period ***Gender:*** Male (163g) /Female (145g). ***Number per Group:*** 5/sex/dose. **Control:** No controls were used ***Method:*** Several groups of 5 rats per sex and dose were treated simultaneously by gavage with preparations of the test substance in suitable vehicle. The concentrations of these preparations were used to achieve comparable volumes per kg body weight. Group-wise documentation of clinical signs was performed over the 14-day study period. The LD50 value was estimated based on the observed mortalities. ***Adverse Effect(s) (brief):*** Green feces were observed in all animals treated 24 hours after application of the test material. Dyspnea was observed in animals of the 1600 mg/kg bw group immediately after treatment but was reversible. No abnormality was found in the organs. The body weights of the individual animals were gathered prior to application of the test material only. No further documentation of body weight data was available. ***Data Quality:*** Data quality cannot be verified because information is from a secondary source; however, secondary source (ECHA) has assigned a reliability (Klimisch) score of 2 (reliable with restrictions). ***Note:*** Biological observations were at 1, 4, hours and at 1-, 4-, 5-, 6-, 7-, 8-, 11-, 12-, and 13-days post-treatment. Biological endpoints included: clinical behavior, body weights, and necropsy.							
ECHA, 1990 (CuPc))	N	Animal	Dermal (occlusive)	NR	24 hr	No observable adverse effect reported	OECD 402/non-GLP
Test article: 29H,31H-phthalocyaninato(2-)-kappa~2~N~29~,N~31~]copper (purity 100%). ***Species (strain):*** Rat (Wistar). **Doses:** 5,000 mg/kg bw in water.. ***Treatment duration:*** Single administration, followed by 14 observation period. ***Gender:*** Male. ***Number per Group:*** 5/dose. **Control:** No controls were used ***Method:*** 24 h before application of the test substance, the fur on the back of the animals was shaved at area about 6 cm x 6 cm. Aliquot part of the test material was applied on the depilated area of skin. The application site was covered by mull, plastic foil and held in contact by plaster (strapping). After 24 h the occlusive dressing and the remains of the test substance were removed. Animals were weighed before application, at 8th day and before euthanasia of animals. Average body weight in a group was calculated from individual body weights. All test animal survivors to the end of the study were sacrificed on the 15th day and gross necropsy was carried out. Normally the nutritious state, body surface, body foramina, thoracic, abdominal and cranial cavity were evaluated. ***Adverse Effect(s) (brief):*** No clinical signs of toxicity were observed during the study period. Body weight gains were within the the physiological range. No individual records of macroscopic changes were found. ***Data Quality:*** Data quality cannot be verified because information is from a secondary source; however, secondary source (ECHA) has assigned a reliability (Klimisch) score of 2 (with restrictions).							
ECHA, 1971c (PcGn))	N	Animal	Oral (gavage)	NR	Single administration	No observable adverse effect reported	OECD 401/non-GLP
Test article: polychloro copper phthalocyanine (purity 100%) in water containing CMC. ***Species (strain):*** Rat (Sprague Dawley). **Doses:** 0, 200, 1600,3200,6,400 mg/kg bw in water. No controls were used. ***Treatment duration:*** Single administration. ***Gender:*** Male/Female. ***Number per Group:*** 5/sex/dose. **Control:** No controls were used***. Method:*** Animals were observed and examined for clinical signs of toxicity during the first hour following application, after 4 and 5 hours and further on day 1, 2, 5, 6, 7, 8, 9, 12, 13 and 14 after dosing. The body weights of the individual animals were gathered prior to application of the test material. Necropsy of survivors performed: Deceased animals and those sacrificed at the end of the observation period (on day 14 after dosing) were necropsied. ***Adverse Effect(s) (brief):*** Green feces were observed in all treated animals 24 hours after application. Dyspnea was observed in 1600 mg/kg bw group immediately after treatment but was reversible. The body weights of the individual animals were gathered prior to application of the test material only. No further documentation of body weight data was available. No abnormality was found in the organs. ***Data Quality:*** Data quality cannot be verified because information is from a secondary source; however, secondary source (ECHA) has assigned a reliability (Klimisch) score of 2 (with restrictions). ***Note:*** Biological observations were at 0.25, 0.5, 1, 2, 4, 5 hours and after 1-, 2-, 3-, 6-, 7-, 8-, 9-, 10-, 13- and 14-days post-treatment. Biological endpoints included: clinical behavior, body weights, and necropsy.							
ECHA, 1980d (PcGn))	N	Animal	Oral (gavage)	>5,000 mg/kg bw (LD_{50})	Single administration	No observable adverse effect reported	Similar to OECD 401/non-GLP
Test article: polychloro copper phthalocyanine (purity 98%) **Doses:** 5,000 mg/kg bw in suspension containing 0.5 % CMC and 1-2 drops Cremophor EL. ***Treatment duration:*** Single administration. ***Species (strain):*** Rat (Sprague Dawley). ***Gender:*** Male (180g) /Female (160g). ***Number per Group:*** 5/sex/dose. **Control:** No controls were used**.** ***Method:*** Animals were observed and examined for clinical signs of toxicity during the first hour following application at 15 min, 30 min and 60 min, after 2, 4 and 5 hours and further on day 1, 2, 3, 6, 7, 8, 9, 10, 13 and 14 after dosing. The body weights of the individual animals were gathered prior to application of the test material and on day 3, 7 and 13 after dosing. Deceased animals and those sacrificed at the end of the observation period (on day 14 after dosing) were necropsied. ***Adverse Effect(s) (brief):*** **LD_{50} >5,000 mg/kg bw.** All animals survived; no mortality was observed. Dyspnea, apathy, green feces as well as a poor general state were observed. The average body weight in the male group increased within the normal range. A slight decrease of the average body weight in the female group was seen on day 13. Autopsy revealed no relevant findings. ***Data Quality:*** Data quality cannot be verified because information is from a secondary source; however, secondary source (ECHA) has assigned a reliability (Klimisch) score of 2 (with restrictions). ***Note:*** Biological observations were at 4, 5 hours and after 1-, 2-, 5-, 6-, 7-, 8-, 9-, 12-, 13- and 14-days post-treatment. Biological endpoints included: clinical behavior, body weights, and necropsy.							

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
ECHA,2001 (PcGn))	N	Animal	Oral (gavage)	>2,000 mg/kg bw (LD_{50})	Single administration	No observable adverse effect reported	OECD 401/GLP
Test article: polychloro copper phthalocyanine (99.04 %) **Doses:** 2,000 mg/kg bw in olive oil ***Treatment duration:*** Single administration, followed by 14 observation period. ***Species (strain):*** Rat (Crj: CD(SD)). 5 weeks ***Gender:*** Male (153 - 160g)/Female (121 - 135g). ***Number per Group:*** 5/sex/dose. **Control:** controls were not defined ***Method.*** Animals were dosed and observed for 14-days. observations daily, weighing immediately before administration then 3, 7 and 14 days after administration. All animals were necropsied. ***Adverse Effect(s) (brief):*** **LD_{50} >2,000 mg/kg bw.** A greenish stool, that matched the color of the test substance, was observed in all rats of both sexes 1 day after administration. Animals gained weight throughout the observation period. No other abnormalities were found. Treatment related adverse effects were not reported. ***Data Quality:*** Data quality cannot be verified because information is from a secondary source; however, secondary source (ECHA) has assigned a reliability (Klimisch) score of 1 (without restrictions).							
ECHA, 1981 (PcGn))	N	Animal	Intraperitoneal	>2,000 mg/kg bw (LD_{50})	Single administration	No observable adverse effect reported	NR, non-GLP
Test article: Heliogengruen D 8730 (solide) (purity ca. 98%). **Doses:** 700, 2000 mg/kg bw in suspension containing 0.5 % CMC and 1-2 drops Cremophor EL. ***Treatment duration:*** Single administration, followed by 14-day recovery period. ***Species (strain):*** Mouse (NMRI). ***Gender:*** Male (30g) /Female (26g). ***Number per Group:*** 5/per sex/ per dose. **Control:** No controls were used ***Method:*** Test groups consisting of 5 animals/sex/group were treated with several doses of an aqueous suspension of the test substance applied intraperitoneally. After exposure, the animals were observed for a period of 14 d within which time signs of clinical toxicity as well as mortality were monitored and recorded. Animals found dead during the observation period were subjected to necropsy. At conclusion, the surviving animals were also sacrificed for the purpose of necropsy. The LD50 value was estimated based on the observed mortalities. ***Adverse Effect(s) (brief):*** **LD_{50} >2,000 mg/kg bw**. Dyspnoea, apathy, spastic walk, stretching, unkempt fur, exsiccosis and poor general condition were noted within the first 5 hours after treatment. Green unkept fur and green urine were noted in the 2000 mg/kg bw group on day 3 and 4. The body weight gain was within the normal range. Autopsy revealed incorporation of substance in the abdomen and coloration of fat tissue. ***Data Quality:*** Data quality cannot be verified because information is from a secondary source; however, secondary source (ECHA) has assigned a reliability (Klimisch) score of 2 (with restrictions).							
Systemic Toxicity (subacute/subchronic/chronic)							
FR 1989 (PcGn and MnGn)	N	Animal	Oral (gavage)	NR	Daily for 13 weeks	No observable adverse effect reported	NR
Test article: PcGn (pigment green 7) and MnGn (pigment blue 15). ***Species (strain):*** Rat and mouse. **Doses:** NR ***Treatment duration:*** 13- week. ***Gender:*** NR. ***Number per Group:*** NR. **Control:** NR ***Method:*** NR ***Adverse Effect(s) (brief):*** No adverse treatment-related effects were observed except for decreased body weight gain in rats exposed to PcGn. ***Data Quality:*** Data quality cannot be verified because information is from a secondary source.							
FR 1989 (PcGn and MnGn)	N	Animal	Oral (gavage)	NR	Daily for 13 weeks	No observable adverse effect reported	NR
Test article: PcG and MnGn. ***Species (strain):*** Rat and mouse. **Doses:** dose(s) not reported ***Treatment duration:*** 13-week. ***Gender:*** NR. ***Number per Group:*** NR. ***Method:*** NR. **Control:** NR. ***Adverse Effect(s) (brief):*** No adverse treatment-related effects were observed except for statistically significant elevations of copper in the liver and kidney exposed to MnGn and in the liver and kidneys of mice exposed to PcGn. ***Data Quality:*** Data quality cannot be verified because information is from a secondary source. ***Note:*** Study was conducted by the National Toxicology Program (NTP).							
ECHA, 1979b (PcGn))	N	Animal	Oral (feed)	4600 (M), 5000 (F) mg/kg bw **(NOAEL)**	Daily for 90 days	No observable adverse effect reported	Similar to OECD 408/non-GLP
Test article: polychloro copper phthalocyanine (purity 97.8%) was mixed with water to facilitate incorporating into feed. **Doses:** 0 (untreated control), 300, 600, 1200 (M), 1250 (F), 2300 (M), 2500 (F) and 4600 (M), 5000 (F) mg/kg bw ***Treatment duration:*** 90-day (daily). ***Species (strain):*** Rat (Fischer 344). ***Gender:*** Male/Female. ***Number per Group:*** 10/sex/dose. **Control:** untreated 10 control rats. ***Method:*** The concentrations of the chemical mixture were the same for male and female rats. All dose levels were prepared on a weight per weight basis. There were 5 dose level groups with 10 individuals of each sex in each dosage and control group. Each dosed group received 90 consecutive days of dosed feed mixture. After one day of observation, the animals were necropsied. Animals were observed twice each day for clinical signs, with at least 6 hours between observations. All observations were recorded daily. Additionally, blood sampling was conducted from 10 control rats, 5 males and 5 females. Copper analyses were completed in the liver and kidney tissues and the formalin preserving those tissues from male rats in the highest dose group (5 % w/w) and control groups. Microscopic examinations were performed on all tissues from all animals in the control group and the highest dose treatment group: Kidney, liver, lung, heart, testis, epididymis, prostate, stomach, thyroid, skin, cecum, pancreas and spleen. ***Adverse Effect(s) (brief):*** **NOAEL=4600 mg/kg/day (males); NOAEL=5000 mg/kg/day (females).** There were dose-related trends in body weights among both male and female rats, when body weights are expressed in percent differentials of weight							

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
gains compared with untreated controls. Male rats at the highest doses of 5.0 % and 2.5 % had differential weight gains of -11 % and -5 % respectively. Female rats at the top doses of 5.0 and 2.5 % had differential weight gains of -9.0 %. There were slight trends in patterns of diet consumption between dosed and control rats of both sexes, with dosage groups eating about 1 g more of the higher concentrations of the dosed feed. This may be an adjustment in diet intake to offset the relatively high portion of non-caloric dyestuff present in the diet. Daily average diet consumption for female rats ranged from 11.5 g (controls) to as much as 12.7 g (1.25 %); for male rats, the range was from 14.6 g (controls) to 16.6 g (2.5 %). No systemic uptake after ingestion. No clinical chemistry, hematology, or urinalysis were conducted, and no organ weights were taken. Treatment, including highest dose, did not result in an observable adverse effect. Highest dose was NOAEL (4600 mg/kg/day for males and 5000 mg/kg/day for females). ***Data Quality:*** Data quality cannot be verified because information is from a secondary source; however, secondary source (ECHA) has assigned a reliability (Klimisch) score of 2 (with restrictions).							
ECHA, 1979c (PcGn))	N	Animal	Oral (feed)	16000 mg/kg/day (Males) **(NOAEL)** 20000 mg/kg/day (Females) **(NOAEL)**	Daily for 90 days	No observable adverse effect reported	Similar to OECD 408/non-GLP
Test article: Pigment Green 7, C.I. 74260 (purity 97.8%) **Doses:** 0, 1000, 2000, 4000, 8000 or 16000 mg/kg/day; Females: 0, 1200, 2500, 5000, 10000 and 20000 mg/kg/day was mixed with water to facilitate incorporating into feed. ***Treatment duration:*** 90-days. ***Species (strain):*** Mouse (B6C3F1) 8.5 weeks. ***Gender:*** Male (20 - 24 g) /Female (15 - 18 g). ***Number per Group:*** 10/sex/dose. **Control:** untreated diet. ***Method:*** Five dose levels of 0.0, 0.3, 0.6, 1.25, 2.5 and 5.0 % in feed were used in this study (approx. 0, 1000, 2000, 4000, 8000 or 16000 mg/kg/day for males, [based on 8.2 g/d average food consumption, 0.026 kg average bw] and approx. 0, 1200, 2500, 5000, 10000 and 20000 mg/kg/day for females [based on 7.7 g/d average food consumption, 0.019 kg average bw]). The selected doses were prepared by mixing weighed portions of Purina Lab Chow in meal form with weighed portions of the chemical. Each dosed group received dosed feed mixture on 91 consecutive days. Mice were necropsied on day 92 and 93. Animals were observed twice each day for clinical signs, with at least 6 hours between observations. All clinical signs were recorded daily. Additional studies included blood sampling for the animal disease screening program from 10 control mice, 5 males and 5 females. Microscopic examinations were performed on following organs from all animals in the control group and the highest dose treatment group: Kidney, liver, lung, heart, epididymis, stomach, thyroid, skin (only in control group: bone marrow, urinary bladder, testis). Copper analyses were completed in the liver and kidney tissues and the formalin preserving those tissues from male mice in the highest dose group (5 % w/w) and control groups. ***Adverse Effect(s) (brief):*** NOAEL=16,000 mg/kg/day (males); NOAEL= 20,000 mg/kg/day (females). Treatment, including highest dose, did not result in an observable adverse effect. Highest dose was considered to be NOAEL (16,000 mg/kg/day for males and 20,000 mg/kg/day for females). ***Data Quality:*** Data quality cannot be verified because information is from a secondary source; however, secondary source (ECHA) has assigned a reliability (Klimisch) score of 2 (with restrictions).							
(JECDB (date not reported) (CuPc))	N	Animal	Oral (gavage)	40 mg / kg **(NOEL)**	28-day	mild effect on the erythrocyte	NR
Test article: Copper phthalocyanate. **Doses:** 0, 40, 200 and 1000 mg / kg of copper phthalocyanine blue in corn oil as a solvent. ***Treatment duration:*** 28 days, followed by with a recovery period of 14 days was set up in the 1,000 mg / kg group and the control group. ***Species (strain):*** Rat (Wistar). 5-weeks. ***Gender:*** Male (101.8 to 120.0g) /Female (95.3 to 111.1g). ***Number per Group:*** 10/sex/dose. **Control:** untreated group ***Method:*** General conditions were observed every morning and dead and moribund animals were necropsied immediately after discovery. Body weight and food intake were measured twice a week (three times only in the first week of males). At the end of the 14-day recovery period, 5 patients in each of the control and L, M, and H groups, and 5 patients in the recovery control group and recovery H group 24 hours after the final administration, 1.7% of the body weight from the orbital plexus under ether anesthesia. The following amounts of blood were collected and hematological and serum biochemical tests were performed. After blood collection, the animals were exsanguinated from the carotid artery and pathologically examined. Blood was collected from the jugular vein under ether anesthesia at the same time for 5 patients in each group different from the animals that underwent these tests, and blood coagulation ability tests were performed. In addition, urinalysis (pH, protein, urobilinogen, ketone bodies, glucose, occult blood) was performed using urolabustics for 5 patients in each group by forced urination on the day before the final administration day. Animals killed by jugular artery bleeding were promptly necropsied to weigh the brain, heart, lungs, liver, kidneys, spleen, testes, ovaries, adrenal glands, salivary glands (part of the submandibular and sublingual glands) and thymus. In addition to these, the pituitary gland, eyeball, thymus, testis, and macroscopically altered organs / tissues were excised and fixed with 10% neutral buffered formalin. Paraffin sections were prepared according to a conventional method for the heart, liver, spleen, kidney, adrenal gland, and organs / tissues with macroscopic changes, stained with hematoxylin / eosin, and histopathological examination was performed. The kidneys were examined by PAS staining. ***Adverse Effect(s) (brief):*** NOEL =40 mg / kg. No difference was observed between the control group and the administration group in terms of body weight and food intake, and no effect of administration was observed. Hematological examination after 28 days of administration showed a significant decrease in RBC and a tendency to decrease Hb and PCV in the 200 and 1,000 mg / kg groups of males, and the change was very small. A dose correlation was found in these animals. In addition, a slight increase in erythroblasts in the female							

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
recovery 1,000 mg / kg group and an increase in the actual weight and specific weight of the spleen in the male recovery 1,000 mg / kg group suggest a weak effect on the erythrocyte system. Histopathological examination showed no changes that seemed to be due to the specimen. In the male recovery 1,000 mg / kg group, an increase in the actual weight of lungs, spleen, testis, adrenal glands, and salivary glands was observed, and a slight increase in specific weight was also observed. From the above results, it was considered that the effect of repeated oral administration of copper phthalocyanate for 28 days was only the mild effect on the erythrocyte system observed from the 200 mg / kg group, and the NOEL was 40 mg / kg. ***Data Quality:*** Data quality cannot be verified because information is from a secondary source; however, secondary source has assigned a reliability (Klimisch) score of 1 (without restrictions). ***Note***: Biological endpoints included: reproductive ability/delivery, maternal behavior, viability, clinical signs, body weight change and autopsy of pups. *OECD Preliminary Reproduction Toxicity Screening Test. ***Reference:*** JECDB Phthalocyanine Blue. Japan Existing Chemical Data Base (JECDB).							
Reproductive/Developmental Toxicity							
(JECDB (date not reported) (CuPc))	N	Animal	Oral (gavage)	1000 mg/kg/day **(NOEL)**	Male, 42 days Female, from 14 days before mating to day 3 of lactation	No observable adverse effect reported	NR
Test article: Copper phthalocyanine blue. (purity: 99.55%) ***Doses:*** 0, 40, 200 and 1000 mg / kg / day in 0.5% CMC-Na solution. Controls were administered with a 0.5% CMC-Na solution ***Treatment duration***: Male, 42 days; Female, from 14 days before mating to day 3 of lactation. ***Species (strain):*** Rat (Crj: CD (SD) SPF) 8-weeks. ***Gender:*** Male/Female. ***Number per Group:*** 12/sex/dose. ***Control:*** were administered with a 0.5% CMC-Na solution, for a total of 4 groups. ***Method:*** The administration period is 14 days before mating and mating period until mating is established for males, 46 days after the start of administration for mating cases, 14 days before mating and mating period until mating is established for females, and mating. The established cases were the gestation period and the period up to 3 days of nursing. The number of animals was 12 for each male and female per group. Grouping was performed by a weight-based stratified random sampling method so that the weight of each group was uniform on the final day of acclimatization (the day before the start of administration). All patients were measured 1 day after administration (before administration), 2, 5, 7, 10 and 14 days after administration, every 7 days thereafter (including the end date of administration), and further on the day of autopsy. In addition, the amount of weight gain and the rate of weight gain were calculated. All cases were measured 1 day after administration (before administration), 2, 5, 7, 10 and 14 days after administration, and thereafter on the same day as the body weight measurement day (including the end date of administration) except for the mating period. Males on day 14 and females in the same study group were allowed to live together one-on-one in the evening for up to 14 days. Mating was established when sperm were confirmed in the vagina of the partner female, and pregnancy was established when implantation marks were confirmed in the uterus of the partner female. The cases in which copulation was established were exsanguinated under ether anesthesia on the day after the 46th day of administration, and the cases in which copulation was not established were lethal under ether anesthesia, and the organs and tissues of the whole body were visually observed. In all cases, at autopsy, liver, kidney, spleen, heart, lung, brain, pituitary gland, adrenal gland, thyroid gland, epithelial body, thymus, mesenteric lymph node, pancreas, tongue, mandibular lymph node, submandibular gland, tongue Lower gland, parotid gland, mammary gland, skin, eyeball, harder gland, thymus and femoral bone (including bone marrow), spinal cord (cervical), skeletal muscle (lateral broad muscle), thoracic aorta, laryngeal, trachea, bronchus, esophagus , Gastric (antrum, glandular stomach), duodenum, empty intestine, ileum, cecum, colon, rectum, bladder, testis, upper body, sperm sac (including coagulation glands), prostate and abnormal findings 10% neutral buffer It was fixed with a formalin solution and stored. The eyeball and Harder gland were fixed and preserved with Davidson's solution, and the testis and epididymis were fixed and preserved with Buan's solution. Of the excised organs, the testes and epididymis of all cases were embedded in paraffin and then sliced to prepare hematoxylin / eosin-stained specimens, which were examined microscopically. In females, for all cases, administration 1 day (before administration), administration 2, 5, 7, 10 and 14 days, pregnancy 0, 1, 3, 5, 7, 10, 14, 17 and 20 days, nursing 1 and 4 days In addition, the amount of weight gain and the rate of weight gain during the administration period, the gestation period, and the nursing period were calculated All cases were measured on 1 day of administration (before administration), 2, 5, 7, 10 and 14 days of administration, 1, 3, 5, 7, 10, 14, 17 and 20 days of pregnancy, and 1 and 4 days of nursing. For all cases, the delivery status, maternal behavior, the number of surviving and dead pups, the sex of the offspring and the external table were observed. In addition, the implantation rate, the birth rate, the delivery rate, the birth rate, the nursing rate and the sex ratio were calculated. The gestation period was calculated as 21 days. In all cases, at the time of autopsy, the organs and tissues similar to those of males, as well as the ovaries, uterus, vagina, and abnormal findings were fixed with 10% neutral buffered formalin and stored. The eyeball and Harder gland were fixed and preserved with Davidson's solution. Of the excised organs, the ovaries of all cases and the abnormal findings (spleen and mammary gland) of the 40 and 200 mg / kg groups were sliced after paraffin embedding, and hematoxylin / eosin-stained or PAS-stained specimens were prepared and microscopically examined. In offspring, In all cases, survival and death were confirmed once a day from the day of delivery to the day of autopsy (4 days of nursing), and the general condition and external surface were observed. The number of nursing days was calculated with the delivery date as 0 days of nursing. In addition, the neonatal survival rate was calculated in units of one litter. All cases were measured on days 1 and 4 of nursing. In addition, the body weight value was shown as an average value in units of one belly for each sex. Debilitated and dead cases with a poor prognosis were immediately necropsied, and the whole body was fixed with 10% neutral buffered formalin and stored. In other cases, the extracorporeal surface (including the oral cavity) was observed on the 4th day of nursing, euthanasia was performed by the carbon dioxide inhalation method, and the organs and tissues of the whole body were visually observed. For cases with abnormal findings, the whole body was fixed and preserved with a 10% neutral buffered formalin solution. ***Adverse Effect(s) (brief):*** NOEL =1,000 mg/kg/day. In general condition observation and autopsy, both males and females had blue-green or gray-blue coloration of the contents of the stomach and intestinal tract of 200							

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
mg / kg in all cases of 40 mg / kg or more. It was observed in a small number of cases and almost all cases in the 1000 mg / kg group. These were due to the fact that the test substance had a blue color. Body weight transition, food intake, organ weight, autopsy and histopathological examination showed no effect of administration of the test substance in both males and females. From the above, it was suggested that the no-effect level (NOEL) of repeated administration of copper phthalocyanate in this screening study was 1000 mg / kg / day for both males and females. No effects of test substance administration were observed in fertility tests, observation of labor and maternal behavior, survival of newborns, observation of general condition, body weight transition, and autopsy. From the above, it was suggested that the no-effect level (NOEL) of copper phthalocyanate on the reproduction and development of offspring of male and female animals in this screening test was 1000 mg / kg / day for both males and females. ***Data Quality:*** Data quality cannot be verified because information is from a secondary source; however, secondary source has assigned a reliability (Klimisch) score of 1 (without restrictions). ***Note***: Biological endpoints included: reproductive ability/delivery, maternal behavior, viability, clinical signs, body weight change and autopsy of pups. *OECD Preliminary Reproduction Toxicity Screening Test. ***Reference:*** *JECDB Phthalocyanine Blue. Japan Existing Chemical Data Base (JECDB): https://dra4.nihs.go.jp/mhlw_data/jsp/FileListPageENG.jsp?parameter_csno=147-14-8 .*							

II.2 Other Health Effects

Pigment	Endpoint	Outcome	Reference
CuPc	Irritation (skin)	No observable adverse effect reported	Similar to (ECHA, 1971b); non-GLP
Test item: CuPc. ***Dose***: Test sample: 1 ml of pigment (diluted 50% in H2O); ***Treatment duration:*** 1, 5, 15 min and 26 hr (occlusive, shaved); Observation Period: 1, 24, 48 hr and days 6 and 8. ***Species (strain):*** Rabbit (Vienna White). ***Gender:*** Male/ female ***Number per Group:*** 2/ per sex/ per dose. ***Control:*** none. ***Method:*** White Vienna rabbits were used. Usually, 2 animals were treated for 1, 5, 15 min and 20 h using occlusive conditions. An application site of 2.5 cm x 2.5 cm was covered with the liquid test substance. In addition, skin tissue from the ear was tested by wrapping the ear. These results from the ear, however, would not be taken into account for evaluation as they do not represent testing of the dorsal/lateral flank of the back. After the application time, the skin was washed with water which sometimes contained a mild detergent. The animals were observed for 8 days. Skin reaction was recorded after 1 h, 24 h, 48 h, 72 h, 96 h and after 7 and 8 days. Non-Published (secondary source); Non-compendial / non-guideline. ***Adverse Effect(s) (brief):*** No observable erythema or edema was reported at any reading time point and after any of the treatment durations. ***Data Quality:*** ECHA Reliability 2 (reliable with restrictions)			

Note: Feinman and Doyle (1988) mention a case of occupational allergy to Ingrain Blue 2, a precursor in the manufacture of Pigment Blue 15, reported in a 1982 edition of Color Index, Third Edition (2nd revision).

Note: ECHA database of registered substances includes three reports of non-published guideline sensitization data (two mouse local lymph node assay and one guinea pig maximization test). All three reports negative results for sensitization for the test samples. The test sample identity is reported by a tradename, rather than a chemical name or CAS number. Therefore, the details of these test are not included in this toxicity profile.

Section III. Dose-Response Assessment

III.1 Derivation of Provisional Tolerable Intake

Contact Duration	Exposure Route	Patient	POD	MF	pTI
Permanent	Parenteral	All	40 mg/kg/d	270	0.148 mg/kg/d

pTI = POD ÷ MF

Note: When a supplier-specific color additive safety data sheet (SDS) does not report the presence of a carcinogen or non-carcinogen impurity, and SDS complies with Globally Harmonized System (GHS) hazard communication limits (i.e., <0.1% and <1%, respectively), then this pTI is low enough to be protective for naturally occurring impurities which could be present in the color additive. Processes used to color a polymer system involve diluting the pigment and its impurities by at least 50x (i.e., the CHRIS calculator is valid when the maximum concentration of pigment in a colored polymer is 2%, i.e., 1 ÷ 2% = 50); therefore, the concentration of naturally occurring impurities in the finished colored polymer will be significantly lower compared to the concentration of these substances in the pigment (i.e., 0.002% (i.e., 0.1% ÷ 50) for carcinogens and 0.02% (i.e., 1% ÷ 50) for non-carcinogens). Unknown impurities are addressed by the CHRIS calculator.

III.2 Derivation of a Modifying Factor

Health Effect Study	Biological Endpoint	Exposure Route	POD Type	POD Value	UF_1	UF_2	UF_3	MF
(JECDB (year not reported) (CuPc))	Systemic Tox	Oral	NOEL	40	3	3	30	270

MF = modifying factor (UF1 x UF2 x UF3)

III.3 Toxicological Uncertainties Applied to POD

Uncertainty	Justification	Source(s)
UF_1	(3) Due to pigment's moderately large molecular size*, not metabolized by the body non-absorbed by oral route (1), and absence of pigment found in blood after *ip* injection (1).	(Kurliandskii *et al.*, 1988; Kurlandsky *et al.*, 1993)
UF_2	(3) Due to pigment's moderately large molecular size*, not metabolized by the body, non-absorbed by oral route (1), and absence of pigment found in blood after *ip* injection (1).	(Kurliandskii *et al.*, 1988; Kurlandsky *et al.*, 1993)
UF_3 **Error! Bookmark not defined.**	(30) Account for low data quality, and differences in exposure route and duration	(Kurlandsky *et al.*, 1993; JECDB)

UF_1, UF_2, and UF_3, are used to extrapolate the POD to the general human population (including pediatric) exposed parenterally for a lifetime.

III.4 Critical Health Effect Study Design and Outcome

JECDB (date not reported) Phthalocyanine Blue. Japan Existing Chemical Data Base (JECDB). http://dra4.nihs.go.jp/mhlw_data/home/file/file147-14-8.html. Webpage accessed: January 21, 2017.			
Test Substance	Copper, [29H, 31H-Phthalocyanate(2-)] / Pigment Blue-15 (CAS # 147-14-8)		
Species	Rats (Wistar)	Frequency	once per day
Gender	Male/Female	Duration	28 days
Age	NR	#/Group	10/sex/dose
Route	Oral	Protocol Guideline	See Notes
Dose(s)	0, 40, 200, 1,000 mg/kg/day	Statistical Method(s)	NA
Observed Responses	No changes in general condition, body weight gain or food consumption were detected in any of the groups. After the 28 days of administration, a significant decrease in red blood cell count (RBC) and decrease of hemoglobin (Hb) and packed cell volume (PCV) were detected in the 200 and 1000mg/kg male groups. These slight changes were dose dependent. After the recovery period, significant increase of erythroblasts was detected in the 1000 mg/kg female group. Additionally, increases of absolute organ weights of lung, spleen, adrenal and salivary gland and a tendency for increased relative organ weights of the spleen were evident in the 1000 mg/kg male group. No histopathological changes due to administration of phthalocyanine blue were detected. These findings suggest that phthalocyanine blue slightly affects red blood cells in rats at 200 and 1000 mg/kg. POD: 40 mg/kg/day. Reference is a secondary source and absent methodology information.		
Notes:	Guidelines for 28-Day Repeat Dose Toxicity Test of Chemicals (Japan).		

*CAS number, supplier, and purity not reported;

TITANIUM DIOXIDE

CAS # 13463-67-7

Toxicological Profile Summary

Titanium dioxide (TiO_2; C.I. Pigment White 6) (CAS # 13463-67-7) is a widely used inorganic, white, odorless pigment because of its light scattering properties, chemical stability and biological inertness [77,80]. Titanium oxide is naturally occurring and produced via the treatment of the titanium oxide ore with chlorine gas or sulfuric acid. The particle size of titanium dioxide assessed in this report is typically between >200 nm to < 300 nm (i.e., pigment).[76] TiO_2 is insoluble in water, organic solvents and physiological conditions. Reported hazard information includes an adverse health effect study, supporting studies, and secondary sources. Titanium dioxide, administered at high oral doses (1000 mg/kg/d) to rodents/rats, is reported not to elicit observable systemic toxic effects. For assessing titanium dioxide exposure that will be without appreciable harm to health, the calculated provisional tolerable intake (pTI) value for parenteral systemic toxicity is 1 mg/kg/day. This value is based on the lowest reported point-of-departure (1000 mg/kg/day, 40 days, rodent with a modifying factor of 1000 (i.e., 10 x 10 x 10) to address the following sources of uncertainty: variation among humans (10), variation between species (10), and data quality/route-to-route extrapolation (10).

NOTE: This pTI is protective for the color additive including any impurities listed in Section I.1 "Substance Identity," as the impurities likely will be present at a very low percentage of the color additive, and the quantity of the impurity will be at an even lower percentage in the final device (see Section III, Table III.1 footnote). Unknown impurities are addressed by the CHRIS calculator.

Section I. Substance Information

I.1 Substance Identity

	Descriptor
Synonyms[77]	Dioxotitanium oxide, Pigment White 6, Titania, Titanic oxide, Titanium oxide, Titanium (IV) oxide, Titanium peroxide
Formula[77]	TiO_2
Molecular Weight	79.87 g/mol
Type[78],[79]	Mono constituent substance. Inorganic. Noncombustible solid
Physical Form[78]	Odorless white powder
Physical/Chemical Characteristics[79],[80]	Melting point: 1855°C Boiling point: 2500–3000°C @ 101.3–101.325 kPa Density: 4.26 g/cm^3 Odor: 100% odorless Density: 3.9–4.17 g/cm^3 @ 20 °C

[76] Webber, T. G. Coloring of Plastics. Edited by Thomas G. Webber. New York, NY: JOHN WILEY & SONS, 1979.
[77] ChemIDplus. n.d. "Titanium Dioxide - GWEVSGVZZGPLCZ-UHFFFAOYSA-N - Titanium Dioxide [USP] - Similar Structures Search, Synonyms, Formulas, Resource Links, and Other Chemical Information." Accessed October 1, 2020. https://chem.nlm.nih.gov/chemidplus/rn/13463-67-7.
[78] CDC. 2019. "CDC - NIOSH Pocket Guide to Chemical Hazards - Titanium Dioxide." October 30, 2019. https://www.cdc.gov/niosh/npg/npgd0617.html.
[79] ECHA. 2020. "Titanium Dioxide - Brief Profile - ECHA." 2020. https://echa.europa.eu/brief-profile/-/briefprofile/100.033.327.
[80] IARC. 2010. "Carbon Black, Titanium Dioxide, and Talc. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. VOLUME 93." WORLD HEALTH ORGANIZATION. INTERNATIONAL AGENCY FOR RESEARCH ON CANCER. http://monographs.iarc.fr/ENG/Monographs/vol93/mono93.pdf.

	Descriptor
	Titanium dioxide is insoluble in water. It is soluble in hot concentrated sulfuric acid and alkalis.
Production[81]	Five stages in the manufacture of pigmentary titanium dioxide: Titanium dioxide ore is converted to either aqueous titanyl sulfate solution or anhydrous titanium tetrachloride. These intermediates are converted to crystalline, size-specific pigmentary particles of titanium dioxide-rutile or titanium dioxide-anatase. Pigment is coated and, in some cases, involves a grinding step. Pigment is then filtered, washed and dried. Pigment agglomerates may be ground to reduce their size without breaking the primary titanium dioxide particles
Impurities[80]	Natural rutile, anatase and brookite contain impurities of up to ~2% that include iron, chromium, vanadium, aluminum, niobium, tantal, hafnium and zirconium (Heaney & Banfield 1993) and account for slight variations in density, color and indices of refraction. Since most commercial titanium dioxide is manufactured from natural material by dissolution of the parent mineral and reprecipitation as fine particles with the structure of anatase or rutile (referred to as titanium dioxide-anatase or titanium dioxide-rutile), most but not all of these chemical impurities are generally removed.
Other[80]	An ultraviolet (UV)-activated catalyst, and organic polymers that are in contact with it degrade under UV radiation. Scattering of light by TiO_2 is maximized in particles that are 0.2–0.3 μm in diameter, and most commercial products that are used as pigments have modal primary particle sizes within this range.

Physical Form = gas, liquid, solid (at room temperature); Type = gas, liquid, or solid (include temp & pressure if available); RT = room temperature

I.2 Molecular Descriptors

Name	CAS #	Formula	Mass	Structure
Titanium dioxide	13463-67-7	O_2-Ti	79.865	O=Ti=O

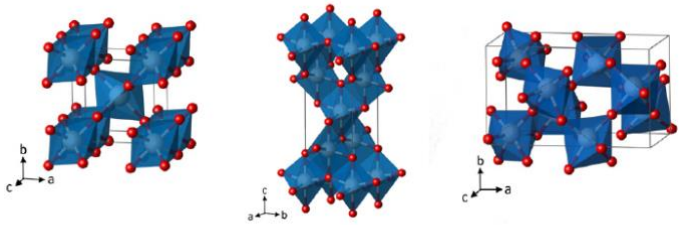


Figure I.1. Schematic of Titanium Dioxide Molecular [82]

Titanium dioxide exists in three different crystallographic structures: rutile, anatase and brookite. Rutile forms white tetragonal crystals; anatase forms brown tetragonal crystals; and brookite forms white orthorhombic crystals.

[81] Braun, Juergen H., Andrejs Baidins, and Robert E. Marganski. "TiO2 Pigment Technology: A Review." Progress in Organic Coatings 20, no. 2 (May 4, 1992): 105–38. https://doi.org/10.1016/0033-0655(92)80001-D.

[82] OECD SIDS. 2013. "SIDS Initial Assessment Profile - Titanium Dioxide," 8.

Section II. Hazard Identification

Health effect data, supporting studies, and secondary sources were reviewed, see tables in sections II.1, and II.2for details.

Multiple published studies examined the toxicity, genotoxicity, and carcinogenicity of TiO_2. In these studies, microparticles of TiO_2 were administrated by several routes (e.g., oral, intraperitoneal, and inhalation) using multiple model systems (mouse, drosophila, and rat). The duration of these studies ranged from 24hrs to 2.49 years. There were no reported acute toxicities observed in the summarized studies.

Acute oral studies mainly produced results negative for adverse effects regardless of administration concentration. In sub-acute inhalation studies inflammation was observed in multiple studies. Genotoxicity studies primary yielded ambiguous or negative results with a lack of adverse effects regardless of administration concentration. Reproductive and developmental toxicity testing displayed no adverse effects in treated test animals at the highest dose tested.

Genotoxicity was not observed in various *in vitro* systems, including Syrian hamster embryo cells, salmonella typhimurium (strains: TA97, TA98, TA100, TA1535, TA1537, TA1538), Escherichia coli (strain: WP2 urvA), L5178Y mouse lymphoma cells, and Chinese hamster ovary K5 cells. Multiple reports suggest exposure to TiO_2 can lead to accumulation of the particles in various organ systems (e.g., lung, spleen, kidney, and liver) and tissues. However, there was no correlation between particle deposition and biologically relevant pathological or functional changes. There were no consistent or biologically important changes in survival, body weight gains, hematologic, clinical chemistry parameters or histopathology observed in the reviewed studies. Results suggest that the carcinogenic effects appear to be route specific (inhalation) resulting from lung overload.

II.1 Systemic, Genotoxicity, Carcinogenicity, Reproductive/Developmental Toxicity

ADVERSE HEALTH EFFECT STUDIES

Source Citation	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
Systemic Toxicity (acute)							
Wang et al. (2007)	ND	Animal	Oral	5,000 mg/kg bw **(LOAEL)**	Single	No observable adverse effect reported	OECD TG 420
Test article: Titanium dioxide (0 and 155 nm; >92%) ***Dose: Methods:*** In this oral mouse study titanium dioxide was administered by single exposure to 5,000 mg/kg suspended in 0.5 % HPMC. The following parameters were examined: body weight, organ weight (liver, spleen, and kidney), blood biomarkers, titanium content, and histopathology. ***Species (strain):*** CD-1 Mouse. ***Gender:*** Male and female. ***Number per group:*** (8-9/sex/ per group). ***Control:*** and 0.5 % HPMC. ***Adverse Effect(s) (brief):*** **LOAEL= 5,000 mg/kg bw.** There were no significant alterations in body or organ weight observed in all animals during a 14 days observation period. There were significant increases of titanium dioxide concentration in spleen and brain observed in the treatment group. Histopathological results showed neuron vacuoles in the hippocampus, swelling in the renal glomerulus, hydropic degeneration and spotty necrosis in liver cells. Authors do not report that these effects were treatment related. There were no abnormal pathology changes in the heart, lung, testicle (ovary), and spleen tissues. TiO2 was mainly retained in the spleen and brain tissues. ***Conclusion:*** No obvious acute toxicity was observed after a single oral exposure to 5 g/kg TiO2 particles. It was shown that TiO2 particles could be transported to other tissues and organs after uptake by gastrointestinal tract. Particle disposition did not result in any functional changes. ***Data Quality:*** Data quality cannot be verified because information is from a secondary source. ***Note:*** none ***Reference:*** *Wang, Jiangxue, Guoqiang Zhou, Chunying Chen, Hongwei Yu, Tiancheng Wang, Yongmei Ma, Guang Jia, et al. "Acute Toxicity and Biodistribution of Different Sized Titanium Dioxide Particles in Mice after Oral Administration." Toxicology Letters 168, no. 2 (January 30, 2007): 176–85. https://doi.org/10.1016/j.toxlet.2006.12.001.*							
Bernard et al. (1990)	Yes	Animal	Oral (feed)	500 mg/kg/day **(NOAEL)**	130 weeks	No observable adverse effect reported	NR
Test article: titanium dioxide ***Dose:*** 0, 1.0, 2.0, or 5.0% (100, 200 or 500 mg/kg/day) titanium dioxide (TiO2) coated mica (1:1 blend of titanium dioxide mica and Flamenco superpearl; Particle size: 10 to 35 mm; flat platelets) . ***Test article purity***: 28% TiO2 and 72% mica. A purity of 100% was assumed for purposes of diet formulation. ***Control:*** Control was 1% corn oil **Methods*:*** In							

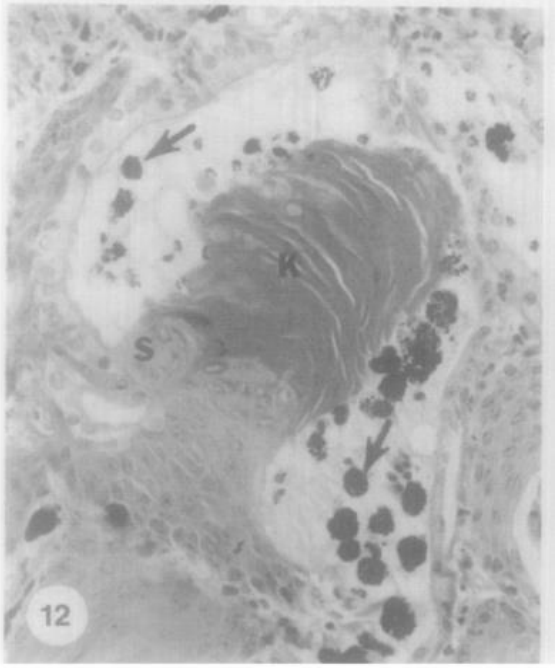
Source Citation	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
this study 130-week carcinogenesis study rats were exposed orally to diets containing the test material or control. Body weights, food consumption, clinical observation, and physical examinations were recorded weekly for the first 14 wks, then every 4 wks thereafter. Ophthalmoscopic evaluation were performed prior to the start of the study, during week 52 and 104. Clinical pathology profiles were developed for subgroup of 10 animals per sex during weeks 27 and 53. Blood and urine was collected from fasted subgroup rats during wk 53. The subgroups were sacrificed during wk 53 for necropsy. Organ/body weight ratios were determined for brain, heart, liver, spleen, kidney, testes (with epididymites), both adrenals, thyroid, ovaries, and pituitary. The control and high dose groups received complete histopathologic evaluation. All surviving animals were sacrifice for complete necropsy after 130 wk. ***Species (strain):*** F344 Rat. ***Gender:*** Male and female. ***Number per Group:*** (60/sex/per dose) ***Adverse Effect(s) (brief):*** > NOAEL=500 mg/kg/day At week 130 the absolute body weights, and body weight gains were comparable across groups. TiO2-coated mica had no significant effect on survival. From week 2 the only clinical sign observed was in animals of the high dose, which consisted of silver-colored feces. There was an increase in hyperplasia in the adrenal medullary (no evidence of progression of these proliferative changes to pheochromocytoma) and grossly observed cataracts (not substantiated with histopathologic evaluation) occurred in males after 5.0% exposure. Incidence of mononuclear cell leukemia in male mice surviving to 130 weeks was significantly increased after 5.0% exposure; however, disease in F344 rats increases dramatically with age confounding the result interpretation. Furthermore, MCL is a rapidly fatal disease in rats, and a treatment-related increase in incidence would be expected to decrease the survival of dosed animals, which was not seen. Functional change of the animals, organ, or tissue is not reported. ***Conclusion***: There were no consistent or biologically important changes in survival, body weight gains, hematologic or clinical chemistry parameters or histopathology. There was no treatment-related carcinogenic effects observed. ***Data Quality:*** ToxRTool. Score of 21 (score of 1, reliable without restrictions). (LCS) ***Reference:*** *Bernard, Bruce K., Merrill R. Osheroff, Andreas Hofmann, and John H. Mennear. "Toxicology and Carcinogenesis Studies of Dietary Titanium Dioxide-coated Mica in Male and Female Fischer 344 Rats." Journal of Toxicology and Environmental Health 29, no. 4 (April 1, 1990): 417–29. https://doi.org/10.1080/15287399009531402.*							
Genotoxicity							
Driscoll et al. (1997)	ND	Animal	Intratracheal instillation	100 mg/kg bw **(NOAEL)**	2 consecutive days	Increased mutation frequency, Inflammation	NR
Test article: titanium dioxide . ***Dose:*** saline suspensions of 10 and 100 mg/kg of titanium dioxide (given as two equal doses of 5 or 50 mg/kg on consecutive days) (Particle size: median diameter=0.18 mm; surface area=8.8 m^2/g).titanium dioxide. . ***Species (strain):*** F344 Fischer Rat. ***Gender:*** Female. ***Number per Group:*** (9/ per dose) and (12/ control). ***Control:*** Saline (control) ***Methods:*** In this study rats were exposed to TiO_2 by intratracheal instillation for two consecutive days and effects were examined 15 months post-exposure. Animals were intratracheally instilled with pyrogen-free sterile saline (control Mutagenic activity of inflammatory cells and particles group) or saline suspensions of titanium dioxide anatase. A dosing volume of 2.0 ml/kg body weight was used for all intratracheal instillations. Fifteen months after dust-exposure animals were sacrificed. The lungs and heart were removed en bloc and the lungs perused. BAL was performed by cannulating the trachea and infusing the lungs five times. Cell differentials were performed. The hprt Clonal selection assay was performed. Freshly isolated alveolar type II cells were cultured. After 14–21 days in culture the cells were fixed and immunostained with an antibody to cytokeratins 8, 18 and 19 and 6TG-resistant cytokeratin staining colonies of .50 cells counted. Mutation frequencies were calculated. Histology was performed on groups of three rats/treatment group following exposure. Rat lung cells obtained by BAL of rats 15 months after saline or particle exposure were evaluated for their ability to cause mutation in rat alveolar epithelial cells in vitro. Experiments were conducted to characterize the cell-derived mediators that contributed to the mutagenic effects on RLE-6TN cells. ***Adverse Effect(s) (brief):*** **LOAEL = 100 mg/kg/day. NOAEL = 10 mg/kg/day.** The number of neutrophils in bronchoalveolar lavage fluid 15 months after intratracheal instillation of 100 mg/kg titanium dioxide increased. The number of lymphocytes increased after exposures to 10 & 100 mg/kg titanium dioxide. The number of macrophages in bronchoalveolar lavage fluid 15 months after intratracheal instillation of 100 mg/kg titanium dioxide decreased. *Hprt* mutation frequency was increased in alveolar type II cells from rats (*in vivo)* exposed to 100 mg/kg titanium dioxide. However, the *in vitro* mutagenic effect of 100 mg/kg titanium dioxide on BAL cells (50:1 ratio) from exposed rats on the rat lung epithelial cell line, RLE-6TN was slightly elevated, but not significant. Histology results showed lesions observed in titanium dioxide-exposed rats were similar in both high and low dose groups consisting of a minimal centriacinar alveolitis, and accumulation of particle-laden macrophage in the alveoli and interstitium. Other changes observed were like those in the control animals. ***Conclusion:*** These studies demonstrate titanium dioxide particles produce significant neutrophilic inflammation and this is associated with increased mutation in rat alveolar type II cells in vivo. However, the same mutagenic effects were not observed in *in vitro*. ***Data Quality:*** NR ***Reference:*** *Carcinogenesis Vol 18 Issue 2 Reference: Driscoll, K. E., L. C. Deyo, J. M. Carter, B. W. Howard, D. G. Hassenbein, and T. A. Bertram. "Effects of Particle Exposure and Particle-Elicited Inflammatory Cells on Mutation in Rat Alveolar Epithelial Cells." Carcinogenesis 18, no. 2 (February 1, 1997): 423–30. https://doi.org/10.1093/carcin/18.2.423.*							
Foureman et al. (1994)	ND	*Drosophila melanogaster*	Oral (feeding); Injection	1500 ppm **(oral; NOAEL);** 5680 ppm **(injection ; NOAEL).**	72 hours	No observable adverse effect reported	NR

Source Citation	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
Test article: Seventy compounds, including titanium dioxide, ***Dose:*** feeding (0, 1500 ppm for titanium dioxide) or 24 hr after injections (0, 5680 ppm for titanium dioxide) or inhalation. Controls were treated with the solution used to dissolve the test substance. ***Species (strain):*** Canton-S males and *Basc* females Drosophila melanogaster. ***Gender:*** Male (for treatment) and female (for mating). ***Number per Group:*** (10 males and 20 females per vial). ***Control:*** untreated group***. Methods:*** In this study TiO_2 were tested for the ability to induce sex-linked recessive lethal (SLRL) mutations in postmeiotic and meiotic germ cells for male *Drosophila melanogaster.* The test substance was administered oral (feeding) and injections. F, cultures were scored as presumptive lethals if the number of wild-type males was 0, 1, or <5% of the number of Base males (or Basc/+ females). All putative lethals were confirmed through an additional generation. If the SLRL test performed after a feeding exposure gave a negative result, additional tests were made using an injection exposure. If these, too, gave a negative result. the compound was non-mutagenic. ***Adverse Effect(s) (brief):*** **NOAEL> 1500 ppm (oral); NOAEL> 5680 ppm (injection).** The feeding exposures were found nonmutagenic based on the sex-linked recessive lethal (SLRL) mutation test. There is no evidence in the results (0.07% lethals in the treated and 0.03-0.05 lethals in the control) that titanium dioxide is mutagenic***:*** Under the experimental conditions, titanium dioxide was determined to be non-mutagenic. ***Data Quality:*** NR ***Reference***: *Environmental and Molecular Mutagenesis Vol 23 Issue 3 Reference: Foureman, P., J. M. Mason, R. Valencia, and S. Zimmering. "Chemical Mutagenesis Testing in Drosophila. X. Results of 70 Coded Chemicals Tested for the National Toxicology-Program." Environmental and Molecular Mutagenesis 23, no. 3 (1994): 208–27. https://doi.org/10.1002/em.2850230310.*							
Shelby et al. (1993)	ND	Animal	Intraperitoneal injection	1500 mg/kg bw **(NOAEL)**	3 days	Ambigiuos	NR
Test article: TiO_2 ***Dose:*** (0, 250, 500, 1000, 1500 mg/kg bw), were tested after three daily exposures. ***Control:*** Corn oil used as controls. ***Species (strain):*** B6C3F1. ***Gender:*** Male. ***Number per Group:*** (5 animals/dose group)**.. *Methods:*** In this study TiO2 (along with 48 other compounds) were used in the micronuclei (MN) test using bone marrow cell of mice. In the initial MN study, B6C3F1mice were all treated by intraperitoneal (IP, injection) 0.4 ml per mouse. Dosing occurred three times at 24 hours intervals. Animals were monitored twice daily, and 48 hr after the third treatment, the surviving mice were euthanized by C02 asphyxiation. ***Adverse Effect(s) (brief):*** **NOAEL >1500 mg/kg.** After 24 hrs of exposures, the initial MN experiment showed a significant trend with the effect at the highest dose (1000 mg/kg bw), being significant. In a second trial using high dose of 1500 mg/kg, the frequency of MN-PCE was significantly elevated in the low and middle dose, but not the high dose. A trend analyses were conducted after the highest dose was deleted. The results were concluded to be positive. However, the protocol employed here is not adequate to detect all chemicals that induce cytogenetic damage in mouse bone marrow cells and a more comprehensive detection of such chemicals will require that the protocol be supplemented with an additional protocol. Under the experimental conditions, titanium dioxide induced significantly elevated levels of micronuclei in the bone marrow cells of mice. However, the results are not dose dependent. ***Data Quality:*** NR ***Reference:*** *Environmental and Molecular Mutagenesis Vol 21 Reference: Shelby, M. D., G. L. Erexson, G. J. Hook, and R. R. Tice. "Evaluation of a Three-Exposure Mouse Bone Marrow Micronucleus Protocol: Results with 49 Chemicals." Environmental and Molecular Mutagenesis 21 (1993): 160-179 (cited in Babich 2010).*							
Shelby and Witt (1995)	ND	Animal	Intraperitoneal injection	2,500 mg/kg bw **(NOAEL)**	1 or 3 days	No observable adverse effect reported	NR
Test article: TiO_2 ***Dose:*** 0, 250, 500, 1000, 1500 mg/kg bw for micronucleus; 0, 625, 1250, 2500 mg/kg bw for chromosomal aberration, were tested. ***Treatment durtion:*** 1-3 days***. Species (strain):*** B6C3F1 Mouse ***Gender:*** Male. ***Number per Group:*** (8 animals/dose group) for ABS; (5 animals/dose group) for Micronucleus. ***Contorl:*** untreateg group. ***Methods:*** In this study TiO2 (along with 64 other compounds) were used in the chromosomal aberration test (ABS) and the micronuclei (MN) test using bone marrow cell of mice. In the ABS study male B6C3F1 mice received a single intraperitoneal injection with the chemical dissolved in corn oil or PBS (injection volume = 0.4 ml). Animals were subject to two different sacrifice times; The first used a harvest time of 17 hr (standard protocol) and the second, conducted when no evidence of aberration induction was seen at 17 hr, used a harvest time of 36 hr (extended protocol). In the MN study, B6C3F1mice were all treated by intraperitoneal (IP, injection) 0.4 ml per mouse. Dosing occurred three times at 24 hours intervals. Animals were monitored twice daily, and 48 hr after the third treatment, the surviving mice were euthanized by C02 asphyxiation. ***Adverse Effect(s) (brief):*** **MN: NOAEL > 2500 mg/kg.** After 24 hrs of exposures, the initial MN experiment on titanium dioxide showed a significant trend with the effect at the highest dose (1000 mg/kg bw) significantly elevated; however, the effects observed were small. In a second trial on titanium dioxide (0, 500, 1000, 1500 mg/kg bw), frequency of MN-PCE was significantly elevated in 1,000 mg/kg bw, but not in the high dose 1500 mg/kg bw. These results were not dose-dependent and only of minor significance. In chromosomal aberration tests, titanium dioxide was clearly negative at 17- and 36-hour sampling times at all concentrations examined. Under the reported experimental conditions, titanium dioxide did not induce chromosome aberrations in the bone marrow cells of the mouse. ***Data Quality:*** NR ***Reference:*** *Environmental and Molecular Mutagenesis Vol 25 Reference: Shelby, M. D., and K. L. Witt. "Comparison of Results from Mouse Bone Marrow Chromosome Aberration and Micronucleus Tests." Environmental and Molecular Mutagenesis 25 (1995): 302-313 (cited in Babich 2010).*							
Casto, Meyers, and DiPaolo (1979)	ND	Syrian hamster embryo cells	Adenovirus Cell culture.	> 12.5 mM (**NOAEL)**	No observable adverse effect reported	NR
Test article: titanium dioxide ***Dose:*** 0, 1.56, 3.12, 6.25, and 12.5 mM in acetone:water (1:1) (38 chemicals tested in this study, one of them being titanium dioxide) ***Treatment duration:*** 48 hrs. ***Species (strain):*** primary Syrian hamster embryo cells. ***Number per***							

Source Citation	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
Group: 2 plates (prior to inoculation); 5 – 10 plates (5 hrs after virus inoculation). ***Control:*** 5 plates medium only. ***Methods:*** transformation assay (SA7) for 18hr prior to inoculation or at 5 hrs after virus inoculation. After pretreatment, rinsed and inoculated with simian adenovirus. When treated after virus inoculation, titanium dioxide remained in the medium for 48 hrs. Enhancement was expressed as the ratio between the TF of treated, surviving cells and the TF of control cell. The increased TF was considered statistically significant at the 5 or 1% confidence level if the enhancement ratio exceeded the appropriate value obtained from the Lorenz table. ***Adverse Effect(s) (brief):*** **NOAEL = 12.5 mM.** The enhancement ratio of virus transformation of Syrian hamster cells is 0.8 at 12.5 mM. Titanium dioxide failed to enhance viral transformation. ***Data Quality:*** ND. ***Reference:*** *Casto, Bruce C., Judy Meyers, and Joseph A. DiPaolo. "Enhancement of Viral Transformation for Evaluation of the Carcinogenic or Mutagenic Potential of Inorganic Metal Salts." Cancer Research 39, no. 1 (January 1, 1979): 193–98.*							
Dunkel et al. (1985)	ND	Cell culture	Ames		NR	No observable adverse effect reported	NR
Test article: Titanium dioxide ***Dose:*** chemicals were tested only to a dose of 333 pg/plate. ***Species (strain):*** Salmonella typhimurium TA98, TA100, TA1535, TA1537, and TA1538 cells; E. coli WP2 uvrA cells ***Number per Group:*** Triplicate. All plates were prepared in triplicate. ***Control:*** concurrent positive and negative controls. ***Methods:***. Salmonella mutagenicity assay. 60 chemicals tested in this study, one of them being titanium dioxide; concentrations not reported) with and without S9 (either negative and positive controls not run or the positive control did not induce a positive response in Salmonella). ***Adverse Effect(s) (brief):*** **NOAEL = NR.** Titanium dioxide resulted in no positive mutagenic responses and toxicity were observed at any dose level under this test conditions. A negative response with and without metabolic activation. ***Data Quality:*** ND. ***Reference:*** *Dunkel, Virginia C., Errol Zeiger, David Brusick, Elena McCoy, Douglas McGregor, Kristien Mortelmans, Herbert S. Rosenkranz, and Vincent F. Simmon. "Reproducibility of Microbial Mutagenicity Assays: II. Testing of Carcinogens and Noncarcinogens in Salmonella Typhimurium and Escherichia Coli." Environmental Mutagenesis 7, no. S5 (January 1, 1985): 1–19. https://doi.org/10.1002/em.2860070902.*							
Ivett et al. (1989)	ND	Chinese Hamster Overy Cells	Sister chromatid exchange; Chromosomal aberrations		> 25 µg/mL (±S9)	No observable adverse effect reported	NR
Test article: titanium dioxide ***Dose:*** 0, 15, 20, and 25 µg/mL in medium ***Treatment duration:*** 2 hrs with and 25 hrs without S9 for the sister chromatid exchange assay. 2 hrs with and 8 hrs without S9 for the chromosomal aberration assay. ***Species (strain):*** Chinese hamster overy cells. ***Number per Group:*** Two independent experiments. ***Control:*** Solvent and positive controls were run concurrently with each trial. ***Methods:*** 15 chemicals tested in this study, one of them being titanium dioxide at 37°C for 2 hrs with and 25 hrs without S9 for the sister chromatid exchange assay and for 2 hrs with and 8 hrs without S9 for the chromosomal aberration assay. Medium was used as a negative control; positive control was mitomycin C and cyclophosphamide. In the SCE assay, an increase of 20% or greater increase in SCE per chromosome over the solvent control was considered significant. ***Effect(s) (brief):*** **NOAEL = 25 µg/mL.** Titanium dioxide did not induce sister chromatid exchange or chromosomal aberrations; the high dose was limited by solubility. In the first aberration trial with activation, there was a positive response at 20 µg/mL; however, this response was not repeatable in the subsequent study. ***Data Quality:*** ND. ***Reference****: Ivett, J. L., B. M. Brown, C. Rodgers, B. E. Anderson, M. A. Resnick, and E. Zeiger. "Chromosomal Aberrations and Sister Chromatid Exchange Tests in Chinese Hamster Ovary Cells in Vitro. IV. Results with 15 Chemicals." Environmental and Molecular Mutagenesis 14, no. 3 (1989): 165–87.*							
Kanematsu, Hara, and Kada (1980)	ND	*B. subtillis* H17 and M45; *E. coli* strains TA98, TA100, TA1535, TA1537, and TA1538	Rec assay; Reversion assay		> 0.5 M	No observable adverse effect reported	NR
Test article: titanium dioxide ***Dose:*** 0.001 – 10 M in distilled water. ***Treatment duration:*** 1 to 3 days ***Species (strain):*** Bacillus subtilis H17 (rec+) and M45 (rec-) strains; E. coli TA98, TA100, TA1535, TA1537, TA1538 strains. ***Number per Group:*** NR. ***Control:*** untreated group. ***Methods:*** (127 chemicals tested in this study, one of them being titanium dioxide) incubated at 4°C for 24 hrs and then at 37°C overnight for the rec assay while incubated at 37°C for 3 days for the reversion assay. ***Adverse Effect(s) (brief):*** **NOAEL = 10 M.** Negative results were obtained for titanium dioxide in the present study (Rec assay and reversion assay). ***Data Quality:*** ND. ***Reference****: Kanematsu, Nobutake, Masako Hara, and Tsuneo Kada. "Rec Assay and Mutagenicity Studies on Metal Compounds." Mutation Research/Genetic Toxicology 77, no. 2 (February 1, 1980): 109–16. https://doi.org/10.1016/0165-1218(80)90127-5.*							
Miller, Pujadas, and Gocke (1995)	ND	Chinese hamster ovary cells	Micronucleus test		> 10 µg/mL	No observable adverse effect reported	OECD TG 473
Test article: titanium dioxide ***Dose:*** 0, 0.025, 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5 and 10 µg/mL in DMSO ***Treatment duration:*** for 48 ***Species (strain):*** CHO K5 cells. ***Number per Group:*** NR. ***Control:*** untreated group. ***Methods:*** 48 hrs at 37°C without S9, and 0,							

Source Citation	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
0.25, 0.5, 1, 2.5, 5 and 10 µg/mL titanium dioxide in DMSO for 3 hours with S9 (1% DMSO vehicle control; positive control was cyclophosphamide). ***Adverse Effect(s) (brief):*** **NOAEL = 10 µg/mL.** Titanium dioxide did not increase the frequency of micronuclei with or without metabolic activation at any concentration. It is stated that it was almost impossible to dissolve the compound. Precipitation was seen on the microscopic slides in the form of large crystals at concentrations of 0.5 µg/mL or higher. Titanium dioxide was negative in this *in vitro* micronucleus test. ***Data Quality:*** ND. ***Reference:*** *Miller, Beate M., Eva Pujadas, and Elmar Gocke. "Evaluation of the Micronucleus Test in Vitro Using Chinese Hamster Cells: Results of Four Chemicals Weakly Positive in the in Vivo Micronucleus Test." Environmental and Molecular Mutagenesis 26, no. 3 (January 1, 1995): 240–47. https://doi.org/10.1002/em.2850260309.*							
Myhr, Caspary, and Holden (1991)	ND	L5178Y mouse lymphoma cells	Mutation experiment		50 µg/mL (±S9) (NOAEL)	No observable adverse effect reported	NR
Test article: titanium dioxide ***Dose:*** 0, 1.56, 3.13, 6.25, 12.5, 25, and 50 µg/mL in culture medium ***Treatment duration:*** 11 – 12 days ***Species (strain):*** L5178Y mouse lymphoma cells (clone 3.7.2C). ***Number per Group:*** Duplicate or triplicate. ***Control:*** untreateg group. ***Methods:*** Cell culture for 11 – 12 days at 37°C (without S9 mix, positive control was methyl methanesulfonate, and with S9, 3-methyl-cholanthrene was positive control). (If no clear mutagenic response obtained without S9, retested with S9). ***Adverse Effect(s) (brief):*** **NOAEL = 50 µg/mL.** No evidence for mutagenesis or significant toxicity was obtained. An isolated increase in mutant frequency (MF) at the TK locus appeared for one dose in the second S9 trial, for unknown reason, but otherwise the MFs in the treated cultures remained at the background level. ***Data Quality:*** ND. ***Reference:*** *Myhr, Brian C., William J. Caspary, and H. E. Holden. "Chemical mutagenesis at the thymidine kinase locus in L5178Y mouse lymphoma cells: Results for 31 coded compounds in the national toxicology program." Environmental and Molecular Mutagenesis 18, no. 1 (January 1, 1991): 51–83. https://doi.org/10.1002/em.2850180109.*							
Türkez and Geyikoğlu (2007)	ND	Human whole blood cultures (WBCs)	Oxidative stress markers; Sister chromatid exchange assay; Micronuclei assay		> 1 µM	Oxidative stress; Increased sister chromatid exchange and micronuclei frequencies	OECD TG 473
Test article: titanium dioxide ***Dose:*** 0, 1, 2, 3, 5, 7.5, 10 µM in DMSO ***Treatment duration:*** for 72 hours at 37°C. ***Species (strain):*** Human whole blood cells. ***Gender:*** NR (non-spoking healthy donors of 22 – 30 years). ***Number per Group:*** four experiments. ***Control:*** untreated group. ***Methods:*** For the studies, stock solution of the TiO2 was prepared with sterile dimethyl sulfoxide (DMSO) and subsequently the standard solutions of the chemical at diverse concentrations (1, 2, 3, 5, 7.5 and 10mM) were prepared by diluting the stock solution. Blood samples (5–10 mL) were obtained through arm vein puncture from four non-smoking healthy donors, aged 22–30. After supplementation with different concentrations of TiO2, the blood was incubated for 1 h at 37°C to adjust body conditions, except for testing SCE and MN (for 72 h at 37°C). The control samples of each volunteer were incubated and treated equally as the samples, but without TiO2 addition. CAT activity was determined. GR catalyzed NADPH oxidation was measured in spectrophotometer at saturating substrate concentrations (100mM NADPH, 1 mM GSSG). MN test was performed by adding cytochalasin B (final concentration of 6mg/mL) after 44 h of culture. At the end of the 72-h incubation period, experimental data were analyzed using one-way analysis of variance to determine whether any treatment significantly differed from controls and/or from each other. Significant differences between the controls and/or treated samples were confirmed by using Fisher's least significant difference and Student's t tests. ***Adverse Effect(s) (brief):*** **NOAEL = 1 µM.** The activities of antioxidant enzymes in erythrocytes showed significant decreases with increasing doses of titanium dioxide. At 10 µM titanium dioxide, the activities of oxidative stress markers, glutathione peroxidase (GSH-Px), glutathione reductase (GR), catalase (CAT), and superoxide dismutase (SOD), were reduced by 32, 12, 21 and 26%, respectively. A minimum of 30 s-division metaphases per subject was scored for sister chromatid exchange (SCE) assay. At least 1,000 binucleated lymphocytes were examined per concentration in the presence of one, two or more micronuclei (MN). Titanium dioxide was able to induce genotoxic effects, as observed by the significant increases found in SCE frequency (from 2 µM upwards, 2-fold increase at 10 µM) as well as in MN frequency (from 5 µM upwards, 4-fold increase at 10 µM). Treatments with titanium dioxide promoted oxidative stress in human whole blood cultures with an increase in concentration. ***Data Quality:*** ND. ***Reference:*** *Türkez, Hasan, and Fatime Geyikoğlu. "An in Vitro Blood Culture for Evaluating the Genotoxicity of Titanium Dioxide: The Responses of Antioxidant Enzymes." Toxicology and Industrial Health 23, no. 1 (February 2007): 19–23. https://doi.org/10.1177/0748233707076764.*							
Zeiger et al. (1988)	ND	*Salmonella typhimurium* TA97, TA98, TA100, TA1535, TA1537	Ames		NR	No observable adverse effect reported	NR
Test article: Titanium dioxide (CASRN 13463-67-7) with and without S9 metabolic activation (300 chemicals tested in this study, one of them being titanium dioxide) for 2 days at 37°C. ***Dose:*** *NR* ***Control:*** untreated group ***Methods:*** The test chemical (0.05 ml), Salmonella culture (0.10 ml), and S-9 mix or buffer (0.50 ml) were incubated at 37"C, without shaking, for 20 min. The histidine-							

Source Citation	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
independent (his') colonies arising on these plates were counted following two days incubation at 37°C. Concurrent solvent and positive controls were run with each trial. The positive controls in the absence of metabolic activation were sodium azide (TA1535 and TA loo), 9-aminoacridine (TA97 and TA 1537), and 4-nitro-o-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene. ***Adverse Effect(s) (brief):*** **NOAEL/LOAEL = NR.** No mutagenicity of titanium dioxide was observed with and without metabolic activation. Titanium dioxide may not induce mutagenicity in salmonella typhimurium after 2 days of exposures. ***Data Quality:*** ND. ***Reference:*** *Zeiger, Errol, Beth Anderson, Steve Haworth, Timothy Lawlor, and Kristien Mortelmans. "Salmonella Mutagenicity Tests: IV. Results from the Testing of 300 Chemicals." Environmental and Molecular Mutagenesis 11, no. Suppl. 12 (1988): 1–157.*							
Carcinogenicity							
Lee, Trochimowicz, and Reinhardt (1985)	ND	Animal	Inhalation	250 mg/m^3 **(LOAEL)** ; 50 mg/m^3 **(NOAEL)**	2 years	Tumors	NR
Test article: Titanium dioxide ***Dose:*** 0, 10, 50, 250 mg/m^3 ***Treatment duration:*** for 6 hr/day, 5 days/week for 2 years (1.5 to 1.7 mm; 84% <13 mm). ***Species (strain):*** CD Rat. ***Gender:*** Male and female. ***Number per Group:*** (100/sex/group). ***Control***: Air used as vehicle. ***Methods:*** In this two-year inhalation carcinogenicity study rats were exposed to TiO2 using a dust generation system. Five animals per dose and group were sacrificed at 3 and 6 months of exposure, and subsequently, 10 animals per dose and groups were scarified after one year. All animals were subjected to gross and microscopic evaluation. ***Adverse Effect(s) (brief):*** **NOAEL = 50 mg/m^3.** There were no abnormal clinical signs, body weight changes, or excess mortality in any exposed group. In the lungs of rats exposed to 10 mg/m3, white foci (< 1 mm) were scattered sparsely throughout the pleural surface with dense accumulation of white foci in the periphery of the lobes. These white foci increased significantly in number and size (~2 mm) at 50 mg/m3. At 250 mg/m3, the lungs were voluminous, showed a white "paintbrushed' appearance, contained rough pleural surfaces, and failed to collapse. There was a significant increase in lung weight for animals exposed at the mid, and high dose compared to controls. The high dose lung weights were more than twice the weight of the controls. The tracheobronchial lymph nodes were markedly enlarged in a dose-related fashion and showed a white"chalky mass" appearance. Exposed groups showed slight increase in the incidence of pneumonia, tracheitis, and rhinitis with squamous metaplasia in the anterior nasal cavity. The severity of the lesion was dose dependent. Lungs of rats exposed to 10 mg/m3, the inhaled particulates were mostly phagocytosed by interalveolar macrophages (dust cells) in a few alveolar ducts and adjoining the alveoli in an acinus. The alveoli showed slight hyperplasia of the cubidal lining cells. The lungs of the 50 mg/m3, dust cell aggregates were increased and were confined to alveolar ducts and adjacent alveoli. Alveolar walls enclosing dust cells showed hyperplasia of the alveolar lining cells. Cholesterol granulomas increased in numbers. The incidence of focal pleurisy was increased significantly. At 250 mg/m3, Bronchioloalveolar adenomas and cystic keratinizing squamous cell carcinomas occurred, while no compound-related lung tumors were found in rats exposed to either 10 or 50 mg/m^3. The lung tumors were different from common human lung cancers in terms of tumor type, anatomic location, tumorigenesis, and were devoid of tumor metastasis; therefore, the biological relevance of these lung tumors and other pulmonary lesions for man is negligible. **This figure represents pathologic evidence of carcinogenetic of TiO2 in mouse lung tissue.**							

Source Citation	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
 FIG. 12. Alveolar wall is lined with ciliated columnar cells showing squamous cell metaplasia (S) with massive keratin formation (K). The dilated air space is filled with mucinous secretion and dust cells (arrows) (250 mg/m^3, HE stain, ×450). ***Data Quality:*** Data quality is not assessed based on overloading of the lungs in this study. ***Reference:*** *Lee, K. P., H. J. Trochimowicz, and C. F. Reinhardt. "Transmigration of Titanium Dioxide (TiO2) Particles in Rats after Inhalation Exposure." Exp Mol Pathol 42, no. 3 (1985): 331–43.*							
NCI (1979)	ND	Animal	Oral (feed)	7500 mg/kg/d	103 weeks	No observable adverse effect reported	NR
Subchronic study: Test article : 98% TiO_2 ***Dose:*** 6, 250, 12500, 25000, 50000 or 100000 ppm ***Methods:*** A oral 14-day repeat dose study in rodents was conducted as a dose range-finding study**.** Test animals were dosed via diet at 6, 250, 12500, 25000, 50000 or 100000 ppm, while controls received a basal diet. The animals were dosed for 13-weeks consecutively. ***Species (strain):*** F344 Rat & B6C3F1 Mouse ***Gender:*** Male and female. ***Number per Group:*** (10 animals/ per sex/ per dose). ***Contorl:*** untreated group. ***Adverse Effect(s) (brief):*** NOAEL = 100000 ppm. In both the rat and mouse studies, there were no mortality, no treatment related changes in body weight, and no gross or microscopic pathology changes, observed compared to controls. 50000 ppm was selected as the high dose for the chronic exposure study. **Chronic study: *Test article:*** Titanium dioxide antase; (Rats) 0, 25000 ppm (3750 mg/kg/d), 50000 ppm (7500 mg/kg/d) titanium dioxide for 103 weeks, 7 days a week; (mice) 0, 25000 ppm (3750 mg/kg/d), 50000 ppm (7500 mg/kg/d) titanium dioxide for 103 weeks, 7 days a week. Corn oil used as vehicle. ***Test article*** p***urity***: 98% TiO_2 ***Methods:*** In this two-year carcinogenicity study rats and mice where fed diets containing various dose of TiO_2 anatase or control diets ad libitum. The test animals were observed twice daily for sign of toxicity. Clinical signs and presence of palpable masses were recorded weekly. Mean body weight and food consumptions were recorded bi-weekly for the first 12 weeks and monthly thereafter. The pathologic evaluation consisted of gross and microscopic examination of the major tissues/ major organs (brain, pituitary , spinal cord, esophagus, trachea, salivary glands, mandibular lymph nodes, thyroid, parathyroid, heart, thymus, lungs and mainstem bronchi, liver, gallbladder (mice only), pancreas, spleen, kidney, adrenal, stomach, small intestine, colon, urinary bladder, prostate or uterus, testes or ovaries, sternebrate, femur, or vertebrae marrow, mammary gland, tissues masses), and all gross lesions. The examined tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with H&E. ***Species (strain):*** F344 Rat & B6C3F1 Mouse ***Gender:*** Male and female. ***Number per Group:*** 50 animals per sex per dose. ***Adverse Effect(s) (brief):*** Rats: The test substance had no effects on mean body weights in male or females compared to controls. The observed clinal signs where alopecia, sores, lacrimation, protruding, and pale eye. Observations in the dosed group were comparable to controls. All dosed animals had white feces. There was no dose dependent effects related to mortality observed in treated animals. Pathological changes were observed. In the male rats, pheochromocytomas of the adrenal medulla and fibromas of the subcutaneous tissue were observed with slightly greater frequency in dosed groups; however, the number of neoplasms was compatible with incidences of these tumors in historical-control rats of this age and strain. In the female rats, endometrial stromal polyps were observed more frequently in dosed groups than in control groups, but the incidence of lesions is comparable with that in historical controls. Thus, these lesions are not considered to be related to administration of the test chemical. Based on the histopathologic							

Source Citation	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Protocol Guideline
examination, titanium dioxide was neither toxic nor carcinogenic to Fischer 344 rats under the conditions of this bioassay. There was no significant correlation compared to controls. Thus, these tumors of the thyroid were not considered to be related to the administration of the test chemical. ***Conclusion:*** Based on the reported results, under the conditions of the bioassay, TiO2 was not carcinogenic by oral route for Fischer 344 rats or B6C3F1 mice. ***Data Quality:*** Not reported. ***Reference*** NCI. 1979. "*Bioassay of Titanium Dioxide for Possible Carcinogenicity. CAS No. 13463-67-7. NCI-CG-97." National Cancer Institute (NCI) Carcinogenesis Technical Report Series 97: 1–123.*							
Bischoff and Bryson (1982)	Yes	Animal	Intraperitoneal injection	25 mg (**NOAEL**)	Once	No observable adverse effect reported	NR
Test article: titanium dioxide ***Dose:*** 25 mg and ***Species (strain):*** Marsh-Buffalo Mouse. ***Test article purity***: 58% titanium ***Gender:*** Male. ***Number per group:*** (32/per dose) and (30/controls). ***Control:*** Saline . ***Methods:*** In this 18-month rat study, animals received intraperitoneal injections of titanium dioxide (100 mg TiO2 suspended in 1 ml saline). The test substance was prepared by mortar and pestle in an isotonic solution. Each animal was injected with the TiO2 suspension or saline. The experiment was terminated after 18-months, animals were sacrificed, and autopsy was performed. ***Adverse Effect(s) (brief):*** **NOAEL = 25 mg.** There was an accumulation of test material in the intraperitoneal, muscle wall and gut. There were no association of neoplastic development with experimental treatment. There were no significant pathological findings in treated animals when compared to controls. At the termination of the experiment, the histologic finding, demonstrated lack of inflammatory response. While local deposits of TiO_2 were found in necropsy (ex., mesentery, gut, striated muscle, colon, and peritoneal wall) there was no evidence of increased incidences of tumors in treated animals compared to controls for up to 18 months. A nonsignificant association of tumorigenesis with experimental treatment indicated a lack of carcinogenic potential for titanium dioxide. ***Data Quality:*** NR. ***Reference:*** *Bischoff, F., and G. Bryson. "Tissue Reaction to and Fate of Parenterally Administered Titanium Dioxide. I. The Intraperitoneal Site in Male Marsh-Buffalo Mice." Research Communications in Chemical Pathology and Pharmacology 38, no. 2 (November 1982): 279–90.*							

SUPPORTING STUDIES

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
Distribution							
Huggin,1996	ND	Animal	intravenous injection	250 mg per kg.	single intravenous injection	NA	NR
Test article: Titanium dioxide (particle size was 0.2 to 0.4 µm;TiO2, 99%; Pb 0.0016%.). ***Dose:*** the usual dose was 200 to 250 mg per kg. 5% dextrose as vehicle. ***Species (strain):*** Rat (Sprague-Dawely). ***Gender:*** Female (145-155g). ***Number per Group:*** 8/per group ***Control:*** untreated group ***Methods:*** In this study a known amount of TiO2 was injected intravenously as a suspension freshly prepared for each experiment. TiO2, 2.5 g, was added to 100 ml of 5% dextrose and dispersed by shaking for 1 hr. Aliquots of each suspension were taken for chemical analysis. For routine work particle size was estimated by microscopy. The suspensions were injected slowly (1 to 2 min) in a caudal vein; the usual dose was 200 to 250 mg per kg. Samples of whole blood were obtained by cardiac puncture. Animals were decapitated. Chemical analyses for TiO2 were always multiple when the amount of available tissue permitted. The reported values are expressed as mg of TiO2 per g of fresh tissue (wet weight). Tissue Distribution of Injected Titanium Dioxide.--Normal female rats age 50 days received a single i.v. injection of TiO2, 250 mg per kg. Autopsy was performed on groups of 8 animals at intervals thereafter. In one experiment the animals were killed soon after the injection. Celiac Lymph Nodes.--A series of experiments was carried out to determine the effects of removal of spleen or of a large fraction of liver upon the number and content of TiO2 in celiac lymph nodes. Surgical operations were performed at age 50 days; at various intervals thereafter a suspension of TiO2, 250 mg per kg was injected intravenously and tissues were harvested 24 hr after the injection. Control rats were not operated upon. ***Results (brief):*** The following fractions of the injected dose of TiO2, were found in liver: at 5 min, 69%; at 15 min, 78%. 6-hr group: the highest concentration of TiO2 was found in liver, followed by spleen. Titanium was not concentrated in any of the lymph nodes of the celiac group. Values of TiO2, 0.4 to 0.5 mg per g, were found in all of the lymph nodes which were studied including celiac, iliac and mediastinal nodes. 12-hr group: concentration of TiO2 in celiac lymph nodes was observed in this group. Some of the celiacs were pearl-gray in color and in them the concentration of TiO2 was, on average, 1.2 mg per g. In iliac lymph nodes of the same animals, the TiO2 content was 0.43 mg per g. 24-hr group: The highest concentration of TiO2 was found in white celiacs, followed by that in liver while in third place was spleen. 1-yr group: The concentration of TiO2 was measured in several tissues. The highest concentrations of TiO2, were found in celiacs; mediastinal lymph nodes lateral to the thymus; spleen; liver. The concentration of TiO2 in white celiacs exceeded by 18 times that in the mediastinal lymph nodes and these occupied rank 2 in titanium concentration. The celiac lymph nodes were intensely white, but they were not enlarged; 28 white celiac lymph nodes from 5 rats weighed, on average, 11.6 + 1.6 mg, a value							

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
rather similar to controls. On microscopy, it was found that the largest amount of TiO2 was located in the medulla of the lymph nodes. Effect of Splenectomy or Partial hepatectomy on Celiac Lymph Nodes: In 8 rats, 5 to 7 white celiac lymph nodes were found. In most cases 3 nodes were found on the right side of abdomen and 2 to 4 white celiacs on the left side. The content of TiO2 was measured in various organs and the results were expressed in percentage of the total injected dose: liver 76 to 82 %; spleen 1.5 to 3.4 %; celiacs 0.05 to 0.08 %. In normal rats injected TiO2 always had higher concentration in liver than in spleen; the ratio of TiO2 spleen/liver was 0.41. Splenectomy: In 8 rats injected with TiO2 1 to 21 days after removal of the spleen, 5 to 7 white celiac lymph nodes were found in normal rats. The concentration of TiO2 in liver of splenectomized rats was like that of normal controls. Partial hepatectomy: Excision of median and left lateral hepatic lobes had profound effects on the distribution of injected TiO2; these changes were impressive 24 hr after removal of the liver. The number of white celiacs was reduced to 2 lymph nodes; these were located only on the right side of the abdomen around the epiploic foramen. There were no white celiacs on the left side of the retroperitoneal space. The regenerating liver was intensely white from its ingestion of TiO2. As in the intact normal control, 80 % of the dose of TiO2 was found in the regenerating hepatic lobes even though the total amount of liver was less than in normal controls. In rats injected with TiO2, white celiacs on the left side of abdomen became evident first on day 7 after hepatectomy. On day 21 the number and site of white celiac lymph nodes were reminiscent of those of controls. In the rat, the greatest accumulation, in any anatomical structure, of titanium dioxide following its intravenous injection was found in two small clusters of lymph nodes in upper abdomen behind the peritoneum. These are the lymph nodes of the liver. This extraordinary quantitative characteristic of the abdominal clusters is attributed to their topography which results in progressive filtration of particulate matter from hepatic lymph. ***Data Quality:*** NR ***Reference:*** *Huggins, Charles B., and Jeffrey P. Froehlich. "High Concentration of Injected Titanium Dioxide in Abdominal Lymph Nodes." Journal of Experimental Medicine 124, no. 6 (December 1, 1966): 1099–1106. https://doi.org/10.1084/jem.124.6.1099.*							
Jani, 1993	ND	Animal	Oral Gavage	12.5 mg kg 1	Once	NA	NR
Test article: Titanium dioxide (500 nm) ***Dose:***12.5 mg kg 1 ***Species (strain):*** Rat (Sprague-Dawely). ***Gender:*** Female (150g). ***Number per Group:*** 6/per group ***Control:*** untreated group ***Methods:*** Titanium dioxide (rutile) particles of nominal size 500 nm were administered as a 0.1 ml dose of a 2.5% w/v suspension (12.5 mg kg 1) to female Sprague Dawley rats, by oral gavage daily for 10 days. Organs and tissues such as Peyer's patches, small intestine, colon, mesentery network and nodes, peritoneal tissue, liver, spleen, heart and kidney were removed for histology, scanning electron microscopy, and spectroscopic analysis for titanium, using the technique of inductively coupled plasma atomic emission spectroscopy. The animals were weighed daily and kept in individual metabolic cages to ease the collection of urine and feces and to prevent coprophagia. After the final dose was administered, the animals were kept for 24 h in a particle-free environment to clear the gastrointestinal tract of an unabsorbed particles. Before being killed with ether, the animals were fasted for 15 h to clear the gut of food particles. Stomach, intestine (with mesentery network), colon, peritoneal tissue, liver, spleen, kidney, heart, and lungs were carefully removed, weighed, and stored in a 10% v/v formalin solution in individual pots, avoiding cross contamination between samples. ***Results (brief):*** Histological and chemical analysis showed the presence of titanium dioxide particles in all the major tissues of the gut associated lymphoid tissue (GALT), and demonstrated that 500 nm titanium dioxide particles were translocated to systemic organs such as the liver and the spleen. Titanium dioxide particles were also found in the lung and peritoneal tissues but were not detected in the heart or the kidney. The authors report 6.5% of the total dose of titanium dioxide particles in the 500 nm size range administered orally over 10 days is absorbed. ***Data Quality:*** NR ***Reference:*** *Jani, Praful U., David E. McCarthy, and Alexander T. Florence. "Titanium Dioxide (Rutile) Particle Uptake from the Rat GI Tract and Translocation to Systemic Organs after Oral Administration." International Journal of Pharmaceutics 105, no. 2 (May 2, 1994): 157–68. https://doi.org/10.1016/0378-5173(94)90461-8.*							
Wang, 2007	ND	Animal	Oral Gavage	5 g/kg bw	single oral gavage	NA	OECD 420
Test article: Titanium dioxide fine TiO2 (155 nm). ***Dose:*** 5 g/kg bw ***Species (strain):*** Mouse (CD-1). ***Gender:*** Male/Female (19 g). ***Number per Group:*** 40/per sex/ (8-10/ per group) ***Control:*** untreated group ***Methods:*** To evaluate the toxicity of TiO2 particles, the acute toxicity of fine TiO2 particles (155 nm). Due to the low toxicity, a fixed large dose of 5 g/kg body weight of TiO2 suspensions was administrated by a single oral gavage according to the OECD procedure. A 0.5% hydroxypropylmethyl cellulose K4M (HPMC, K4M) was used as a suspending agent. A 3 g of each TiO2 powder was dispersed onto the surface of 0.5%, w/v HPMC solution (12 ml), and then the suspending solutions containingTiO2 particles were treated by ultrasonic for 15–20 min and mechanically vibrated for 2 or 3 min. The sizes of particles were tested using transmission electron microscopy (TEM). The sizes observed by TEM are in coincidence with the nominal sizes. The size of fine TiO2 was 155±33 nm. Before treatment, animals were fasted overnight. After vigorous stirring, TiO2 suspension (single dose of 5 g/kg body weight) was given to mice by a syringe via gastrointestinal tract in a minute. Control mice were given 0.5% HPMC. Food and water were provided 2 h later. ***Results (brief):*** After 14-days mice exposed to TiO2, particles showed no obvious symptoms of acute toxicity. No							

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
changes in the serum LDH, alpha-HBDH enzymes, or BUN and CK levels compared with the control in the fine group. There was no difference in tissue TiO2 accumulation when compared to control for the fine group. In addition, no pathology change (heart, lung, testicle (ovary), and spleen tissues) were observed in the treatment group. Biodistribution experiment showed no difference for TiO2 in the treatment group compared to control. ***Data Quality:*** NR ***Reference:*** *Wang, Jiangxue, Guoqiang Zhou, Chunying Chen, Hongwei Yu, Tiancheng Wang, Yongmei Ma, Guang Jia, et al. "Acute Toxicity and Biodistribution of Different Sized Titanium Dioxide Particles in Mice after Oral Administration." Toxicology Letters 168, no. 2 (January 30, 2007): 176–85. https://doi.org/10.1016/j.toxlet.2006.12.001.*							

SECONDARY SOURCES

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
Systemic Toxicity (acute)							
ECHA, 1971	ND	Animal	Oral (gavage)	> 25000 mg/kg bw **(LD_{50})**	Once	No observable adverse effect reported	NR
Test article: Titanium dioxide 50/50 mixture with mono and bis butyl phosphate. ***Dose:*** 0, 2250, 5000, 7500, 11000, 17000, or 25000 mg/kg bw was treated once. Corn oil used as vehicle. ***Species (strain):*** Rat (ChR-CD). ***Gender:*** Male. ***Number per Group:*** 5 animals per sex per dose. ***Control:*** untreated group ***Methods: Survivors were sacrificed 14 days later.*** Oral (gavage). ***Adverse Effect(s) (brief):*** **LD_{50} > 25000 mg/kg bw.** Weight loss for 1-2 days at 2250 mg/kg and above. The test substance caused diarrhea (compound and/or metabolite excreted with feces) on day of dosing and wet perineal area on day after dosing at 5000 mg/kg and above**.** Approximate Lethal Dose (ALD) is reported to be greater than 25,000 mg/kg of body weight. ***Data Quality:*** *ECHA Reliability 2 (reliable with restrictions)*							
ECHA (n.d.)	ND	Animal	Oral (gavage)	> 5000 mg/kg bw **(LD_{50})**	Once	Lethality	OECD 420
Test article: Titanium dioxide ***Dose:*** suspended in 0 or 5000 mg/kg (in 0.5% HPMC) was administered by a syringe via the gastrointestinal tract in a minute. Hydroxypropylmethyl cellulose (HPMC) was used as vehicle. ***Species (strain):*** Mouse (CD-1). ***Gender:*** Male and female. ***Number per Group:*** 10 animals per sex per dose. ***Control:*** untreated group ***Methods:*** Animals were fasted overnight. TiO2 suspension (in 0.5% HPMC) was given by a syringe via the gastrointestinal tract in a minute. Food and water were provide 2h later. Symptoms and mortality were observed and recorded during the first 24 h. 14 days after dosage the animals were sacrificed and subjected to gross pathological examination. Results were expressed as mean ± standard deviation (S.D.). Multigroup comparisons of the means were carried out by one-way analysis of variance (ANOVA) test. Dunnett's test was used to compare the differences between the experimental groups and the control group. Student's t-test was used to compare the means of each nano-group and the corresponding fine group. The statistical significance for all tests was set at $p < 0.05$. ***Adverse Effect(s) (brief):*** **LD_{50} >5000 mg/kg bw.** Morality occurred during the study 2 female mice after 2 days, 1 female mice after 2 days and 1 male mouse after 3 days. No abnormal clinical signs were observed during treatment of the test animals. No obvious differences were found in the body weight under the conditions of this study. The oral LD50 for titanium dioxide was greater than 5000 mg/kg for male and female mice. ***Data Quality:*** *ECHA Reliability 2 (reliable with restrictions)*							
ECHA, 1989	ND	Animal	Oral (gavage)	>5000 mg/kg bw **(LD_{50})**	Once	No observable adverse effect reported	Similar to OECD 401; GLP
Test article: Titanium dioxide ***Dose:*** suspension of 0 or 5000 mg/kg (in 0.5% HPMC) was given by a syringe via the gastrointestinal tract in a minute. Water was used as vehicle. ***Species (strain):*** Rat (Sprague-Dawley). 123-169 g ***Gender:*** Male and female. ***Number per Group:*** 5 – 6 animals per sex per dose. ***Control:*** Not specified ***Methods:*** The rats were observed frequently on the day of dosing and once daily for 14 days following dosing. They were weighed immediately prior to dosing, 7 days after dosing and at sacrifice at the end of the 14 day observation period. At the end of the observation period and sacrifice by carbon dioxide asphyxiation, each animal was subjected to a gross post mortem examination. ***Adverse Effect(s) (brief):*** **LD_{50} = >5000 mg/kg bw.** There were piloerection, no deaths following a single oral dose of titanium dioxide at a dose level of 5000 mg/kg bw. No gross postmortem abnormalities were observed. ***Data Quality:*** *ECHA Reliability 2 (reliable with restrictions)*							
ECHA (n.d.)	ND	Animal	Oral (gavage)	>11000 mg/kg bw **(LD_{50})**	Once	No observable adverse effect reported	OECD 425
Test article: Titanium dioxide ***Dose:*** suspension of 0 3400, 5000 or 7500, 11000 mg/kg was orally. Water was used as vehicle. ***Species (strain):*** Rat (Crl:CD (SD)). ***Gender:*** Female. ***Number per Group:*** 1 – 3 animals per dose. ***Control:*** untreated group ***Methods:*** The dosing day was test day 1; postexposure day 14 was test day 15. Following administration of the test substance, rats were observed for clinical signs of toxicity. Surviving rats were weighed and observed daily until signs of toxicity subsided, and then at least 3 times per week throughout the 14 or 15-day observation period. Observations for mortality were made daily							

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
throughout the study. Pathological examinations of the test animals were not performed. ***Adverse Effect(s) (brief):*** **LD_{50} >11000 mg/kg bw.** No clinical signs of toxicity were observed in the treated rats following a single oral dose of titanium dioxide at a dose level of 11000 mg/kg bw. Rats dosed at 3400, 5000 or 7500 mg/kg exhibited weight losses of up to 6% of initial body weight 1 day after dosing. No deaths occurred during the study. ***Data Quality:*** *ECHA Reliability 1 (reliable without restrictions)*							
ECHA (n.d.)	ND	Animal	Oral (gavage)	7500 mg/kg bw **(LD_{50})**	Once	No observable adverse effect reported	OECD 420
Test article: Titanium dioxide ***Dose:*** suspension of 0, 3400, 5000, or 7500 mg/kg was orally. Water was used as vehicle. ***Species (strain):*** Crl:DC BR Rat. ***Gender:*** Male. ***Number per Group:*** 6 animals per dose. ***Control:*** untreated group ***Methods:*** Oral (gavage). ***Adverse Effect(s) (brief):*** **LD_{50}= 7500 mg/kg bw.** No effects were observed following a single oral dose of titanium dioxide at a dose level of 7500 mg/kg bw. ***Data Quality:*** *ECHA Reliability 2 (reliable with restrictions)*							
ECHA (n.d.)	ND	Animal	Inhalation	>5.09 and 3.43 mg/l **(LC_{50})**	Once	No observable adverse effect reported	OECD 403
Test article: Titanium Dioxide Powders, NP 89/117 and NP 89/118 at ***Dose:*** 0, 5.09, and 3.43 mg/L was inhaled for 4 hrs. Air used as vehicle. ***Species (strain):*** Sprague-Dawley Rat. ***Gender:*** Male and female. ***Number per Group:*** 5(female) 6 (male) per dose. ***Control:*** untreated group ***Methods:*** Inhalation (nose). ***Adverse Effect(s) (brief):*** LC_{50}= 5.09 and 3.43 mg/l, respectively. Gross pathology resulted in pale lungs for 3/5 male and 1/5 female animals exposed to NP 89/117 and mottled lungs were recorded for 2/5 male and 3/5 female animals exposed to NP 89/118.Titanium dioxide powders were considered to be non-toxic to rats by the inhalation route at measured atmospheric concentrations of 5.09 and 3.43 mg/L. ***Data Quality:*** *ECHA Reliability 2 (reliable with restrictions)*							
OECD SIDS (2013)	ND	Animal	Oral	> 5000 mg/kg bw **(LD_{50})**	NR	Ambiguous	OECD 420, 425, 401
Test article: Titanium dioxide ***Dose:*** 0, 1750, 2000, 5000 mg/kg. ***Species (strain):*** Mice (CD-1); Rat (Sprague-Dawley) ***Gender:*** Male and female. ***Number per Group:*** 5-10 per group/ per sex. ***Control:*** untreated group ***Methods:*** Oral. ***Adverse Effect(s) (brief):*** **LD_{50} > 5000 mg/kg bw (for male/female mice).** This is a combination of three studies. Significant increase of titanium dioxide in the spleen and brain. Neuron vacuoles in the hippocampus and hydropic degeneration and spotty necrosis in liver cells were observed. In another system, no mortality and body weight changes were observed, and no gross lesions were present in all the animals at necropsy. Temporary grey colored feces observed. ***Data Quality:*** Not reported ***Reference:*** *OECD SIDS. 2013. "SIDS Initial Assessment Profile - Titanium Dioxide," 8.*							
Systemic Toxicity (subacute)							
ECHA, 1996	ND	Animal	Oral (gavage)	2000 mg/kg bw (NOAEL)	14 days	No observable adverse effect reported	OECD 401
Test article: Titanium dioxide ***Dose:*** 0 or 2000 mg/kg bw was treated once daily for 14 days. Arachis oil used as vehicle. ***Species (strain):*** Rat (Sprague-Dawley). ***Gender:*** Male 227-243 g and female. 200-214 g ***Number per Group:*** 5 animals per sex per dose. **Control:** Not specified ***Methods:*** The animals were observed for deaths or overt signs of toxicity 0.5, 1, 2 and 4 hours after dosing and subsequently once daily for 14 days. Individual body weights were recorded prior dosing on day 0 and on days 7 and 14. At the end of the study the animals were killed and subjected to gross pathological examination. This consisted of an external examination and opening of the abdominal and thoracic cavities for examination of major organs. The appearance of any macroscopic abnormalities was recoded. No tissues were retained. ***Adverse Effect(s) (brief):*** NOAEL = **2000 mg/kg bw.** There were no deaths or clinical signs of toxicity. Based on this information, a dose level of 2000 mg/kg body weight was selected for the main study. No signs of systemic toxicity were noted during the study. The acute oral median lethal dose of the test material was found to be greater than 2000 mg/kg body weight. All animals showed an expected gain in body weight during the study. ***Data Quality:*** *ECHA Reliability 2 (reliable with restrictions)*							
Systemic Toxicity (subchronic)							
ECHA, 2011	ND	Animal	Oral (gavage)	≥1000 mg/kg/day **(NOAEL)**	92 – 93 days	No observable adverse effect reported	OECD 408; GLP
Test article: Titanium dioxide ***Dose:*** 0, 100, 300, or 1000 mg/kg/day were administered for 92 days (males) or for 93 days (females). 0.5% aqueous methylcellulose used as vehicle. ***Control***: control (0 mg/kg/day). ***Treatment duration:*** 92-93 days *daily (approximately the same time (± 2 hours))* ***Species (strain):*** Rat (Crl:CD (SD)). ***Gender:*** Male and female. ***Number per Group:*** 10 animals per sex per dose. ***Methods:*** H-29865 was suspended in 0.5% aqueous methylcellulose. Dose suspensions of the test substance were prepared daily and stored at room temperature until used. Animals were dosed at a dose volume of 10 mL/kg body weight. The amount of test substance each animal received was based on the most recently collected body weight and the suspension concentration. Control animals were dosed with the vehicle at a volume of 10 mL/kg of body weight. Beginning test day 35 through the remainder of the study, the dose volume for several male rats was more than 5 mL. The dose for these rats was administered in 2 aliquots at least 15 minutes apart. dosages of 0, 100, 300, and 1000 mg/kg were selected for this study based on a previously conducted 28-day oral gavage study with H-27201 and H-27203 (please refer to Section 7.5.1 Repeated dose toxicity: oral: k_Mayer_2006_28 days). No test substance-related effects were observed on any in-life, clinical pathology, or anatomic pathology							

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
parameter in male rats dosed at 1000 mg/kg for 28 consecutive days. Animals of each sex were selected for use on study based on adequate body weight gain and freedom from any ophthalmology abnormalities or clinical signs of disease or injury. The selected animals were distributed by computerized, stratified randomization into study groups as designated in the Study Design so that there were no statistically significant differences among group body weight means within a sex. The weight variation of selected animals did not exceed ± 20% of the mean weight for each sex. ***Adverse Effect(s) (brief):*** **NOAEL ≥1000 mg/kg/day.** no test substance-related adverse changes in histopathology were observed. No test substance-related gross observations. no test substance-related organ weight changes, no test substance-related effects on neurobehavioral parameters, no treatment-related changes in group mean urinalysis parameters at test day 93/94 in male or female rats. No treatment-related changes in group mean clinical chemistry parameters at test day 93/94 in male or female rats. no treatment-related changes in group mean haematology parameters and group mean coagulation parameters at test day 93/94 in male or female rats. no test substance-related ophthalmological findings were observed in any male or female group. No test substance-related effects were observed on food efficiency. No test substance-related effects were observed on food consumption. No test substance-related effects were observed on body weights or body weight gains. no deaths occurred. No adverse effects were observed in animals dosed in 1000 mg/kg/day. ***Data Quality:*** *ECHA Reliability 1 (reliable without restrictions)*							
ECHA, 2015 .	ND	Animal	Oral (gavage)	≥24000 mg/kg/day **(NOAEL)**	29 days	No observable adverse effect reported	OECD 407; GLP
Test article: Titanium dioxide ***Dose:*** 0 or 24000 mg/kg/day were administered for 29 consecutive days. **Treatment duration:** daily; The rats were dosed by intragastric intubation for 29 consecutive days. Water was used as vehicle. ***Species (strain):*** Rat. (Crl:CD (SD) IGS BR). ***Gender:*** Male. ***Number per Group:*** 5 animals per sex per dose. ***Control:*** untreated group ***Methods:*** The test substance was suspended in NANOpure water and the dosing suspensions were prepared daily. At every weighing, each rat was individually handled and examined for abnormal behavior and appearance. Detailed clinical observations in a standardized arena were also evaluated on all rats. The detailed clinical observation included (but were not limited to) evaluation of fur, skin, eyes, mucous membranes, occurrence of secretions and excretions, autonomic nervous system activity (lacrimation, piloerection, and unusual respiratory pattern), changes in gait, posture, response to handling, presence of clonic, tonic, stereotypical, or bizarre behaviour. Any abnormal clinical signs noted were recorded. A clinical pathology evaluation was conducted on all surviving rats on test day 29. Parameters checked: red blood cell count, absolute reticulocyte count, hemoglobin, platelet count, hematocrit, white blood cell count, mean corpuscular (cell) volume, differential white blood cell count, mean corpuscular (cell) hemoglobin, microscopic blood smear examination, mean corpuscular (cell) hemoglobin concentration, red cell distribution width, prothrombin time and activated partial thromboplastin time. Parameters checked: aspartate aminotransferase, glucose, alanine aminotransferase, total protein, sorbitol dehydrogenase, albumin, alkaline phosphatase, globulin, total bilirubin, calcium, urea nitrogen, inorganic phosphorus, creatinine, sodium, cholesterol, potassium, triglycerides and chloride. After approximately 28 days on study (test day 29), the surviving rats were sacrificed and necropsied for evaluation of repeated dose toxicity. The order of sacrifice for scheduled deaths was stratified among treatment groups. Rats were euthanized by carbon dioxide anesthesia and exsanguination. Gross examinations were performed on all rats. Final body weights and organ weights were recorded. The following tissues were collected from all (15/15) rats: digestive system (liver, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, salivary glands, pancreas), urinary system (kidneys, urinary bladder), respiratory system (lungs, trachea, nose, larynx/pharynx), cardiovascular system (heart, aorta), hematopoietic system(spleen, thymus, mandibular lymph node, mesenteric lymph node, bone marrow (collected with the femur and sternum), Peyer's patches (collected from sections of the digestive tract)), endocrine system (pituitary gland, thyroid gland, parathyroid glands, adrenal glands), nervous system (brain (three sections; including cerebrum, cerebellum, medulla/pons), spinal cord (three levels; cervical, mid-thoracic, lumber) sciatic nerve), musculoskeletal system (skeletal muscle, femur/knee joint, sternum), reproductive system male (testes, epididymides, prostate, seminal vesicles) and miscellaneous (skin, eyes (including retina and optic nerve), gross observations. The following tissues were weighed from rats sacrificed by design at the end of the 28-day repeated dose toxicity study: liver, kidneys, adrenal glands, thymus, brain, spleen, heart, testes, and epididymides. Organ weight ratios (% final body weight, % brain weight) and group mean values were calculated. ***Adverse Effect(s) (brief):*** **NOAEL = 24000 mg/kg/day.** No test substance-related signs were noted on daily or weekly clinical observations during the 28-day oral gavage study. Some non-specific clinical observations were noted in some animals but were not test substance related. These observation which included a neck wound, misshapen ears and hair loss. These types of clinical signs are commonly reported among rats of this sex and age group. One animal, number 305 (H-27201), was found dead at the end of the first week. During the second week, animal number 505 (H-27203) was observed to have breathing noises and was sacrificed. The necropsy report indicated both deaths were attributed to trauma from gavage. The remaining 13 male rats survived until the scheduled sacrifice (test day 29). No statistically significant test substance related effects were observed on mean body weight or body weight gains in the two treatment groups when comparison was made between the groups and when each group was compared to the control. There were no statistically significant differences in food consumption and food efficiency in the two treatment groups when compared against each other, and when compared to the control. There were no statistically significant or treatment-related changes in hematologic parameters in male rats exposed to 24,000 mg/kg TiO2 particles in either group when compared against each other or when compared to the control. There were no statistically significant or treatment-related changes in coagulation parameters in male rats. There were no statistically significant or treatment-related changes in clinical chemistry parameters in male rats. There were no test substance-related effects on organ weights. All individual and mean organ weight differences were spurious and unrelated to test substance administration. There were no test substance-related deaths. Of the 15 rats on study (5 males/dose group), two died before the terminal sacrifice, both due to dosing accidents. Gross examination demonstrated perforation of the esophagus in both rats. There were no test substance-related gross observations in the treatment or the control groups. All gross observations at the terminal necropsy were consistent with normal background lesions in rats of this							

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
age and strain. No adverse effects were observed in animals dosed in 24000 mg/kg/day. ***Data Quality:*** *ECHA Reliability 2 (reliable with restrictions)*							
OECD SIDS (2013)	No	Animal	Oral (gavage)	1000 mg/kg/day **(NOAEL)**	28 days	No effect	OECD 407
Test article: Titanium dioxide ***Dose:*** vehicle control (in 1% methyl cellulose solution) at 0, 250, 500 or 1,000 mg/kg/day for 28 days. Additional recovery groups were the control and high dose and observed for 14 days after treatment. ***Species (strain):*** Rat. ***Gender:*** Male and female. ***Number per Group:*** 5 animals per sex per dose. ***Control:*** untreated group ***Methods:*** Oral (gavage). ***Adverse Effect(s) (brief):*** **NOAEL = 1000 mg/kg/day (for both sexes).** No deaths were observed in either sex. Treatment related effects observed (compound-colored feces, effects on a few functional performance tests, some hematological and clinical chemistry parameters, liver and thymus weight changes) were not toxicologically significant. ***Data Quality:*** NR							
Genotoxicity (in *vitro* or *in vivo*)							
ECHA, 1995	ND	Bacteria	In vitro	5000 µg/ml	Once	No observable adverse effect reported	Similar to OECD 471; GLP
Test article: Chrome antimony titanium buff rutile; ***Dose:*** 100, 250, 500, 1000, 2500 and 5000 µg/plate ***Treatment Duration:*** 48±8 h at 37±2 °C ***Species (strain):*** S. typhimurium TA98, 100, 1535, 1537, 1538 and E. coli WP2uvrA ***Number per Group:*** Three replicates ***Control:*** S9 mix: 2-nitrofluorene (TA98, TA1538), sodium azide (TA1535, TA100), ICR-191 (TA1537), 4-nitroquinoline-M-oxide (WP2uvrA); +S9 mix : 2-Aminoanthracene (six strains) ***Method:*** Ames Assay. TA98, TA100 and WP2 uvrA considered positive if the test substance produced at least a 2-fold increase in revertant per plate over vehicle control and a dose response to increasing concentrations; same criteria for the other strains but 3-fold increase. ***Adverse Effect(s) (brief):*** The results were negative for mutagenic activity with and without metabolic activation in S. typhimurium ***Data Quality:*** Data quality cannot be verified because information is from a secondary source; however, secondary source (ECHA) has assigned a reliability (Klimisch) score of 2 (with restrictions).							
OECD SIDS, 2002	ND	Bacteria	In vitro	5000 ug/plate	NR	No observable adverse effect reported	NR; GLP
Test article: C.I. Pigment Brown 24 96.5 %. Bacteria was dosed at 5000 ug/plate. ***Dose:*** NR ***Treatment Duration:*** NR ***(strain):*** Salmonella typhimurium TA 1535, TA 100, TA 1537, TA 98 ***Gender:*** NA. ***Number per Group:*** NR. ***Methods:*** Ames Assay. **Adverse *Effect(s) (brief):*** Negative for genotoxicity in Salmonella typhimurium with and without metabolic activation. ***Data Quality:*** (4) Data quality cannot be verified because information is from a secondary source							
OECD SIDS, 2002	ND	Mouse Lymphoma Cells"	In vitro	0, 3.13, 6.25, 12.5, 25, 50, 100 µg/ml	NR	No observable adverse effect reported	Similar to OECD 476; GLP
Test article: C.I. Pigment Brown 24 96.5 %. ***Dose:*** 0, 3.13, 6.25, 12.5, 25, 50, 100 µg/ml in DMSO ***Treatment Duration:*** NR (***strain):*** L5178Y mouse lymphoma cells ***Gender:*** NA. ***Number per Group:*** NR. ***Method:*** Mouse lymphoma assay ***Adverse Effect(s) (brief):*** No cytotoxicity at any concentration tested. Results were negative in L5178Y mouse lymphoma cells with and without metabolic activation. ***Data Quality:*** (2) valid with restrictions. Data quality cannot be verified because information is from a secondary source;							
OECD SIDS, 2002	ND	Bacteria	In vitro	≥5000 ug/plate	NR	No observable adverse effect reported	Similar to OECD 471; GLP
Test article: Chrome Antimony Titanate. ***Dose:*** 0, 100, 250, 500, 1000, 2500, 5000 µg/plate ***Treatment Duration:*** NR ***Species*** (strain): Salmonella typhimurium TA 1535, TA 100, TA 1537, TA 98 ***Gender:*** NA. ***Number per Group:*** NR. ***Method:*** Ames assay. ***Adverse Effect(s) (brief):*** **NOAEL ≥5000 ug/plate**. Negative for genotoxicity in Salmonella typhimurium with and without metabolic activation. Data Quality: No 2nd independent trial. Data quality cannot be verified because information is from a secondary source ***Note:*** Critical study for SIDS endpoint							
OECD SIDS, 2002	ND	Mouse Lymphoma Cells	In vitro	0, or 0.78-25 µg/ml	NR	No observable adverse effect reported	Similar to OECD 476; GLP
Test article: Pigment Brown 24 obtained from BASF AG, purity 99.4%. ***Dose:*** 0, or 0.78-25 µg/ml were administered DMSO used as vehicle. ***Treatment Duration:*** NR ***Method:*** MLA test in vitro ***Species(strain):*** L5178Y mouse lymphoma cells ***Gender:*** NA. ***Number per Group:*** NR. ***Adverse Effect(s) (brief):*** **NOAEL=25 µg/ml.** No cytotoxicity at any concentration tested. Results were non-clastogenic in L5178Y mouse lymphoma cells with and without metabolic activation. ***Data Quality:*** (1) valid without restriction. Data quality cannot be verified because information is from a secondary source; ***Note:*** Critical study for SIDS endpoint							

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
ECHA (n.d.)	ND	Animal	Oral (gavage)	≥2000 mg/kg bw **(NOAEL)**	Once	No observable adverse effect reported	OECD 474: GLP
Test article: titanium dioxide ***Dose:*** 0, 500, 1000, and 2000 mg/kg bw in sterile water for injection ***Treatment duration:*** administered (single dose), followed by up to 72hrs. ***Species (strain):*** Rat (Crl:CD (SD)). 8 weeks ***Gender:*** Male (236.5–277.9 g) and female (172.8–196.3g). ***Number per Group:*** 5 – 7 animals per sex per dose. ***Control:*** 5 per sex/Cyclophosphamide (10 mg/kg) ***Methods:*** Whenever feasible, at least 20,000 reticulocytes were analyzed per blood sample for induction of micronuclei, and toxicity as indicated by the frequency of immature erythrocytes (% reticulocytes) among the total erythrocytes (reticulocytes plus normochromatic erythrocytes). The frequency of micronucleated reticulocytes (% micronucleate reticulocytes) was used as a measure of induction of aneugenic or clastogenic alterations by the test substance. All groups from both the 48- and 72-hour time points were analyzed. The highest dose used for the rangefinder for the in vivo micronucleus study was the limit dose of 2000 mg/kg body weight administered by oral gavage. The animals were observed for clinical signs of toxicity and mortality immediately after dosing and daily thereafter for 2 days (until approximately 48 hours post-dosing). There were 3 test substance concentrations for the main study: 500, 1000, and 2000 mg/kg bw. Peripheral blood samples were collected from all animals, at approximately 48- and 72-hours post-dosing for micronucleus evaluation, and from all groups except from the positive control group for possible titanium analysis. The micronucleus evaluation was conducted by flow cytometry using the In Vivo MicroFlow® rat kit. Evaluations were conducted on 5 animals/sex/group with the exception of the 1000 mg/kg bw group, where only 4 rats were evaluated. At the highest concentration, 5 males and 5 females were selected for evaluation based on survival until the scheduled sacrifice and then cage order. Body weight: the animals were weighed prior to treatment and at 24-, 48-, and 72-hours post-dosing. Clinical signs/mortality/moribundity: the animals were observed for clinical signs and mortality/moribundity prior to treatment, approximately 1 hour (±30 minutes) post-dosing, 3-5 hours post-dosing, 24 hours post-dosing, 48-hours post-dosing, and prior to the scheduled sacrifice. Necropsy: animals placed under general anesthesia by inhalation of isoflurane. All animals were sacrificed after the last blood collection on test day 4 with the exception of one male rat of the 1000 mg/kg bw group which was accidentally killed on day 3. This male rat was discarded without any sample collection or any pathological evaluation. At necropsy, blood was removed from the animal by exsanguination. ***Adverse Effect(s) (brief):*** **NOAEL = 2000 mg/kg bw.** There were no obvious changes in body weights or body weight gain in either male or female rats administered the test substance. One male rat of the 1000 mg/kg bw group was accidentally killed on day 3 prior to blood collection; therefore, bodyweights are not available for this animal on day 4. No clinical signs of toxicity were observed at any dose level in male or female rats exposed to the test substances. No abnormalities were detected in the vehicle or positive control groups. No mortality occurred during the study. One male rat of the 1000 mg/kg bw group was accidentally killed on day 3 prior to the blood collection; therefore, there were no clinical observations made on this animal on day 4. No statistically significant increases in the micronucleated reticulocyte frequency were observed in any evaluated test substance-treated group of male or female animals at either time point. There were no statistically significant decreases in %reticulocytes among the total erythrocytes in any test-substance treated group. One male rat of the 1000 mg/kg bw was accidentally killed on day 3 prior to the blood collection; therefore, this animal was not included in the micronucleus evaluation. Both the negative and positive control groups exhibited expected responses consistent with the laboratories historical control data. The positive control group resulted in statistically significant increases in micronucleated reticulocytes 48 hours post-dosing in both male and female animals. A statistically significant decrease in %reticulocytes was observed in the male positive control animals 48 hour post-dosing. Ultrafine titanium dioxide (TiO2 uf-3) did not induce biologically relevant increases in micronucleated reticulocytes in rat peripheral blood (micronucleus assay performed). ***Data Quality:*** *ECHA Reliability 1 (reliable without restrictions)*								
ECHA, 2010	ND	Animal	Inhalation	> 10 mg/m^3	5 days	No observable adverse effect reported	NR
Test article: titanium dioxide ***Dose:*** 0, 0.5, 2, and 10 mg/m^3 via inhalation, ***Treatment duration***: once daily for 6 hours/day for 5 consecutive days. Recovery for 23 days. Air used as vehicle. ***Species (strain):*** Rat (Wistar). ***Gender:*** Male. ***Number per Group:*** 3 animals per dose. ***Control:*** untreated group ***Methods:*** In this study an alkaline comet assay was conducted with broncho-alveolar lavage cells from male Wistar rats. The rats were exposed by head-nose-only inhalation to the test substance T-Lite TM SF for six hours per day on five consecutive days. The dose levels were 0, 0.5, 2 or 10 mg/m^3. The highest dose level group and the control group were examined in the comet assay. Clinical signs: examination of animals for evident signs of toxicity was conducted twice a day during weekdays and once a day. The clinical condition was recorded once during the preflow period and on post-exposure observation days and before, during and after exposure-on-exposure days. Body weight was determined at the start of the preflow, at the start of the exposure and then, once a week as well as prior to gross necropsy or lavage. Body weight change was calculated as the difference between body weight on the respective day and on the day of the first exposure. The animals were killed by exsanguination from aorta abdominalis and vena cava under Narcoren anesthesia on day 5 from the start of exposure or after the recovery period of 23 days. The lung was lavaged by two instillations of physiologic saline. The following parameters were determined: total cell count, macrophages, polymorphonuclear neutrophils, lymphocytes, eosinophils, monocytes and atypical cells. The humoral parameters in the bronchoalveolar fluid (BALF) were examined according to Roche working instructions: protein, lactate dehydrogenase (LDH), alkaline phosphatase (ALP), gglutamyltransferase (GGT), N-acetyl-b-D-glucosaminidase (NAG). The Comet assay was performed with lung cells after alveolar lavage of the 10 mg of T-LiteTM SF per m^3 group and vehicle control group. The lung cells were isolated by means of in situ perfusion and lavage. The cell density and viability were determined by trypan blue vital dye exclusion using a Neubauer cell counter. 10 mL of the single cell suspension (1–3 10^4 viable lung cells per slide) were carefully mixed with 90 mL low melting agarose and layered on an agarose coated slide. After solidification on ice							

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
the slides were transferred to lysis solution (pH 10) for at least 1 hour at about 4°C in the dark. The following steps until neutralization of the slides were carried out sheltered from daylight to prevent UV light-induced DNA damage. After lysis the slides were placed randomly in the electrophoresis chamber (cooled on ice) and were covered with electrophoresis buffer (pH > 13) for 45 minutes to allow DNA unwinding. Afterwards the electrophoresis was conducted (25 V; 300 mA; 0.85 V/cm) for 30 minutes. Then the slides were transferred into neutralization buffer for about 10 min. Subsequently, they were exposed for 1 minute to absolute ethanol. Finally, the slides were air-dried and stored at room temperature until scoring. Means and standard deviations were calculated. Additionally, body weight and body weight change were evaluated by comparison of each group with the control group using Dunnett's test (two-sided) for the hypothesis of equal means (Dunnett 1955, 1964). ***Control:*** not specified. ***Adverse Effect(s) (brief):*** **NOAEC = 10 mg/m^3.** Clinical signs, mean body weights and mean body weight changes of all test groups were not statistically significantly different from the control groups. The following lung effects (at aerosol concentrations of 2 and 10 mg/m^3) were pronounced two days after the last exposure: slight to moderate increases of neutrophils and monocytes, of total protein and of activities of LDH, GGT, ALP and NAG in the broncheoalveolar lavage fluid. These effects were partly reversible within the post-exposure observation period of three weeks. LDH and ALP activities were still significantly changed in the animals exposed to the highest concentration (10 mg/m^3). Therefore, the lungs of those recovery animals and the respective control animals were used for the investigation of DNA single and double strand breaks and alkali-labile DNA sites in the alkaline Comet assay. No effects were seen after exposure to the lowest concentration of 0.5 mg/m3^. For the negative control group low values (mean tail moment range: 0.89–2.13; mean tail length range: 27.0–40.2 mm; mean tail intensity range: 5.15–8.97%) were obtained from the three animals that were in the expected range based on laboratory experience with the Comet assay in Wistar rats in vivo in several tissues. The quality of the preparation of the lung cells was demonstrated by high cell viabilities (93% and above) in the negative control animals. All parameters determined for DNA damage – mean tail moment (0.6), mean tail length (28.3 mm) and mean tail intensity (3.04%) – after test substance inhalation exposure were below the values of the respective negative control group (mean tail moment: 1.82; mean tail length: 35.2 mm; mean tail intensity: 6.50%). Additionally, the numbers of hedgehog images when scoring 100 Comet images per animal were at 15–33% in the negative control group and from 14–23% in the exposed test group indicating. ***Data Quality:*** *ECHA Reliability 2 (reliable with restrictions)* ***Note:*** The alkaline comet assay was performed in alveolar lavage cells three weeks after exposure.							
ECHA, 2013	ND	Human alveolar basal epithelial A549 cells	Mammalian cell micronucleus test	> 512 µg/mL	No observable adverse effect reported	Similar to OECD 487; NR
Test article: titanium dioxide ***Treatment duration:*** exposure duration: 48 hours cells ***Dose:*** at 0, 64, 128, 256, and 512 µg/mL without S-9 metabolic activation. ***Speciec (strain):***Human bronchial epithelial 16 HBE Micronucleus induction by the test substance was not seen in adenocarcinomic human alveolar basal. epithelial A549. ***Number per Group:*** One experiment cultures was performed and each treatment was conducted as duplicate. A longer-term treatment covering 1.5-2 cell cycles was used (exposure duration: 48 hours). Cytochalasin B was added 6 hours after the start of the treatment***. Control:*** Untreated negative controls/ Positive control substance: mitomycin C and Methylmethanesulfonate***. Method:*** The doses of the test substance tested were chosen on the basis of cytotoxicity measurements using cell count relative to control, relative increase in cell counts (RICC), or relative population doubling (RPD), as well as cell viablility tests based on bioreduction of XTT (sodium 2,3,-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[phenylamino)-carbonyl]-2H-tetrazolium) and neutral red uptake. The highest dose was either at the cytotoxicity limit of 55% ± 5% or otherwise justified. Four to six doses were included in the genotoxicity assays to obtain a minimum of 3 analysable doses. In the case low cytotoxicity was determined, the maximum dose was 256 µg/mL (in some cases double this dose) or was based on technical limitations (e.g. inhibition of analysis because cells were covered with the test substance).The cytokinesis block micronucleus assay, using cytochalasin B (Cyt-B) to prevent cytokinesis, was performed. One experiment cultures was performed and each treatment was conducted as duplicate. A longer-term treatment covering 1.5 - 2 cell cycles was used (exposure duration: 48 hours). Cytochalasin B was added 6 hours after the start of the treatment. Evaluation criteria: Positive result was defined as follows: a statistically significant increase with ≥2 doses or a statistically significant increase at the high dose and a dose-dependent increase. A statistically significant result at a single (not the high dose) was considered a weak positive result.The Chi-square test or Fisher's exact test were applied. Dose-responses were assessed by linear regression. Positive result was defined as follows: a statistically significant increase with ≥2 doses or a statistically significant increase at the high dose and a dose-dependent increase. A statistically significant result at a single (not the high dose) was considered a weak positive result. The Chi-square test or Fisher's exact test were applied. Dose-responses were assessed by linear regression. ***Adverse Effects (s) (brief):*** The test substance concentration of four dosings could not be determined. They all were below 32 µg/mL. At least one concentration showed a MNBNC frequency above the MNBNC frequency of the negative control. No further information can be obtained about the remaining concentrations. The micronucleus assay was negative for NM-103 in adenocarcinomic human alveolar basal epithelial A549 cells. The test substance concentrations of 64, 128, and 256 µg/mL MNBNC frequencies were below the MNBNC frequency of the negative control. The MNBNC frequency of the test item concentration of 512 µg/mL was below the MNBNC frequencies of the other test substance concentrations and the negative control. ***Data quality:*** ECHA Reliability 2 (reliable with restrictions) ***Note:*** Micronucleus induction by the test substance was not seen in adenocarcinomic human alveolar basal epithelial A549.							
ECHA, 2013	ND	Bronchial epithelial BEAS 2B	Mammalian cell micronucleus test	> 256 µg/mL	No observable adverse	Simlar to OECD 487; NR

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
						effect reported	
Test article: titanium dioxide. ***Dose:*** 0, 32, 64, 128, and 256 µg/mL without S-9 metabolic activation. Micronucleus induction by the test substance was not seen in human bronchial epithelial BEAS 2B. ***Treatment duration:*** exposure duration: 48 hours). Cytochalasin B was added 6 hours after the start of the treatment. ***Speciec (strain):*** Broncheal epithelial BEAS 2B cells ***Number per Group:*** One experiment cultures was performed and each treatment was conducted as duplicate ***Control:*** untreated group ***Method:*** The doses of the test substance tested were chosen on the basis of cytotoxicity measurements using cell count relative to control, relative increase in cell counts (RICC), or relative population doubling (RPD), as well as cell viablility tests based on bioreduction of XTT (sodium 2,3,-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[phenylamino)-carbonyl]-2H-tetrazolium) and neutral red uptake. The highest dose was either at the cytotoxicity limit of 55% ± 5% or otherwise justified. Four to six doses were included in the genotoxicity assays to obtain a minimum of 3 analysable doses. In the case low cytotoxicity was determined , the maximum dose was 256 µg/mL (in some cases double this dose) or was based on technical limitations (e.g. inhibition of analysis because cells were covered with the test substance). Positive result was defined as follows: a statistically significant increase with ≥2 doses or a statistically significant increase at the high dose and a dose-dependent increase. A statistically significant result at a single (not the high dose) was considered a weak positive result. The cytokinesis block micronucleus assay, using cytochalasin B (Cyt-B) to prevent cytokinesis, was performed.One experiment cultures was performed and each treatment was conducted as duplicate. A longer-term treatment covering 1.5 - 2 cell cycles was used (exposure duration: 48 hours). Cytochalasin B was added 6 hours after the start of the treatment.The Chi-square test or Fisher's exact test were applied. Dose-responses were assessed by linear regression. ***Adverse Effects (s) (brief):*** The micronucleus assay was negative for NM-103 in bronchial epithelial BEAS 2B cells. The doses of 32 and 64 µg/mL of the test substance seemed to produce the same MNBNC frequency, which was below the MNBNC frequency of the negative control. At the concentration of 128 µg/mL, the MNBNC frequency further slightly decreased. The concentration of 256 µg/mL produced a MNBNC frequency below the MNBNC frequencies of the the concentrations of 32, 64, and 128 µg/mL.***Data quality:*** ECHA Reliability 2 (reliable with restrictions) ***Note:*** Micronucleus induction by the test substance was not seen in human bronchial epithelial BEAS 2B.							
ECHA, 2013	ND	Epithelial colorectal adenocarcinoma Caco-2	Mammalian cell micronucleus test	> 256 µg/mL	No observable adverse effect reported	Simlar to OECD 487
Test article: titanium dioxide ***Dose:*** 0, 28, 85, 128, and 256 µg/mL without S-9 metabolic activation ***Treatment duration: Speciec (strain):*** Epithelial colorectal adenocarcinoma Caco-2 cells. Micronucleus induction by the test substance was not seen in epithelial colorectal adenocarcinoma Caco-2..***Number per Group:*** One experiment cultures was performed and each treatment was conducted as duplicate. ***Control:*** mitomycin C and methylmethanesulfonate ***Method:*** The doses of the test substance tested were chosen on the basis of cytotoxicity measurements using cell count relative to control, relative increase in cell counts (RICC), or relative population doubling (RPD), as well as cell viablility tests based on bioreduction of XTT (sodium 2,3,-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[phenylamino)-carbonyl]-2H-tetrazolium) and neutral red uptake. The highest dose was either at the cytotoxicity limit of 55% ± 5% or otherwise justified. Four to six doses were included in the genotoxicity assays to obtain a minimum of 3 analysable doses. In the case low cytotoxicity was determined, the maximum dose was 256 µg/mL (in some cases double this dose) or was based on technical limitations (e.g. inhibition of analysis because cells were covered with the test substance).The cytokinesis block micronucleus assay, using cytochalasin B (Cyt-B) to prevent cytokinesis, was performed. One experiment cultures was performed and each treatment was conducted as duplicate. A longer-term treatment covering 1.5 - 2 cell cycles was used (exposure duration: 48 hours). Cytochalasin B was added 24 hours after the start of the treatment. Positive result was defined as follows: a statistically significant increase with ≥2 doses or a statistically significant increase at the high dose and a dose-dependent increase. A statistically significant result at a single (not the high dose) was considered a weak positive result. The Chi-square test or Fisher's exact test were applied. Dose-responses were assessed by linear regression ***Adverse Effects (s) (brief):*** test substance concentration of one dosing could not be determined. The concentration was below 28 µg/mL and seemed to caused almost the same MNBNC frequncy as the negative control. All test substance concentrations (28, 85, 128, and 256 µg/mL) caused a MNBNC frequency below the MNBNC frequency of the negative control. The concentration of 28 µg/mL showed a decrease of the frequency from the frequency of the negative control, which further decreased when a concentration of 85 µg/mL was applied to the cells. The MNBNC frequency increased at the 128 µg/mL concentraion, but was still below the frequency of the negative control. Then, the frequency decreased again at the 256 µg/mL concentration. ***Data quality:*** ECHA Reliability 2 (reliable with restrictions)***Note:*** Micronucleus induction by the test substance was not seen in epithelial colorectal adenocarcinoma Caco-2							
ECHA,2013	ND	NHEK	Mammalian cell micronucleus test	> 75 µg/mL	No observable adverse effect reported	Simlar to OECD 487; NR
Test article: titanium dioxide ***Dose:*** 0 – 75 µg/mL without S-9 metabolic activation. NHEK cells returned positive findings following a dose dependent increase of micronucleus formation. ***Treatment duration: Speciec (strain)*** NHEK cells***: Number per Group:*** Two independent experiments were performed and each treatment was conducted as duplicate. ***Control:*** Positive control							

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
substance: mitomycin C and methylmethanesulfonate ***Method:*** The doses of the test substance tested were chosen on the basis of cytotoxicity measurements using cell count relative to control, relative increase in cell counts (RICC), or relative population doubling (RPD), as well as cell viablility tests based on bioreduction of XTT (sodium 2,3,-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[phenylamino)-carbonyl]-2H-tetrazolium) and neutral red uptake. The highest dose was either at the cytotoxicity limit of 55% ± 5% or otherwise justified. Four to six doses were included in the genotoxicity assays to obtain a minimum of 3 analysable doses. In the case low cytotoxicity was determined , the maximum dose was 256 µg/mL (in some cases double this dose) or was based on technical limitations (e.g. inhibition of analysis because cells were covered with the test substance). The cytokinesis block micronucleus assay, using cytochalasin B (Cyt-B) to prevent cytokinesis, was performed. Two independent experiments were performed and each treatment was conducted as duplicate. A longer-term treatment covering 1.5 - 2 cell cycles was used (exposure duration: 48 hours). Cytochalasin B was added 6 hours after the start of the treatment. Positive result was defined as follows: a statistically significant increase with ≥2 doses or a statistically significant increase at the high dose and a dose-dependent increase. A statistically significant result at a single (not the high dose) was considered a weak positive result. The Chi-square test or Fisher's exact test were applied. Dose-responses were assessed by linear regression**.** Whether this particular cell line is more susceptible to a physical insult by PSPs was not further discussed by the authors. Based on the overwhelmingly negative findings with other cell lines and primary human lymphocytes used to test the test substance for genotoxicity, the positive finding with NHEK cells is considered as incidental finding with no biological relevance for a human health hazard assessment. ***Adverse Effects (s) (brief):*** The micronucleus assay was positive for NM-103 in NHEK cells.The MNBNC frequencies of the test substance concentrations were higher than the MNBNC frequency of the negative control. A dose dependent increase of micronucleus formation could be observed. ***Data quality:*** ECHA Reliability 2 (reliable with restrictions)**.** ***Note:*** NHEK cells returned positive findings following a dose dependent increase of micronucleus formation. Whether this particular cell line is more susceptible to a physical insult by PSPs was not further discussed by the authors. Based on the overwhelmingly negative findings with other cell lines and primary human lymphocytes used to test the test substance for genotoxicity, the positive finding with NHEK cells is considered as incidental finding with no biological relevance for a human health hazard assessmen**.**							
Reproductive and Developmental Toxicity							
ECHA, 2014	ND	Animal	Oral (feed)	> 1000 mg/kg/d **(NOAEL)**	14 days	No observable adverse effect reported	OECD 443
Test article: titanium dioxide ***Dose:*** 0, 100, 300, 1000 mg /kg/day. ***Species (strain):*** Rat (CD/Crl:CD(SD)). ***Gender:*** Male and female. ***Number per Group:*** 5 animals per sex per dose. ***Control:*** untreated group ***Methods:*** the repeated dose oral administration of TiO2 pg-2 to pregnant female Wistar rats at doses of 100, 300 and 1000 mg/kg/ day from gestation day 5 through gestation day 19 produced no adverse toxicological effects in the females or foetuses or significant developmental effects at any administered dose. ***Adverse Effect(s) (brief):*** **NOAEL = 1000 mg/kg.** No titanium dioxide-related influence was noted on the general toxicity and the reproductive performance of the parental animals of the F0 Generation as well as on the pre- and postnatal development of the F1 pups. No changes were noted during the histopathological examination and the examination of the intestines for aberrant crypt foci. No treatment-related effects on hormone levels (estradiol, estrone and testosterone, plus T3, T4 and TSH) in any of the treatment groups compared to controls. No test item-related influence was noted on the neurological function of the young adult male and female animals of cohort 2A. ***Data Quality:*** ECHA Reliability 1 (reliable without restriction)							
ECHA (n.d.)	ND	Animal	Oral (gavage)	> 1000 mg/kg bw/d **(NOAEL)**	20 days	No observable adverse effect reported	OECD 414;GLP
Test article: titanium dioxide ***Dose:*** 0, 100, 300, 1000 mg /kg/day from gestation day 5 – 19 (once daily). ***Species (strain):*** Rat (Wistar). ***Gender:*** Female. ***Number per Group:*** 24 – 25 animals per dose. ***Control:*** untreated group ***Methods:*** Time schedule: general clinical observations: once a day; morbidity and mortality: twice daily, except during holidays and weekends where the observation was made once daily. Cage side observations checked: spontaneous activity, lethargy, recumbent position, convulsions, tremors, apnoea, asphyxia, vocalisation, diarrhoea, changes in the skin and fur, eyes and mucous membranes (salivation, discharge), piloerection and pupil size, changes in gait, posture, response to handling as well as the presence of clonic or tonic movements, stereotypes, difficult or prolonged parturition or bizarre behaviour. once before the assignment to the experimental groups, on the first day of administration and weekly during the treatment. The sperm positive females were weighed during gestation days 0, 5, 8, 11, 14, 17 and 20. Food consumption for each animal determined: Yes, food consumption of pregnant females was measured on gestation days 5, 8, 11, 14, 17 and 20. Sacrifice on gestation day #20. At the time of termination or death during the study, each dam (presumed pregnant female) was examined macroscopically for any structural abnormalities or pathological changes which may have influenced the pregnancy. Organs examined: immediately after the termination, the uteri were removed, and the pregnancy status of the dams was confirmed. Uteri that appeared non-gravid were further examined by staining with 10 % ammonium sulphide solution to confirm the non-pregnant status. he ovaries and uterine content was examined after termination. Fetal examinations. A statistical assessment of the results of the body weight and food consumption was performed by comparing values of dosed with control animals using a one-way ANOVA and a post-hoc Dunnett Test. Fetal evaluation parameters like external, visceral, craniofacial and skeletal parameters were analyzed using a Chi-square test. The statistics were performed with GraphPad Prism V.6.01 software ($p<0.05$ is considered as statistically significant). For abnormality “pelvic girdle ilium bone offset” the statistical analyses was performed by combining all unilateral and bilateral findings. For abnormality “cervical vertebral centra- unossified” the statistical analyses was performed by combining all the unossified cervical vertebral centra (vertebral centra 1 to 7). Historical control data is provided for the following: mean uterine data; mean litter weight (g) data; foetal external							

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
examination; foetal visceral examination; foetal craniofacial examination; foetal skeletal examination. ***Adverse Effect(s) (brief):*** **NOAEL = 1000 mg/kg.** mortality: none of the animals died due to treatment in the study. However, one female animal in the 100 mg/kg/day group showed signs of paraplegia during the initial days of dose administration. This animal was euthanized on gestation day 10. This finding was considered to be incidental and not related to treatment. clinical observations: no clinical signs of toxicological relevance noted in any of the animals of the treated groups in comparison to the control. body weight development: no treatment related effect noted for body weight and body weight change in the treated groups in comparison to the controls. No treatment related changes noted for terminal and adjusted maternal body weight in the treated groups in comparison to the controls. The statistical analysis of the body weight data showed no statistical significance. no treatment related effect noted for food consumption in treated groups in comparison to the controls. The statistical analysis of the food consumption data showed no statistically significant changes between the treated and the control group. here were no macroscopic findings considered to be related to the treatment in any of the animals of the control and or test item treated groups at necropsy. However, there was a fluid distended uterus observed in one female of the 1000 mg/kg/day group. This animal was non pregnant and the fluid distension could be the normal physiological change of uterus during normal oestrus cycle. Also considering the finding was reported in a single female of the 1000 mg/kg/day group this was not considered to be related to the treatment. none of the females showed signs of abortion or premature delivery prior to the scheduled terminal sacrifice. no treatment related changes noted for the prenatal parameters including the number of corpora lutea, number of implantation sites, early and late resorptions, or pre- and post-implantation loss. However, there was an increase in the number of early resorptions in the 300 mg/kg/day group, which also accounted for the increased total resorption in the 300 mg/kg/day group. There was no statistically significant or dose-related response noted for the increase in early resorptions and these were not considered treatment related. no effects on the pregnancy rate of the animals. The rates in the control and treated groups were as follows: control group: 80%; 100 mg/kg/day group: LD 92%; 300 mg/kg/day group: 83.33% and 1000 mg/kg/day: 83.33%.no treatment related changes noted for the prenatal parameters including the live foetuses, number of dead foetuses, number of male and female foetuses, or sex ratio. In addition, there was a slight decrease in the number of female foetuses in the 1000 mg/kg/day group, but considering the slightly higher number of male foetuses and total live foetuses in the 1000 mg/kg/day group being comparable to the control it was assumed that decreased numbers of female foetuses were compensated by a slightly higher number of male foetuses. In addition the mean values for the numbers of male and female foetuses were within the historical control data range. Hence, the finding was not considered to be associated with treatment. Because foetal sex is determined shortly after conception and well before the onset of dosing on gestation day 5, such changes in sex ratio are not considered to be indicative of a test substance-related effect. The statistical analysis of data showed no statistically significant changes between the treated and the corresponding control group. no effect of treatment noted for the litter data including mean litter weight, total litter weight and male and female litter weight. However, there was a slight but and not statistically significant decrease in female litter weight noted in the 1000 mg/kg/day group (-12.96%). Given that all litter weight data reported were within the historical control data range, the finding was not considered to be associated with the treatment. no external abnormalities considered to be of toxicological relevance noted in any of the treated groups. The statistical analysis showed no significant changes. However, there were a few abnormalities noted in a few isolated foetuses of the control, 300 mg/kg/day and 1000 mg/kg/day groups. The abnormalities were gastroschisis in the control, micrognathia in the 300 mg/kg/day and 1000 mg/kg/day groups and small upper jaw in the 300 mg/kg/day group. The single foetus with micrognathia and small upper jaw in the 300 mg/kg/day group was used for visceral examination and could not be verified by skeletal examination. The foetuses with micrognathia in the 1000 mg/kg/day group were checked during skeletal examination and only 1 of 2 foetus was confirmed with micrognathia and the other looked normal. These abnormalities were observed in a single foetuses of a single isolated female animal from either control or treated groups and therefore were considered to be spontaneous in their origin and unrelated to the treatment. skeletal examination revealed a range of abnormalities in the control and treated groups that were either within historical control ranges recorded for this laboratory; were significantly lower than the corresponding control values; or were seen only in the 300 mg/kg/day or 100 mg/kg/day dose groups and were not dose dependent. There was statistically significant increase in the foetal incidences for unossification of vertebral cervical centrum in the 1000 mg/kg/day groups. However, the percent litter incidence in the 300 mg/kg/day and 1000 mg/kg/day groups was lower than in the concurrent control group. This variation, from the developmental perspective was of minimal significance and is normal in the foetuses of this strain of rats with C-section on gestation day 20. Therefore, this abnormality was not considered to be an adverse effect related to the treatment. internal examinations of the foetal viscera revealed a range of visceral abnormalities in all groups including the control. There were no abnormalities of toxicological relevance. craniofacial examination revealed a range of abnormalities in all groups including controls. These abnormalities either did not differ significantly from control values or did not show dose-related responses and were thus considered to have no relevance to treatment. No adverse toxicological effects in the females or fetuses or significant developmental effects at any administered dose were observed. ***Data Quality:*** ECHA Reliability 1 (reliable without restriction)							
ECHA, 2014	ND	Animal	Oral (gavage)	> 1000 mg/kg/d **(NOAEL)**	20 days	No observable adverse effect reported	OECD 414:GLP
Test article: titanium dioxide ***Dose:*** 0, 100, 300, 1000 mg /kg/day ***Treatment duration***: from gestation day 6 – 20 (once daily). ***Species (strain):*** Rat (Crl:CD (SD)) ***Gender:*** Female. ***Number per Group:*** 22 animals per dose. ***Control:*** untreated group ***Methods:*** Oral (gavage). mortality/moribundity at least once daily; clinical observation on gestation day 4. mortality/moribundity twice daily (morning and evening); clinical observations twice daily on gestation days 6 - 20 (during weighing and at least 2 hours post-dosing) and once on gestation day 21. Sacrifice on gestation day 21. Organs examined: a gross external and a visceral examination were performed immediately after euthanasia. The ovaries and uterine content was examined after termination. Yes, all foetuses classified as live were examined for alterations. External sex was recorded for each live foetus. Soft tissue examinations: Yes, approximately half of the live foetuses. Skeletal examinations: Yes, all live foetuses; The skeletal bodies of all the foetuses and the							

Source	Peer Review	Data Type	Route	Dose	Frequency/ Duration	Response	Quality Guideline
skulls of half the foetuses (foetuses that were not designated for head examination) were examined for alterations. Head examinations: Yes, approximately half of the foetuses. Body weight of each live foetus was recorded. ***Adverse Effect(s) (brief):*** **NOAEL = 1000 mg/kg.** Litter means for numbers of live and dead foetuses as well as for foetal weight and sex ratio were all comparable to control group values for every dose level tested. 100 mg/kg/day dose level: mean foetal weight was significantly increased relative to the control group mean. This increase was considered to be spurious and unrelated to the test substance because it was minimal in magnitude and not dose dependent. Further, an increase in mean foetal weight is not typically considered to reflect an adverse outcome as a decrease in this endpoint might. no test substance-related foetal malformations or variations observed at any dose level tested. The foetal alterations occurred with low frequency across all groups tested and occur with similar frequency in the test facility historical control database. no test substance-related mortality at any level tested; all animals on study survived until scheduled euthanasia. no test substance-related clinical observations at any level tested; the observations that were recorded were unremarkable and occurred infrequently. no test substance-related effects on maternal body weight parameters at any level tested; data for maternal body weights and weight changes were comparable across all dose levels tested. Mean final body weights (absolute or adjusted) GD 21 were within 2% of the control group mean at every dose level tested. no test substance-related effects on maternal food consumption at any level tested. Mean maternal food consumption from GD 6-21 was within 3% of the control group mean for all dose levels tested. 100 mg/kg/day: mean food consumption from gestation days 6-8 was slightly increased relative to the control mean. At 300 mg/kg/day, mean food consumption from days 14-16 and 16-18 of gestation were slightly lower than the respective control means. These instances were statistically significant; however, they are considered spurious and unrelated to the test substance. The changes are minimal in magnitude, variably increased or decreased, not dose dependent, and had no impact on cumulative food consumption values (gestation days 6-21) for these groups. no test substance-related maternal gross postmortem observations at any level tested. All animals appeared normal at the group at necropsy. no test substance-related effects on any reproductive outcome endpoint. litter means for numbers of implantation sites, early and late resorptions were all comparable to control group values for every dose level tested. There was no evidence of either maternal or developmental toxicity at doses up to 1000 mg/kg/day. ***Data Quality:*** ECHA Reliability 1 (reliable without restriction)							
OECD SIDS (2013)	ND	Animal	Oral (gavage)	> 1000 mg/kg/d **(NOAEL)**	40 days	No observable adverse effect reported	OECD 421
Test article: Titanium dioxide ***Dose:*** 0 or 1000 mg/kg/day for a total of 40 days (males: 2 weeks prior to mating, mating period, and 2 weeks post mating period; females: 2 weeks prior to mating, throughout gestation and until 4 days after delivery). Vehicle used was 1% methylcellulose solution. ***Species (strain):*** Rat (Sprague-Dawley). ***Gender:*** Male and female. ***Number per Group:*** 10 animals per sex per dose. ***Control:*** untreated group ***Methods:*** Oral (gavage). ***Adverse Effect(s) (brief):*** **NOAEL = 1000 mg/kg/day (in both sexes).** No treatment-related changes were observed in clinical signs, body weight, food consumptions, necropsy findings and organ weights of testis and epididymis. At histopathological examination of males, tubular cell vacuolation of seminiferous tubule and inflammatory cell foci of prostate were observed in 2 males each and 4 males each in the 0 and 1,000 mg/kg/day groups, respectively. These symptoms were considered to be incident, since these were also found in the vehicle control group and were common lesions in rats. Inflammatory cell foci of epididymis was observed in 3 males of vehicle control group, and tubular atrophy in testis and olgospermia and cellular debris in epididymis were observed in 1 male of the 1,000 mg/kg/day group. These symptoms were not considered to be treatment-related, since these were minimal in severity and the incidence was low. In females, there was no abnormal finding in any groups. No treatment-related effect was seen in the treatment groups on the following parameters examined: gestation length, the number of corpora lutea, implantation sites, delivery index, body weight of pups in both sexes on Day 0 and 4 of lactation, and pre- and post-implantation losses. From observation of live pups at birth, there were no externally malformed pups in any groups. At necropsy of pups, no gross finding was observed in any groups. ***Data Quality:*** Data quality could not be verified because this is secondary source data.							

II.2 Other Health Effects

Pigment	Endpoint	Outcome	Reference
Titanium dioxide	Eye Irritation/Corrosion	Irritation (slight/reversible)	Warheit *et. al.* (2007)
Test article: titanium dioxide (uf-C characterized by 79% rutile & 21% anatase, surface area 38.5 m^2/g, 140nm +/- 44nm (median diameter) ***Dose:*** 0.057 g in water, (0.9 chemical reactivity delta b, 4.80 pH in water). **Post-Treatment Observation Period:** 1, 24, 48, and 72 hours. ***Control:*** Untreated left eye. ***Species (strain):*** Rabbit (New Zealand White). ***Gender:*** Not reported. ***Number per Group:*** 3 animals. ***Methods:*** Eye (OECD TG 405). Approximately 57 mg of H-27416 was introduced into the lower conjunctival sac of the right eye of each rabbit. The left eye of each rabbit was not treated with the test substance and served as a control. The eyes remained unwashed following treatment. Each rabbit was observed for approximately 30 to 60 seconds before being returned to its cage. One rabbit was initially treated. Since no severe irritation or corrosion was observed, 2 additional rabbits were treated to complete the test. The conjunctiva, iris, and cornea of each treated eye were evaluated and scored according to the Draize Scale. ***Adverse Effect(s) (brief):*** uf-C TiO2 particles produced conjunctival redness (score of 1 or 2) in the three treated rabbit eyes, which was reversible by 24 or 48 hours. Fluorescein stain examinations did not reveal any corneal injury. No clinical signs were observed, and no body weight loss occurred. ***Data Quality:*** *ECHA Reliability 1 (reliable without restrictions)*. **Note:** Authors report titanium dioxide (100% rutile) coated with aluminum oxide or silicone/aluminum oxide aggregate in phosphate buffered saline (PBS) resulting in a 14x - 18x larger particles compared to water. *Reference: Warheit, David B., Thomas R. Webb, Kenneth L. Reed, Scott Frerichs, and Christie M. Sayes. "Pulmonary Toxicity Study in Rats with Three Forms of Ultrafine-TiO2 Particles: Differential Responses Related to Surface Properties." Toxicology 230, no. 1 (January 25, 2007): 90–104. https://doi.org/10.1016/j.tox.2006.11.002.*			

Pigment	Endpoint	Outcome	Reference
Titanium dioxide	Eye Irritation/Corrosion	Irritation (slight/reversible)	ECHA, 2004
Date: 2004 ***Test article:*** titanium dioxide. ***Dose:*** 0.1 g ***Treatment duration:*** single administration, 1, 24, 48, and 72 hours. ***Control:*** Untreated left eye. ***Species (strain):*** Rabbit (New Zealand White). ***Gender:*** Female. ***Number per Group:*** 3 animals. ***Methods:*** Eye (OECD TG 405, GLP). The test substance was instilled into the conjunctival sac of the right eye of each rabbit. The upper and lower lids were then gently hold together for about 1 second before releasing, to minimize loss of the test substance. Ocular irritation was evaluated with the illumination of a white light source according to a modified Draize scale for scoring ocular irritation. Fluorescein dye was used at 24 hours to verify the absence of cornel damage. ***Adverse Effect(s) (brief):*** The test item produced conjunctival redness (Score =1 or 2) in the treated eye of all three rabbits. The treated eyes were normal 48 hours after instillation of the test item. Fluorescein stain examinations were negative for corneal injury. ***Data Quality:*** *ECHA Reliability 2 (reliable with restrictions)*. **Note:** Original report is not available.			
Titanium dioxide	Eye Irritation/Corrosion	Irritation (slight/reversible)	ECHA, 1996
Date: 1996 ***Test article:*** titanium dioxide ***Dose:*** 0.098 g. **Treatment duration:** single administration, 1, 24, 48, and 72 hours. ***Control:*** Untreated left eye. ***Species (strain):*** Rabbit (New Zealand White). ***Gender:*** Not reported. ***Number per Group:*** 3 animals. ***Methods:*** Eye (OECD TG 405, GLP). One rabbit was initially treated. A volume of 0.1ml of the test material, which was found to weight approximately 98 mg was placed into the conjunctival sac of the right eye. The upper and lower eyelid were held together for about 1 second, to prevent loss of the test material. Immediately after administration of the test material, an assessment of the initial pain reaction was made. After consideration of the ocular response produced in the first treated animal, 2 additional animals were treated. Assessment of ocular damage/irritation was made according to the numerical evaluation from Draize. ***Adverse Effect(s) (brief):*** 1 hour after application: Residual test material in all treated eyes, minimal conjunctival redness (score=1) and chemosis (score=1) was noted in all treated eyes. 24 hours after treatment: minimal conjunctival redness (score=1) was observed in 2 animals. Treated eyes appeared normal at the 24 or 48-hour observations. ***Data Quality:*** *ECHA Reliability 2 (reliable with restrictions)*. **Note:** Original report is not available.			
Titanium dioxide	Skin Irritation/Corrosion	No observable adverse effect reported	Warheit *et. al.* (2007)
Test article: uf-C titanium dioxide (79% rutile & 21% anatase, surface area 38.5 m^2/g, 140nm +/- 44nm (median diameter), ***Dose:*** 0 or 0.5 g moistened with water (0.9 chemical reactivity delta b, 4.80 pH in water). **Treatment Duration:** 4 hours. Observation Period of 1, 24 and 48 hours. **Control:** Water (vehicle). ***Species (strain):*** Rabbit (New Zealand White). ***Gender:*** Male and female. ***Number per Group:*** 3 animals per dose/sex. ***Methods:*** Skin irritation/corrosion (semi-occlusive of intact (shaved) skin) (OECD TG 404, GLP). Approximately 57 mg of H-27416 was introduced into the lower conjunctival sac of the right eye of each rabbit. The left eye of each rabbit was not treated with the test substance and served as a control. The eyes remained unwashed following treatment. Each rabbit was observed for approximately 30 to 60 seconds before being returned to its cage. One rabbit was initially treated. Since no severe irritation or corrosion was observed, 2 additional rabbits were treated to complete the test. The conjunctiva, iris, and cornea of each treated eye were evaluated and scored according to the Draize Scale. ***Adverse Effect(s) (brief):*** Erythema and edema scores (Draize) were zero (0) at all of the post-treatment observation periods. ***Data Quality:*** *ECHA Reliability 1 (reliable without restrictions)* **Note:** Original report is not available. This study appears to be reported in OECD SIDS (2013). **Note:** Authors report titanium dioxide (100% rutile) coated with aluminum oxide or silicone/aluminum oxide, which is more representative of TiO_2 pigment particles used to color polymer material compared to uf-C, aggregate in phosphate buffered saline (PBS) resulting in a 14x - 18x larger particles compared to water. *Reference: Warheit, David B., Thomas R. Webb, Kenneth L. Reed, Scott Frerichs, and Christie M. Sayes. "Pulmonary Toxicity Study in Rats with Three Forms of Ultrafine-TiO2 Particles: Differential Responses Related to Surface Properties." Toxicology 230, no. 1 (January 25, 2007): 90–104. https://doi.org/10.1016/j.tox.2006.11.002.*			
Titanium dioxide	Skin Irritation/Corrosion	No observable adverse effect reported	ECHA, 2003
Date: 2003 ***Test article:*** titanium dioxide, ***Dose:*** 0 or 0.5 g moistened with deionized water, and 1-inch gauze. **Treatment Duration:** 4 hours. **Observation Period:** 1, 24 and 48 hours. **Control:** Water (vehicle). ***Species (strain):*** Rabbit (New Zealand White). ***Gender:*** Male and female. ***Number per Group:*** 3 animals per dose/sex. ***Methods:*** Skin irritation/corrosion (semi-occlusive of intact (shaved) skin) (OECD TG 404, GLP). Four separate, localized test sites (approximately 6 cm^2 each) were marked on each rabbit's back with a water-insoluble marker. Approximately 0.5 g of each test substance, moistened with approximately 0.2 or 0.3 mL of deionized water to form a thick paste, was applied to the designated test site and covered with a 2-ply, 1-inch square gauze pad. The pad was held in place with non-irritating tape. Removal of the test substance with warm water and soap. Observation of clinical signs. Measuring of body weight. Scoring after Draize. ***Adverse Effect(s) (brief):*** Erythema and edema scores (Draize) were zero (0) at all of the post-treatment observation periods. ***Data Quality:*** *ECHA Reliability 1 (reliable without restrictions)* **Note:** Original report is not available. This study appears to be reported in OECD SIDS (2013).			
Titanium dioxide	Skin Irritation/Corrosion	Irritation (slight/mild/reversible)	ECHA, 1994
Date: 1994 ***Test article:*** titanium dioxide ***Dose:*** 0 or 0.5 g, moistened with deionized water, and 2-inch square gauze. **Treatment Duration:** 4 hours. **Post-Treatment Observation Period:** 1, 24, 48, and 72 hours. **Control:** Water (vehicle). ***Species (strain):*** Rabbit (New Zealand White).. ***Gender:*** Male and female. ***Number per Group:*** 3 animals per dose/sex. ***Methods:*** Skin irritation/corrosion (semi-occlusive of intact (shaved) skin) (OECD TG 404, GLP). An 0.5 g aliquot of the test substance was applied directly to a 2-inch gauze square that had been pre-moistened with deionised water. The gauze square was then placed on			

Pigment	Endpoint	Outcome	Reference
the test side and was held in place with non-irritating tape. Removal of the test substance with warm water and Ivory soap. Evaluation according to Draize ***Adverse Effect(s) (brief):*** In 3/6 animals, no observable dermal irritation occurred during the study. At 1-hour post-treatment, slight erythema (2/6) or mild erythema (1/6). At 24-hour post-treatment, slight erythema (3/6). At 48-hour and 72-hour post-treatment, slight erythema (1/6). No oedema was observed during the study. ***Data Quality:*** *ECHA Reliability 1 (reliable without restrictions)* **Note:** Original report is not available. This study appears to be reported in OECD SIDS (2013).			
Titanium dioxide	Skin Irritation/Corrosion	Irritation (slight/mild/reversible)	EHCA, 1994
Date: 1994 ***Test article:*** titanium dioxide. ***Dose:*** 0 or 0.5 g **Treatment Duration:** 3 minutes (n=1), 1 hour (n=1), or 4 hours (n=3). **Post-Treatment Observation Period:** 1, 24, 48, and 72 hours and then daily for 9 days. **Control:** Not reported. ***Species (strain):*** Rabbit (New Zealand White). ***Gender:*** Not reported. ***Number per Group:*** 1 (3 min and 1 hour) and 3 (4 hour). ***Methods:*** Direct contact with skin (semi-occlusive of intact (shaved) skin) (OECD TG 404, GLP). Any residual test substance was removed by means of a dry compress. The mean score of the values for erythema and oedema recorded for each animal after 24, 48 and 72 hours was calculated. The interpretation of results was carried out according to the classification criteria laid down in Directive 93/21/E.E.C. Commission Directive of 27th April 1933 adapting to technical progress for the eighteenth time Council Directive 67/548/E.E.C. ***Adverse Effect(s) (brief):*** One animal was free of dermal irritation during the study. The other two animals showed slight to moderate erythema (score = 1 or 2) at 1, 24, 48 and 72-hours post-treatment. One animal showed at 1-hour post-treatment a slight oedema. On day 9 all skin reactions were reversible. ***Data Quality:*** *ECHA Reliability 2 (reliable with restrictions)* **Note:** Original report is not available. This study appears to be reported in OECD SIDS (2013).			
Titanium dioxide	Skin Irritation/Corrosion	No observable adverse effect reported	ECHA, 1996
Date: 1996 ***Test article:*** titanium dioxide ***Dose:*** 0 or 0.5 g of moistened with deionized water or olive/corn oil. **Treatment Duration:** 4 hours. **Post-Treatment Observation Period:** 30-60 min, 1, 2, 3 and 10 days. **Control:** Water (vehicle). ***Species (strain):*** Rabbit (strain not reported). ***Gender:*** Male. ***Number per Group:*** 3 or 6. ***Methods:*** Direct contact with skin (semi-occlusive of intact (shaved) skin) (OECD TG 404, GLP). ***Adverse Effect(s) (brief):*** Erythema and oedema scores (Draize) were 0 in all animals. ***Data Quality:*** *ECHA Reliability 2 (reliable with restrictions)* **Note:** Original report is not available.			
Titanium dioxide	Skin Irritation/Corrosion	Irritation (slight/reversible)	ECHA, 2004
Date: 2004 ***Test article:*** titanium dioxide ***Dose:*** 0 or 0.5 g of, 80% moistened with mineral oil. **Treatment Duration:** 4 hours. **Post-Treatment Observation Period:** 1, 24, 48 and 72 hours. **Control:** Mineral oil (vehicle). ***Species (strain):*** Rabbit (New Zealand White). ***Gender:*** Female. ***Number per Group:*** 3. ***Methods:*** Direct contact with skin (semi-occlusive of intact (shaved) skin) (OECD TG 404, GLP). ***Adverse Effect(s) (brief):*** One hour after patch removal, all three sites exhibited very slight erythema (score = 1). All animals were free of dermal irritation by 72 hours. No oedema was observed during the study. ***Data Quality:*** *ECHA Reliability 2 (reliable with restrictions)* **Note:** Original report is not available.			
Titanium dioxide	Skin Irritation/Corrosion	No observable adverse effect reported	ECHA (n.d.)
Date: 1978 ***Test article:*** titanium dioxide ***Dose:*** 0 or 0.5 g. **Treatment Duration:** 24 hours. **Post-Treatment Observation Period:** Immediate and 48 hours. **Control:** Physiological saline. ***Species (strain):*** Rabbit (not reported). ***Gender:*** Not reported. ***Number per Group:*** 6. ***Methods:*** Direct contact with skin (semi-occlusive of intact (shaved) skin). ***Adverse Effect(s) (brief):*** No skin irritation was observed when TiO_2 was tested on the shaved, intact skin of six rabbits ***Data Quality:*** *ECHA Reliability 2 (reliable with restrictions)* **Note:** Original report is not available.			
Titanium dioxide	Skin Irritation/Corrosion	Irritation (slight/reversible)	ECHA (n.d.)
Date: 1975. ***Test article:*** titanium dioxide ***Dose:*** 0 or 0.5 g moistened with distilled water. **Treatment Duration:** 24 hours. **Post-Treatment Observation Period:** Immediate and 48 hours. **Control:** Distilled water (vehicle). ***Species (strain):*** Rabbit (not reported). ***Gender:*** Male. ***Number per Group:*** 6. ***Methods:*** Direct contact with skin (semi-occlusive of intact (shaved) skin). ***Adverse Effect(s) (brief):*** 2 of 6 animals showed very slight skin irritation (erythema score of 1) at 24h observation. At the 48h observation no skin irritation was noted. ***Data Quality:*** *ECHA Reliability 2 (reliable with restrictions)* **Note:** Original report is not available.			
Titanium dioxide	Skin Irritation/Corrosion	Irritation (slight/reversible)	ECHA (n.d.)
Date: 1998 ***Test article:*** titanium dioxide ***Dose:*** 0 or 0.5 ml of. **Treatment Duration:** 4 hours. **Post-Treatment Observation Period:** 1, 24, 48 and 72 hours. **Control:** Distilled water (vehicle). ***Species (strain):*** Rabbit (not reported). ***Gender:*** Female. ***Number per Group:*** 3. ***Methods:*** Direct contact with skin (semi-occlusive of intact (shaved) skin) (OECD TG 404). ***Adverse***			

Pigment	Endpoint	Outcome	Reference
Effect(s) (brief): One animal exhibited slight erythema (score=1, Draize method) at 1 hour after removal and another animal at the 24 hours observation. No other dermal or clinical signs of toxicity were observed during the study. ***Data Quality:*** *ECHA Reliability 2 (reliable with restrictions)* **Note:** Original report is not available.			
Titanium dioxide	Skin Sensitization	No observable adverse effect reported	Warheit *et. al.* (2007)
Test article: titanium dioxide ***Dose:*** 0% (vehicle), 5%, 25%, 50%, or 100% of (*n*=5/group) in *N*,*N*-dimethyl formamide (uf-C characterized by 79% rutile & 21% anatase, surface area 38.5 m^2/g, 140nm +/- 44nm (median diameter), 0.9 chemical reactivity delta b, 4.80 pH in water). **Treatment Period:** Daily for 3 consecutive days. ***Control:*** Two control groups (*n*=5/group). One group treated with 25% hexylcinnamaldehyde (HCA) in 4:1 acetone:olive oil (AOO) as positive control. Second group treated with AOO as a positive control vehicle. ***Species (strain):*** CBA/JHsd mice. ***Gender:*** Female. ***Number per Group:*** 5 animals per group. ***Methods:*** The authors of the report the following method information. "*Animals of each group were injected into the local lymph node of both ears for three consecutive days. On test day 5, mice received ^{3}H-Thymidine by tail vein injection and sacrificed approximately 5 h later. Cell proliferation in the draining auricular lymph nodes of the ears from the test substance groups was then evaluated and compared to the vehicle control group. A stimulation index (SI) was derived for each experimental group by dividing the mean disintegrations per minute (dpm) of each experimental group by the mean dpm of the vehicle control group. Significance was judged at $p < 0.05$ except for dpm data that were judged at $p < 0.01$. Lymph node dpm data were log transformed to obtain normality or homogenous variances. When possible, an EC3 value for the stimulation index data was derived from linear interpolation of points on the dose–response curve immediately above and below the threefold threshold.*" (OECD TG 429). ***Adverse Effect(s) (brief):*** The authors report the following: "*No statistically significant differences in mean body weights and body weight gains compared to the vehicle control group were observed at any test concentration. No clinical signs of toxicity were observed in the study. Statistically significant increases in cell proliferation measurements compared to the vehicle control group were observed at the 50% and 100% test concentrations. Stimulation indices (SIs) of less than 3.0 were observed at all test concentrations of uf-C TiO2 particle-types. Therefore, the EC3 value (the estimated concentration required to induce a threshold positive response, i.e., SI = 3) for the test substance under the conditions of this study was not calculable. A 25% concentration of the positive control, HCA, produced a dermal sensitization response in mice. Therefore, the LLNA test system was valid for this study with ultrafine TiO2 particles. Under the conditions of this study, uf-C TiO2 particles did not produce a dermal sensitization response in mice.*" ***Data Quality:*** *ECHA Reliability 1 (reliable without restrictions)*. **Note:** Authors report titanium dioxide (100% rutile) coated with aluminum oxide or silicone/aluminum oxide aggregate in phosphate buffered saline (PBS) resulting in a 14x - 18x larger particles compared to water. *Reference: Warheit, David B., Thomas R. Webb, Kenneth L. Reed, Scott Frerichs, and Christie M. Sayes. "Pulmonary Toxicity Study in Rats with Three Forms of Ultrafine-TiO2 Particles: Differential Responses Related to Surface Properties." Toxicology 230, no. 1 (January 25, 2007): 90–104. https://doi.org/10.1016/j.tox.2006.11.002.*			
Titanium dioxide	Skin Sensitization	No observable adverse effect reported	ECHA (n.d.)
Date: 1994. ***Test article:*** titanium dioxide ***Dose:*** 0, 50, or 100% . For occlusive, exposure was for 6 hrs. Physiological saline used as vehicle. ***Species (strain):*** guinea pig (Hartley). ***Gender:*** Male. ***Number per Group:*** 20 animals per dose. ***Control:*** A group of 10 guinea pigs (vehicle control); a 0.4 ml portion of saline was applied. A group of 5 guinea pigs (positive control); a 0.4 ml portion of 0.1% l-chloro-2,4-dinitrobene (DNCB) w/v in 50% ethanol: 0.9% saline was applied. ***Methods:*** Skin sensitization (epicutaneous, occlusive), Buehler test (OECD TG 406). Guinea pigs were closely clipped over the induction site on their left flank (approximately 1.5 X 1.5 inches) one day prior to initiation and repeated as necessary. once a week for three weeks, for a total of three, six-hour applications. 0.4 g moistened with 0.4 ml 0.9% saline. The test site was examined following removal of the patch and scored for irritation according to the Scale for Evaluation of Dermal Irritation at approximately 24 and 48 hours from the initial patch application. After the third induction application, the animals were rested for approximately 14 days. At the termination of the rest period, a challenge application, using the procedures described previously, was applied to a naive challenge site (on the clipped right flank). The challenge application remained on for approximately 6 hours. Upon removal of the challenge application, the contact area was wiped with deionized water and marked with a color marker to indicate the challenge application site. Dates of application and observations were recorded. Approximately 24 and 48 hours after the challenge application, the sites were examined for dermal irritation and/or signs of elicited sensitization ***Adverse Effect(s) (brief):*** Sensitization response did not occur in any of the animals treated with titanium dioxide at either of the time points. Titanium dioxide did not elicit a dermal sensitization reaction in the study. The positive control (l-chloro-2,4-dinitrobenzene), when compared to the dermal reaction elicited on the naive animals at challenge, did elicit a dermal sensitizer in the study. ***Data Quality:*** *ECHA Reliability 1 (reliable without restrictions)*			

Section III. Dose-Response Assessment

III.1 Provisional Tolerable Intake Values

Contact Duration	Exposure Route	Patient	POD	MF	pTI
Long-term	Parenteral	All	1,000 mg/kg/d	1000	1 mg/kg/d

pTI = POD ÷ MF

Note: When a supplier-specific color additive safety data sheet (SDS) does not report the presence of a carcinogen or non-carcinogen impurity, and SDS complies with Globally Harmonized System (GHS) hazard communication limits (i.e., <0.1% and

<1%, respectively), then this pTI is low enough to be protective for naturally occurring impurities which could be present in the color additive. Processes used to color a polymer system involve diluting the pigment and its impurities by at least 50x (i.e., the CHRIS calculator is valid when the maximum concentration of pigment in a colored polymer is 2%, i.e., 1 ÷ 2% = 50); therefore, the concentration of naturally occurring impurities in the finished colored polymer will be significantly lower compared to the concentration of these substances in the pigment (i.e., 0.002% (i.e., 0.1% ÷ 50) for carcinogens and 0.02% (i.e., 1% ÷ 50) for non-carcinogens). Unknown impurities are addressed by the CHRIS calculator.

III.2 Modifying Factor for POD

Critical Study	Critical Health Effect	Exposure Route	POD Type	POD Value	UF_1	UF_2	UF_3	MF
OECD SIDS (2013)	Reproductive and developmental toxicity (repeated exposure)	Oral	NOAEL	1,000 mg/kg/d	10	10	10	1000

MF = modifying factor (UF_1 x UF_2 x UF_3)

III.3 Toxicological Uncertainties Applied to POD

Uncertainty	Justification
UF_1 **Error! Bookmark not defined.**	(10) Default value to account for susceptible individuals.
UF_2 **Error! Bookmark not defined.**	(10) Default value to account for differences between species.
UF_3 **Error! Bookmark not defined.**	(10) Default value to account for differences in exposure route and data quality.

UF_1, UF_2, and UF_3, are used to extrapolate the POD to the general human population (including pediatric) exposed parenterally for a lifetime.

III.4 Critical Health Study Design and Outcome

OECD SIDS (2013) SIDS Initial Assessment Profile – Titanium Dioxide.
https://hpvchemicals.oecd.org/ui/handler.axd?id=1F6BE49D-48B6-4909-8E0D-A6F5E48503D3.

Test Substance	Titanium dioxide		
Species	Sprague-Dawley Rat	Frequency	Daily
Gender	Males and females	Duration	40 days
Age	Not reported	#/Group	10 animals/sex/dose
Route	Oral (gavage)	Protocol Guideline	OECD 421
Dose(s)	0, 1000 mg/kg/d	Statistical Method(s)	Not reported
Observed Responses	No treatment-related changes were observed in clinical signs, body weight, food consumptions, necropsy findings and organ weights of testis and epididymis. At histopathological examination of males, tubular cell vacuolation of seminiferous tubule and inflammatory cell foci of prostate were observed in 2 males each and 4 males each in the 0 and 1000 mg/kg/day groups, respectively. These symptoms were considered to be incident, since these were also found in the vehicle control group and were common lesions in rats. Inflammatory cell foci of epididymis were observed in 3 males of vehicle control group, and tubular atrophy in testis and olgospermia and cellular debris in epididymis were observed in 1 male of the 1000 mg/kg/day group. These symptoms were not considered to be treatment-related, since these were minimal in severity and the incidence was low. In females, there was no abnormal finding in any groups. No treatment-related effect was seen in the treatment groups on the following parameters examined: gestation length, the number of corpora lutea, implantation sites, delivery index, body weight of pups in both sexes on Day 0 and 4 of lactation, and pre- and post-implantation losses. From observation of live pups at birth, there were no externally malformed pups in any groups. At necropsy of pups, no gross finding was observed in any groups.		
Notes:	OECD SIDS Document: https://hpvchemicals.oecd.org/ui/handler.axd?id=1F6BE49D-48B6-4909-8E0D-A6F5E48503D3.		

Zinc Oxide

CAS # 1314-13-2

Toxicological Profile Summary

Zinc oxide (CAS # 1314-13-2) is an inorganic pigment that is a white or yellowish-white powder or hexagonal crystals. Reported hazard information were found in secondary sources. The absence of original reports of toxicological data for zinc oxide is likely due to its long history of use, which includes coloring in the early 19th Century.[83] Zinc oxide, administered at oral doses (up to ~ 320 mg/kg/d) to rodents, is reported not to elicit observable systemic effects.[84] For assessing Zinc oxide exposure that will be without appreciable harm to health, the calculated provisional tolerable intake (pTI) value for parenteral systemic toxicity is 0.053 mg/kg/d. This value is based on the lowest reported point-of-departure (160 mg/kg/d, oral, 28-day, rodent) with a modifying factor of 3000 (i.e., 10 x 10 x 30), to address the following sources of uncertainty: variation among humans (10), variation between species (10), and data quality/route-to-route extrapolation (30).

NOTE: This pTI is protective for the color additive including any impurities listed in Section I.1 "Substance Identity," as the impurities likely will be present at a very low percentage of the color additive, and the quantity of the impurity will be at an even lower percentage in the final device (see Section III, Table III.1 footnote). Unknown impurities are addressed by the CHRIS calculator.

I.1 Substance Identity

	Descriptor
Material Characterization	
Synonyms[85]	Zinc oxide
Formula[85]	ZnO
Molecular Weight[85]	81.41 g/mol
Physical Form[85]	Solid powder
Type[85]	
Physical/Chemical Characteristics[86]	Zinc oxide is a white or yellowish-white powder or hexagonal crystals with a bitter taste. The melting point is 1974°C The density is 5.6 g/cm^3 It is insoluble in water and alcohol and is soluble in acids and alkalies Zinc is a bluish-white, lustrous metal; distorted hexagonal close-packed structure Zinc when heated to 100-150°C becomes malleable, at 210°C becomes brittle and pulverizable Zinc has a boiling point of 907°C and a melting point of 419.53°C. Zinc is insoluble in water and is soluble in acids and alkalies

[83] Zinc White: History of Use URL http://www.webexhibits.org/pigments/indiv/overview/zincwhite.html
[84] ECHA (1968). Zinc oxide. (1314-13-2).Developmental toxicity / teratogenicity-008: https://echa.europa.eu/registration-dossier/-/registered-dossier/16139/7/9/3/?documentUUID=5ad0d2c7-4a04-471d-9640-44e9f1cff5ed
[85] HSDB (2014). HSDB: ZINC OXIDE CASRN: 1314-13-2.
[86] HSDB (2010). HSDB: ZINC, ELEMENTAL CASRN: 7440-66-6.

Section II. Hazard Identification

Health effect data, supporting studies and secondary sources were reviewed, see tables in sections II.1 and II.2 for details

Brief descriptions of acute systemic, genotoxicity, repeat dose systemic toxicity, and developmental/reproductive toxicity were reported. These studies used rats, rabbits, sheep, and animal cells to assess the toxicity of zinc oxide. The oral acute toxicity data resulted in no adverse effects in high doses up to 5000 mg/kg bw in rats[87].

II.1 Systemic, Genotoxicity, Carcinogenicity, Reproductive/Developmental Toxicity

ADVERSE HEALTH EFFECT STUDIES

None reported, see Section III.4 for the critical adverse health effect study.

SUPPORTING STUDIES

None reported.

SECONDARY SOURCES

Source	Peer Review	Data Type		Route	Dose	Frequency/ Duration	Response	Quality Guideline
Systemic Toxicity (acute, subacute)								
ECHA 1993	ND	Animal		Oral (gavage)	ND	28 days	No observable adverse effect reported	Non-guideline study; non-GLP
Test article: Zinc oxide. **Dose:** 240 mg/kg b.w. zinc oxide in water via intraruminal intubation 3 times a week (Monday, Wednesday, Friday); 18 controls were used. ***Species (strain):*** Romney-cross sheep ***Gender:*** Male. ***Number per Group:*** 42 per dose ***Methods:*** Oral (water). A 28-d repeated dose study was conducted in sheep to evaluate the toxicity of the test material. The test material was administered thrice a wk by intraruminal intubation and animals were evaluated till 112 d after the start of dosing. Body weights were examined prior to treatment and once at 1, 2, 3, 4, 8, 12 and 16 wk. Two separate samples of pancreas were examined microscopically. Plasma zinc concentration was analyzed from Day 4 until the end of the dosing period. Liver, kidney and pancreas tissue samples were analyzed for Zinc concentration at necropsy. Six sheep were necropsied each at 4, 7, 14, 21, 28, 56 and 112 days after start of exposure. ***Adverse Effect(s) (brief):*** **No NOAEL.** In treated sheep no mortality occurred, no statistically significant mean body weight reductions changes, and no clinical signs of toxicity were observed. However, pancreatic lesions were observed in many exposed sheep. After 4 weeks, fully develop lesions were evident, and as early as day 7 necrosis of the pancreatic duct epithelium, periductular inflammation, and interlobular fat necrosis were observed. Edema, lobular cystic change, atrophy, fibrosis and ductular hyperplasia were also observed at a later day. Elevated plasma zinc levels (2.0-2.5 µg Zn/mL) were observed during the dosing period, which declined to non-statistically significant levels as compared to controls after the 2 wk of dosing. Organ zinc levels in liver, kidney and pancreas were elevated during the first 3 wk of dosing (> 800 µg Zn/g dry matter basis) but declined to non-statistically significant levels as compared to the controls after the 4 wk of dosing. Based on these observations, the pancreas was identified as the target organ of toxicity when sheep were exposed orally to a large dose of zinc oxide. No NOAEL was able to be identified. ***Data Quality:*** *ECHA Reliability 2 (reliable with restrictions)*								
Genotoxicity								
ECHA, 2010,	ND	Bacteria		ND	5000 µg/plate **(NOAEL)**	Once	No observable adverse effect reported	OECD 471; NR
Test article: zinc oxide. ***Dose:*** 0, 20, 100, 500, 1000, 2500, and 5000 µg/plate; controls were used in DMSO ***Species (strain):*** *S. typhimurium* (TA 1535, TA 1537, TA 98, TA 100 and TA 102) ***Gender:*** ND ***Number per Group:*** NR ***Methods:*** ND ***Adverse Effect(s) (brief):*** **NOAEL =5000 µg/plate**. In a bacterial reverse mutation assay, no cytotoxicity was observed, and zinc oxide was not genotoxic in *S. typhimurium* (TA 1535, TA 1537, TA 98, TA 100 and TA 102) with and without metabolic activation. ***Data Quality:*** *ECHA Reliability 2 (reliable with restrictions)*								
Reproductive/Developmental								
ECHA, 1968	ND	Animal		Oral	160 mg/kg/day **(NOAEL)**	Up to 21 day	Teratogenic	Non-guideline study; non-GLP

[87] ECHA, 2012

Source	Peer Review	Data Type		Route	Dose	Frequency/ Duration	Response	Quality Guideline
Test article: zinc oxide. ***Dose:*** 0, 0.2% (~160 mg/kg/day) or 0.4% (~320 mg/kg/day) in feed in four separate experiments. The percentages of zinc oxide in feed were converted into mg/kg/day dose based on rat feed consumption of 20 grams/day and rat body weight of 0.25 kg. Amounts of 0.2 and 0.4% zinc were mixed with basal diet, containing 7.5 and 230 ppm of copper and iron respectively. Rats were exposed daily. ***Species (strain):*** nulliparous Rat. ***Gender:*** female. ***Number per Group:*** NR ***Methods:*** Oral. Rats were exposed daily zinc oxide in feed in four separate experiments, with differing durations: experiment 1 from 0 days of age of the fetus until fetal age of 15 and 16 days; experiment 2 from 0 days of age of the fetus until fetal age of 18 and 20 days; and experiments 3 and 4 21 days before mating until fetal age of 15 and 16 days. Following exposure, a normal estrous cycle and normal mating with fertilization and implantation were observed in maternal females. Animals were sacrificed for postmortem examination on 15 and 16 for experiment 1, 18 and 20 for experiment 2, 36 and 37 for experiment 3 and 4. Livers were collected for examination. The degree of resorption, grossly visible abnormalities of fetus, and growth were examined. ***Adverse Effect(s) (brief):*** **NOAEL=0.2% (~160 mg/kg/day)**. In 9-11 dams' variable degrees (4-29%) of death and resorption occurred in fetuses from mothers fed 0.4% test material for 15, 16, 18 and 20 d; 100% resorption occurred in the 15 and 16 d old fetus of mothers fed 0.4% zinc beginning at 21 d before breeding. No external abnormalities were observed in the fetuses of exposed mothers. Growth reduction in terms of dry matter or variable degrees of death and resorption occurred in the fetuses of maternal rats exposed to 0.4%; 100% resorption occurred in the 15- and 16-day old fetus of maternal rats exposed to 0.4% beginning 21 days before breeding. The fetuses of maternal rats exposed to 0.2% beginning at 21 days before breeding developed normally. Based on these results, zinc oxide was classified as teratogenic at high oral doses in the rat. ***Data Quality:*** *ECHA Reliability 2 (reliable with restrictions)*								

II.2 Other Health Effects

Pigment	Endpoint	Outcome	Reference
zinc oxide	Sensitization	No observable adverse effect reported	ECHA, 1999a
Test article: zinc oxide (purity 99.9%). **Dose:** 0 or 2% zinc oxide via intradermal injection (induction concentration) in water. ***Species (strain):*** Dunkin-Hartley guinea pigs ***Gender:*** female. ***Number per Group:*** 10 per test ***Methods:*** guinea pig maximization test. In this study experimental animals (10 in each test) were intradermally injected with a 2% concentration and epidermally exposed to a 2% concentration (i.e., the highest practically feasible concentration). Control animals (5 in each test) were similarly treated, but with vehicle (water) alone. Approximately 24 hours before the epidermal induction exposure all animals were treated with 10% SDS. Two weeks after the epidermal application all animals were challenged with a 50% test substance concentration and the vehicle. ***Adverse Effect(s) (brief):*** In this test, white staining of the treated skin by the test substance was observed in some animals 24 and 48 hours after challenge. Overall, 0% sensitization was observed in both experimental and control animals. No skin reactions were evident. ***Data Quality:*** *ECHA Reliability 1 (reliable without restriction)*			
Eye Irritation			
Zinc oxide	Eye irritation	No observable adverse effect reported	ECHA, 2011
Test article: Zinc oxide **Dose:** 12 mg in 0.5 µL water was administered to a reconstructed three-dimensional human cornea model. ***Species (strain):*** EpiOcular™. ***Gender:*** ND ***Number per group:*** 2 tissues per dose. ***Methods:*** EpiOcular™ eye irritation test: The potential of NM-110 Zinc Oxide to cause ocular irritation was assessed by a single topical application of 50 µL bulk volume (about 12 mg) of the test substance to a reconstructed three-dimensional human cornea model (EpiOcular™). Tissue destruction was determined by measuring the metabolic activity of the tissue after exposure/post-incubation using a colorimetric test using the EpiOcular™ eye irritation test. The duration of the exposure was 90 minutes, after which it was washed with sterile PBS; an observation period of 18 hours occurred. Mean tissue viability (% of negative control) was determined. ***Adverse Effect(s) (brief):*** Zinc oxide does not show an eye irritation potential in the EpiOcular™ eye irritation test under the test conditions chosen. The mean tissue viability (% of negative control) was 98, indicating no eye irritation potential. ***Data Quality:*** *ECHA Reliability 1 (reliable without restriction)*			

Section III. Dose-Response Assessment

III.1 Provisional Tolerable Intake Values

Contact Duration	Exposure Route	Patient	POD	MF	pTI
Permanent	Parenteral	All	160 mg/kg/d	3000	0.053 mg/kg/d

pTI = POD ÷ MF

Note: When a supplier-specific color additive safety data sheet (SDS) does not report the presence of a carcinogen or non-carcinogen impurity, and SDS complies with Globally Harmonized System (GHS) hazard communication limits (i.e., <0.1% and <1%, respectively), then this pTI is low enough to be protective for naturally occurring impurities which could be present in the color additive. Processes used to color a polymer system involve diluting the pigment and its impurities by at least 50x (i.e., the CHRIS calculator is valid when the maximum concentration of pigment in a colored polymer is 2%, i.e., 1 ÷ 2% = 50); therefore, the concentration of naturally occurring impurities in the finished colored polymer will be significantly lower compared to the

concentration of these substances in the pigment (i.e., 0.002% (i.e., 0.1% ÷ 50) for carcinogens and 0.02% (i.e., 1% ÷ 50) for non-carcinogens). Unknown impurities are addressed by the CHRIS calculator.

III.2 Modifying Factor for POD

Critical Study	Biological Endpoint	Exposure Route	POD Type	POD Value	UF_1	UF_2	UF_3	MF
ECHA, 1968	Reproductive / developmental	Oral	NOEL	160 mg/kg/d	10	10	30	3000

MF = modifying factor (UF_1 x UF_2 x UF_3)

III.3 Toxicological Uncertainties Applied to POD

Uncertainty	Justification
UF_1 Error! Bookmark not defined.	(10) Default value to account for susceptible individuals.
UF_2 Error! Bookmark not defined.	(10) Default value to account for differences between species.
UF_3 Error! Bookmark not defined.	(30) Default value to account for differences in exposure route, data quality, and duration.

UF_1, UF_2, and UF_3, are used to extrapolate the POD to the general human population (including pediatric) exposed parenterally for a lifetime.

III.4 Critical Health Study Design and Outcome

Zinc Oxide ECHA, 1968: Developmental toxicity / teratogenicity:008			
Test Substance	Zinc oxide		
Species	nulliparous Rat	Frequency	Daily
Gender	females	Duration	Up to 21 day
Age	NR	#/Group	Not reported
Route	Oral (feed)	Protocol Guideline	Not reported
Dose(s)	0, 160 mg/kg/d or 320 mg/kg/d	Statistical Method(s)	Not reported
Observed Responses	In 9-11 dams administered 320 mg/kg/d zinc oxide for 15, 16, 18 and 20 d prior to mating, death and resorption occurred in fetuses (4-29%) is reported. Similarly, 100 % resorption occurred in 15 and 16 d old fetus of mothers fed 0.4 % zinc beginning at 21 d before breeding. No external abnormalities were observed in the fetuses of exposed mothers. The lower dose (i.e., 160 mg/kg/d) did not result in reported observable adverse effects.		
Notes:	The study is limited by the low number of treatment doses.		

* Technical grade sample produced by Bayer AG under the trade name Light Yellow 3R. (CAS number and purity not reported.)

Solvent Violet 13

CAS # 81-48-1

Toxicological Profile Summary

Solvent Violet 13 (CAS # 84-65-1) is an organic dye substance which is part of the Anthraquinones Group. It is a solid and is insoluble in water and soluble in acetone, toluene, and benzene. It is maufactured through a process of treatment of quinizarin, Leuco-quinizarin, or 1-bromo(or chloro)-4-hydroxy-anthraquinone with P-toluidine, followed by sulfonation. Inadequate toxicological data (i.e., point-of-departure) is available for derving a chemical specific pTI value. For assessing exposure that will be without appreciable harm to health, the provisional tolerable intake (pTI) for parenteral systemic toxicity is 0.0015 mg/kg/day. This value is based on a noncarcinogenic threshold of toxicological concern (TTC) value (ISO TS 21726, 2019) because this CA is non-mutagenic (see Section II. Hazard Identification).

NOTE: This pTI is protective for the color additive including any impurities listed in Section I.1 "Substance Identity," as the impurities likely will be present at a very low percentage of the color additive, and the quantity of the impurity will be at an even lower percentage in the final device (see Section III, Table III.1 footnote). Unknown impurities are addressed by the CHRIS calculator.

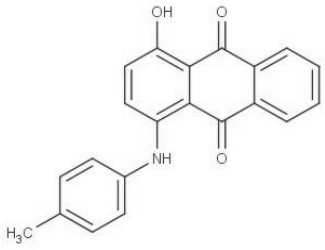
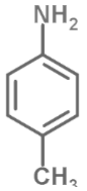
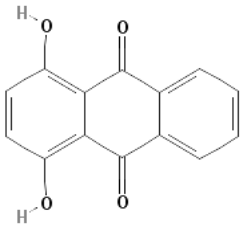
Section I. Substance Information

I.1 Substance Identity

	Descriptor
Synonyms [88], [89]	1-hydroxy-4-(*p*-toluidino)anthraquinone; 1-hydroxy-4-(*p*-toluidino)anthraquinone ; 1-hydroxy-4-(*p*-toluidino)anthraquinone; D&C Violet 2; Disperse Blue 72; Irisol base; Qinezerin blue; Alizurol purple; Quinizarin blue
Commercial Name[89]	11092 Violet; Ahcoquinone Blue IR Base; Alizarine Irisol R Base; Alizarine Violet 3B Base: C.I. 60725; C.I Solvent Violet 13; Disperse Blue 72; Irisol Base; Oil Violet IRS; Oil Violet ZIRS; Waxoline purple A; Alizurol Purple SS; Solvent 13
Formula[88]	$C_{21}H_{15}NO_3$
Molecular Weight[88]	329.355 g/mol
Physical Form[88]	Solid (at 20°C)
Physical/Chemical Characteristics[90]	Deep yellow red – black, crystal powder
Production[90]	Manufactured by either condensation of quinizarin with *p*-toluidine or by condensation of 1-hydroxy-halogenoanthroquinone with *p*-toluidin
Impurities[91]	Quinizarin, a decomposed dye produced as a heat byproduct, and *p*-toluidine
Other[88]	Organic pigment
Related[88]	Other anthraquinone dyes
Surrogates	none

[88] ChemIDplus. Substance Name: p-Toluidine. Found at: https://chem.nlm.nih.gov/chemidplus/rn/106-49-0 (Accessed on 4/5/2017).
[89] Spectrum Chem. Validated by Sonia Owen on 11/15/2011. Found at: https://www.spectrumchemical.com/MSDS/D2555.pdf (accessed on 4/5/2017)
[90] Federal Register. Found at: https://www.gpo.gov/fdsys/pkg/FR-1994-03-14/html/94-5788.htm (Accessed on 4/7/2017).
[91] Tomihata, Kenji, Masakazu Suzuki, Hidetaka Sato, and Masashi Kitagawa. "Sensitizer Contained in Heat-decomposed Dye." Journal of Biomedical Materials Research 54, no. 4 (2001): 531–39.

Molecular Descriptors

Name	CAS #	Formula	Mass	Structure
Solvent Violet 13	81-48-1	$C_{21}H_{15}NO_3$	329.355 g/mol	
Impurities				
***p*-Toluidine**	106-49-0	C_7H_9N	107.156 g/mol	
Quinizarin	81-64-1	$C_{14}H_8O_4$	240.214 g/mol	

Section II. Hazard Identification

Health effect data, supporting studies, and secondary sources were reviewed, see tables in sections II.1 and II.2 for details.

Acute oral LD_{50} values for Solvent violet 13 exceed 2000 mg/kg bw in rats. No clinical observations were reported for the acute lethality studies[71].

Chronic exposure in rodents (18 or 19.5 months) resulted in no adverse reactions that could be attributed to the color additives in doses up to 1% in 0.1mL[92].

The Chronic carcinogenic potential of Quinizarin Blue was determined to be non-carcinogenic, in an 18-month skin painting study in mice. In this study, there were no significant differences in tumor type, incidence, or degree of effects treatment with Quinizarin Blue[92]. Subchronic, reproductive/developmental, and irritation where not addressed due to lack of available data. In vitro genotoxicity test using solvent violet 13 report negative result for mutagenic activity in Salmonella typhimurium[93].

In a 45-day repeat dose reproductive toxicity, no changes were observed in the, mating performance, fertility, gestation lengths, or sex ratio for the offspring at 1000 mg/kg/day. Based on the results of this study, the NOAEL for the adult animals and offspring was also considered to be 1000 mg/kg/day (highest dose tested)[94].

[92] Carson, S. (1984) Skin Painting Studies in Mice on 11 Fd&C and D&C Colors: Fd&C Green no. 3, Red No. 2, Red No. 4, Yellow No. 6, and External D&C No. 7, D&C Orange No. 4, Violet No. 2, Red No. 17, Red No. 34, and Yellow No. 7. J Toxicol. Cutaneous Ocul. Toxicol., 3, 309–331.
[93] Brown, J.P. and Brown, R.J. (1976) Mutagenesis by 9, 10-anthraquinone derivatives and related compounds in Salmonella typhimurium. Mutation Research/Genetic Toxicology, 40, 203–224.
[94] ECHA, 2018

II.1 Systemic, Genotoxicity, Carcinogenicity, Reproductive/Developmental Toxicity

ADVERSE HEALTH EFFECT STUDIES

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Quality Guideline
Systemic Toxicity (acute)							
Marhold 1986	Y	Animal	Oral	5000 mg/kg **(LD_{50})**	NR	Unknown	NR
Test article: Quinizarin Blue/81-48-1 (purity not reported). ***Dose:*** 5000 mg/kg. No data was provided about controls. ***Treatment duration:*** NR***. Species (strain):*** Rat (strain not reported). ***Gender:*** Not reported. ***Number per Group:*** Not reported. ***Method:*** NR. ***Control:*** untreated site of the same animal ***Adverse Effect(s) (brief):*** LD_{50} = 5000 mg/kg. Details of toxic effects not reported other than lethal dose value. ***Data Quality:*** Not reported. ***Note:*** Not available in English. ***Reference:*** *Marhold, J.P. 1986 "Prehled Prumyslove Toxikologie; Organicke Latky,", Czechoslovakia, Avicenum, Vol. -, pg. 1329.*							
Genotoxicity							
Brown and Brown 1976	Y	Bacteria	Ames	10 and 20 µg/plate	NR	Negative	NR
Test article: 1-Hydroxy-4-*p*-toluidine-AQ (D & C Violet 2) (purity not reported). ***Dose:*** Plates were dosed at 10 and 20 µg/plate. ***Treatment duration: Species (strain):*** Salmonella typhimurium TA1535, TA100, TA1537, TA1538, TA1978 and TA98. ***Control:*** 2-anthramine (1 pg/plate), positive control/ untreated group. ***Method***: MST: spot testing of materials applied to the agar surface in 6 mm paper concentration discs (100--200 #g test substance in 10--20 /~1 DMSO/disc). Positive controls used in addition to 2-anthramine were compounds directly mutagenic for the following tester strains: EMS, TA1535, MMS, TA100, 9-aminoacridine, TA1537, 4-NQNO, TA1538 and TA98. Sterility controls were prepared for each "S-9 mix", DMSO, and each test solution. All plates were incubated 72 h at 35°C. DRT: Zones (diameters) of growth inhibition (or killing) were measured for the pair of tester strains following 24 h incubation at 35°C. For comparing induction of reverse mutation, plates were prepared exactly as described above for the pour plate test. The number of revertant colonies per test plate were compared with the 210 mean of control plates (~,N=4) for a given assay and the pooled variance for all assays. ***Adverse Effect(s) (brief):*** The authors report, which includes D & C Violet 2), "*Furthermore, no AQ derivative containing solely secondary amine groups exhibited a clearly positive effect.*" The positive genotoxic response was observed for 10 and 20 µg/plate. ***Data Quality:*** Not reported. ***Reference:*** *Brown, J.P. and Brown, R.J. (1976) Mutagenesis by 9, 10-anthraquinone derivatives and related compounds in Salmonella typhimurium. Mutation Research/Genetic Toxicology, 40, 203–224.*							
Carcinogenicity							
Carson 1984	Y	Animal	Dermal	1% in 0.1mL	18 months	Negative	NR
Test article: Quinizarin Blue (CAS# 81-48-1). ***Dose:*** 1% of test substance in 0.1 mL of 0.1% Na Lauryl Sulfate or distilled water ***Treatment duration:*** Once weekly; followed by up 18-month observation period. ***Species (strain):*** Mice (Swiss-Webster). ***Gender:*** Male and female. (17 to 25 g) ***Number per Group:*** 20-21 per sex/ per treatment group. ***Control:*** three series included a vehicle control: distilled water ***Method***: once per week for 18 months to depilated 6 cm^2 skin area of mice. Survival, body weight, and palpable growths were followed for the 18-month period. Microscopic examination which initially involved approximately 50% of the treated animals was extended to include all tumors and grossly abnormal tissues and organs. Seventeen hundred Swiss-Webster mice with an initial weight ranging from 17 to 25 g were used in this study. Mean body weight was determined at 3,6, 9, 12, 15, and 18 months; Series A was the only group determined at 19.5 months. All groups were equally divided as to sex. Initially, the hair on the dorsal area of each animal was clipped with an animal clipper free of lubricating oil. Subsequent periodic clipping was performed according to the rate of hair growth. An area of approximately 6 cm2 was treated twice weekly. Each mouse was observed daily for behavior, survival and visible or palable growth. Records included observations on the incidence, size, and description of any such growths. All surviving animals were terminated after approximately 18 months, when a marked increase of geriatric mortality became apparent. The mice in Series A had proceeded to 19.5 months on test when the decision was made to terminate the study. All mice were necropsied after they died or were sacrificed. Organs were fixed in 10% formalin solution after recording any gross pathological findings. The initial microscopic examinations of the tissues listed below involved approximately 50% of the treated animals in this group but was enlarged in a supplemental series to include histological examinations carried out on all tumors and all grossly abnormal organs or tissues. (Brain, Thymus, pituitary, thyroid, spleen, kidney, axillary lymph node, adrenal, stomach, small intestine, large intestines, urinary bladder. Axillary lymph node, ovary, skin from area of treatment, any tissue masses, grossly abnormal organs or tissues. Each color was dissolved or dispersed in either water or aqueous sodium lauryl sulfate solution (0.1%) for application to the depilated skin of mice once a week for approximately 18 months. To adequately evaluate responses of the test animals, each of the three series included a vehicle control: distilled water in Series A and Series B, and 0.1 % solution of sodium lauryl sulfate in Series C. In Series A, B, and C, 100 female and 100 male mice were used in each control group for a total of 600 control mice. ***Adverse Effect(s) (brief):*** No adverse reactions or pathological changes were observed following weekly dermal applications for 18 or 19.5 months to the dorsal area of mice that could be attributed to the color additives. No treatment related changes to behavior, body weight, survival rate, lymphomas, gross macroscopic or histopathology of major organs. ***Data Quality:*** Not reported. ***Note***: Initial weight of mice ranged from 17 to 25 g. These data are useful to address long-term dermal exposure to Solvent Violet 13 where contact is less than daily. ***Reference:*** *Carson, S. (1984) Skin Painting Studies in Mice on 11 Fd&C and D&C Colors: Fd&C Green no. 3, Red No. 2, Red No. 4, Yellow No. 6, and External D&C No. 7, D&C Orange No. 4, Violet No. 2, Red No. 17, Red No. 34, and Yellow No. 7. J Toxicol. Cutaneous Ocul. Toxicol., 3, 309–331.*							

These data are of insufficient quality for deriving a tolerable intake but may be useful for determining hazard potential in a weight of evidence approach; Data Type: human, animal, PBPK/ADME, mechanistic, human case reports, review, other; Y = yes; N = no; NR= Not reported

SUPPORTING STUDIES

In Silico			
D&C Violet No. 2	In Silico	Positive	Sosted et al. 2004

Test article: Solvent Violet 13 (81-48-0). ***Methods:*** TOPS-MODE QSAR model to estimate the likely sensitization potency for a chemical. The higher the number, the more likely the chemical is a sensitizer. The predicted sensitization potency number represents an arbitrary relative ranking scheme. A list of hair dye ingredients was compiled from 3 main sources: 1). The Inventory of Cosmetics Ingredients (INCI) found on The European Commissions homepage. This list covers 261 hair dye substances. 2). European Commission Directive 26 on the approximation of the laws of the member states relating to cosmetic products. This is a list of 61 newly regulated hair dye substances. 3). Provisional quantitative list of hair dyes used in hair coloring products on the European market year 2002, a list of 89 substances. Structures identified were then imported into a molecular spreadsheet TSAR. Simplified Molecular Input Line Entry Specifications (SMILES, www.daylight. com), which are 1-dimensional representations of chemical structures, were generated. This list was filtered in order to remove any further duplicate structures, such as salt-containing ingredients (HCl and SO4), leaving 229 hair dye substances. The TOPS-MODE QSAR model was used in order to estimate the likely sensitization potency in 1 of 3 bands: * strong/moderate sensitizers, * weak sensitizers and * extremely weak or non-sensitizer. The European Cosmetic Toiletry and Perfumery Association has generated tonnage data for use in the EU Commission for prioritizing hair dyes in risk assessment and risk management. The cluster analysis provided a means of grouping substances according to their chemical properties such that a representative diverse subset could be selected for further work. As little is understood about the chemical drivers or the specific mechanisms of hair dye substances allergy, the approach to calculating as much chemical information about these hair dye substances as possible should encompass those features important in sensitization. TOPSMODE descriptors accounting for hydrophocity, molar refractivity, polarizability, charges, polar surface area, molecular weight and van der Waals radii were calculated for the 229 substances. The algorithm applied was that of K-means, as implemented in STATISTICA. The algorithm was set to define 10 clusters, as 10 substances would be a reasonable number on which to focus any further work. Each cluster contained other 'similar' chemicals. In this case, similarity is in the context of the descriptors selected, rather than similarity in their likely sensitization response, although, if we have captured those descriptors that are relevant for sensitization; then, it is plausible that the sensitization mechanisms for chemicals within a class might be the same. K-means starts with k random clusters (in this case 10), and then moves substances between those clusters with the goal of minimizing variability within clusters and maximizing variability between clusters. ***Results (brief):*** The predicted sensitization potency was 21.9 and predicted to be a weak sensitizer. ***Data Quality:***NR. ***Note:*** This model was chosen, as it was a general model and not restricted to 1 chemical class. It was readily accessible and could be useful in risk evaluation. *Reference: Sosted, H., D. A. Basketter, E. Estrada, J. D. Johansen, and G. Y. Patlewicz. "Ranking of Hair Dye Substances According to Predicted Sensitization Potency: Quantitative Structure-Activity Relationships." Contact Dermatitis 51 (2004): 241–54.*

SECONDARY SOURCES

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Quality Guideline
Systemic Toxicity (acute)							
ECHA,1982	NR	Animal	Oral (gavage)	>5000 mg/kg bw **(LD_{50})**	single dose	Negative	Similar to OECD 401; NR

Test article: 1-hydroxy-4-[(4-methylphenyl)amino]-9,10-anthraquinone. ***Dose:*** 5000 mg/kg bw in polyethylene glycol. ***Treatment duration:*** Single administration, followed by 14-day observation period. ***Species (strain):*** Rats (SPF-bred Wistar). 9- weeks ***Gender:*** Male (180g)/ female (158g) ***Number per Group***: 5/per sex/ per dose. ***Control:*** None. ***Method:*** A single dose of 5000 mg/kg bw of the test substance was applied to 5 male and 5 female Wistar rats per gavage. The animals were inspected several times on the day of administration, and twice daily during the following 14-day observation period (once on weekends and bank holidays). During inspections, the type, onset, duration, and intensity of clinical signs were recorded, and dead animals removed if necessary. The animals were individually weighed at application, after one week and at the end of the 14-day observation period. Animals sacrificed at the end of study were randomly necropsied. ***Adverse Effect(s) (brief):*** LD_{50} > 5000 mg/kg bw. No morality, no change in body weight, no clinical signs of toxicity, and no abnormal gross pathological findings were observed in treated rats. ***Data Quality:*** ECHA Reliability 1 (reliable without restriction)

ECHA,1993	NR	Animal	Oral (gavage)	>5000 mg/kg bw **(LD_{50})**	single dose	Negative	Non-guideline study; non-GLP

Test article: 1-hydroxy-4-[(4-methylphenyl)amino]-9,10-anthraquinone. ***Dose:*** 5000 mg/kg bw in polyethylene glycol. ***Treatment duration:*** Single administration, followed by 14-day observation period. ***Species (strain):*** Rats (Wistar). 7- weeks ***Gender:*** Male (150-200g) ***Number per Group***: 10/ per dose ***Control:*** None. ***Method:*** A single dose of 5000 mg/kg bw of test substance was applied once to 10 male Wistar rats per gavage. The animals were inspected several times on the day of administration, and twice

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Quality Guideline
daily during the following 14-day observation period (once on weekends and bank holidays). During inspections, the type, onset, duration, and intensity of clinical signs were recorded and dead animals were removed if necessary. The time of death of deceased animals was recorded. The animals were weighed individually at the time of administration, and per group after one week and at the end of the observation period. ***Adverse Effect(s) (brief):*** LD_{50} > 5000 mg/kg bw. No morality, no clinical signs of toxicity, and no abnormal gross pathological findings were observed in treated rats. ***Data Quality:*** ECHA Reliability 1 (reliable without restriction)							
Systemic Toxicity (subacute or subchronic)							
ECHA, 2017	NR	Animal	Oral (gavage)	1000 mg/kg/day **(NOEAL)**	28- day (daily)	Negative	OECD 407; GLP
Test article: 1-hydroxy-4-[(4-methylphenyl)amino]-9,10-anthraquinone / 1,4-bis[(4-methylphenyl)amino]-9,10-anthraquinone/ unknown impurity. ***Dose:*** 0, 250, 500 or 1000 mg/kg/day in Arachis oil BP. The volume of test and control item administered to each animal was based on the most recent scheduled body weight and was adjusted at weekly intervals. ***Treatment duration:*** The test item was administered daily, for twenty-eight consecutive days, by gavage. ***Species (strain):*** Rats (Wistar) 6-8 weeks ***Gender:*** Males (223 to 273g), females (163 to 193g) ***Number per Group***: 5/ per sex / per dose. (Controls: 5 / per negative) ***Control:*** Control animals were treated in an identical manner with 4 mL/kg of Arachis oil BP ***Method:*** All animals were examined for overt signs of toxicity, ill-health or behavioral change immediately before dosing, up to thirty minutes post dosing and one hour after dosing during the working week. Individual body weights were recorded on Day 1 and at weekly intervals thereafter. Body weights were also performed prior to terminal kill. Food consumption was recorded for each cage group at weekly intervals throughout the study. Food conversion efficiency was calculated retrospectively. Water intake was observed daily, for each cage group, by visual inspection of the water bottles for any overt changes except during Week 3 where water intake was measured gravimetrically. Prior to the start of treatment and on Days 7, 14, 21 and 25, all animals were observed for signs of functional/behavioral toxicity. Functional performance tests were also performed on all animals during week 4, together with an assessment of sensory reactivity to different stimuli. Observations were carried out from approximately two hours after dosing on each occasion. Each animal was individually assessed for sensory reactivity to auditory, visual and proprioceptive stimuli. Hematological and blood chemical investigations were performed on all animals from each test and control group at the end of the study (Day 28). Microscopic examination was conducted. ***Adverse Effect(s) (brief):*** **NOAEL=1000 mg/kg/day.** No mortality, changes in body weight, food consumption, water consumption, occurred because of treatment. Fur staining by the test item was apparent for all treated males and for 1, 2 and all females at 250, 500 and 1000 mg/kg/day respectively. Additionally, dark/blue staining of the feces was frequently observed from Day 5 to termination for both sexes at 250, 500 and 1000 mg/kg/day. Necropsy of treated animals revealed several organ/tissues showing blue discoloration or blue colored contents including the aorta, bone and bone marrow (sternum), adipose tissue, mammary gland, mesenteric lymph nodes, mandibular lymph nodes, skin, stomach, ileum, caecum and thyroid/parathyroid. For animals at 1000 mg/kg/day, mean erythrocyte count, hemoglobin and hematocrit values were statistically significantly lower than control ($p<0.05$). In the absence of any supporting histopathological change for the bone marrow, this finding was considered likely to be incidental and would be insufficient to represent an adverse effect of treatment. Assessment of the animals in a standard arena did not reveal any obvious adverse effects of treatment at dosages of 250, 500 or 1000 mg/kg/day. Based on the results of this study the No Observed Adverse Effect Level (NOAEL) for the oral administration of over twenty-eight consecutive days was 1000 mg/kg/day. ***Data Quality:*** ECHA Reliability 1 (reliable without restriction)2 (reliable with restrictions). ***Note:*** The test article used in this study was a mixture of 1-hydroxy-4-(p-toluidino)anthraquinone (CAS # 81-48-1; molecular formula C21H15NO3) and 1,4-bis(p-tolylamino)anthraquinone (CAS # 128-80-3; molecular formula C28-H22-N2-O2). Insufficient information is reported to verify the reported NOAEL is representative of Solvent Violet 13 (CAS # 81-48-1). Therefore, the reported NOAEL is not recommended for deriving a pTI value for Solvent Violet 13 (CAS # 81-48-1).							
Genotoxicity (in vitro or in vivo)							
ECHA,2007	NR	Bacteria	Cell culture	5000 µg/plate	48hrs	Negative	OECD 471; GLP
Test article: C.I.: Solvent Violet 13. ***Dose:*** Plates were dosed at Pre-Experiment / Experiment I: 0, 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate. Experiment II: 0(untreated control), 33; 100; 333; 1000; 2500; and 5000 µg/plate. ***Treatment duration:*** 48hrs. ***Species (strain):*** Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA 100, and the Escherichia coli strain WP2 uvrA. ***Gender:*** NA ***Number per Group***: two independent experiments.***: Control:*** sodium azide, Methylmethanesulfonate, 4-nitro-o-phenylene-diamine, 4-NOPD; 2-aminoanthracene, 2-AA. / untreated. ***Method:*** The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate. The test item was tested at the following concentrations. ***Adverse Effect(s) (brief)****:* **NOAEL=5000 µg/plate.** The test substance was negative with or without the S-9 fraction in *Salmonella typhimurium.* ***Data Quality:*** ECHA Reliability 1 (reliable without restriction)							
ECHA, 2016	NR	Animal Cell	Cell culture	400.0 µg/ml	4hrs	Negative	OECD 476; GLP
Test article: 1-hydroxy-4-[(4-methylphenyl)amino]-9,10-anthraquinone / 1,4-bis[(4-methylphenyl)amino]-9,10-anthraquinone/ unknown impurity. ***Dose:*** Plates were dosed at 0 (untreated control), 3.1, 6.3, 12.5, 25.0, 50.0, 100.0, 200.0 and 400.0 µg/ml Positive and negative controls were used. ***Treatment duration:*** 4hrs. ***Species (strain):*** Chinese hamster lung fibroblasts (V79)							

Source	Peer Review	Data Type	Route	Dose	Frequency / Duration	Response	Quality Guideline
Number per Group: Two independent experiments. ***Control:*** Concurrent solvent and positive controls were treated in parallel. ***Method:*** in vitro mammalian forward mutation assay ***Adverse Effect(s) (brief):*** No cytotoxicity was observed in concentrations tested up to precipitating concentrations. The test substance was negative with or without the S-9 fraction in Chinese hamster lung fibroblasts (V79). ***Data Quality:*** ECHA Reliability 1 (reliable without restriction)							
ECHA, 2001	NR	Animal	Intraperitoneal injected	2000 mg/kg/day	48hrs	Negative	Similar to OECD 475; GLP
Test article: Solvent violet 13. ***Dose:*** Rats were injected with doses 0, 500, 1000, 2000 mg/kg/day**.** Corn oil was the vehicle. ***Treatment duration:*** 24 or 48 hours. ***Species (strain):*** Mouse (NMRI). 6-weeks ***Gender:*** Male (150-200g) ***Number per Group***: 5/ mice per sampling time/ per treatment group. (Controls: 5 / per negative / per positive). ***Method:*** Four groups each comprising 5 males, received an intraperitoneal injection. Two groups were dosed with 2000 mg/kg body weight, one group was dosed with 1000 mg/ g body weight and one group was dosed with 500 mg/kg body weight. A vehicle treated group served as negative control, a group treated with an intraperitoneal injection of cyclophosphamide (CP) at 50 mg/kg body weight served as positive control. Bone marrow of the groups treated was sampled 24 or 48 hours after dosing. Bone marrow from the negative control group was harvested at 24 hours after dosing only and bone marrow from the positive control group was harvested at 48 hours after dosing only. ***Adverse Effect(s) (brief):*** **NOAEL=2000 mg/kg/day.** After dosing, the animals of the dose level of 2000 mg/kg body weight showed the following toxic signs: lethargy, rough coat and a hunched posture. No effects were observed in any other treatment dose. The mean number of micronucleated polychromatic erythrocytes scored in treated groups were compared with the corresponding solvent control group. The test substance was determined to be not mutagenic ***Data Quality:*** ECHA Reliability 2 (reliable with restrictions)							
Reproductive/ Developmental Toxicity							
ECHA, 2018	NR	Animal	Oral (gavage)	1000 mg/kg/day **(Adult NOEAL);** 1000 mg/kg/day **(Offspring; NOEAL)**	56-days (female). 42-days (males)	Negative	OECD 421; GLP
Test article: 1-hydroxy-4-[(4-methylphenyl)amino]-9,10-anthraquinone / 1,4-bis[(4-methylphenyl)amino]-9,10-anthraquinone/ unknown impurity. ***Dose:*** 0, 250, 500 or 1000 mg/kg/day in Arachis oil BP. Control animals were treated in an identical manner with 4 mL/kg of Arachis oil BP. The volume of test and control item administered to each animal was based on the most recent scheduled body weight and was adjusted at weekly intervals. ***Treatment duration:*** approximately six weeks (males) and up to eight weeks (females) (including a two-week pre-pairing phase, pairing, gestation and early lactation for females). ***Species (strain):*** Rats (Wistar) 6-8 weeks ***Gender:*** Males (279 to 346g), females (199 to 236g) ***Number per Group***: 12 / per sex / per dose. ***Controls:*** 12 / per negative ***Method:*** All animals were examined for clinical signs of toxicity immediately before dosing, soon after dosing, and one hour after dosing. Individual body weights were recorded on Day 1 (prior to dosing) and then weekly for males until termination and weekly for females until pairing. During the pairing phase females were weighed daily until mating was confirmed. Body weights were then recorded for females on Days 0, 7, 14 and 20 post coitum, and on Days 1, 4, 7 and 14 post-partum. Bodyweights were recorded at terminal kill. The epididymides, testes, seminal vesicles (with coagulating gland) and prostate were removed from terminal kill adult males, dissected free from fat and weighed before fixation. The stage of the estrous cycle was recorded for each day. Animals were sacrificed on day 44 or 45 (adult males) and day 14 (adult females' post-partum). The number of live and dead offspring was recorded. The number of offspring born, number of offspring alive recorded daily and reported on days 1, 4, 7 and 13 post-partum, sex of offspring on Days 1, 4 and 13 post-partum, clinical condition of offspring from birth to Day 13 post-partum v. Individual offspring weights on Days 1, 4, 7 and 13 post-partum (litter weights were calculated retrospectively from this data). Thyroid/parathyroid were dissected free from fat for terminal kill animals from both sexes and weighed post-fixation. Histopathology was concluded (epididymidesn, prostate, glans Penis, seminal vesicles (with coagulating gland), gross lesions, testes, LABC (levator ani bulbcavernous) muscle, thyroid/parathyroid, ovaries, uterus/cervix (with oviducts), mammary gland, vagina, pituitary. ***Adverse Effect(s) (brief):*** Staining of the fur was observed for five males and seven females at 250 mg/kg/day, most males and all females at 500 mg/kg/day from Day 8 and all animals at 1000 mg/kg/day. Dark feces were observed in 1000 mg/kg/day animals at stages throughout the study. There was no effect of treatment on body weight and body weight gain of males. There was no effect of treatment on body weight and body weight gain of females during the pre-pairing, gestation, or lactation phases. Females at 1000 mg/kg bw showed a non-significant change in body weight gain. No changes in estrous cycles during the pre-pairing phase. There were no histopathological changes of reproductive tissues were observed at the highest dose tested. No changes were observed in the, mating performance, fertility, gestation lengths, or sex ratio for the offspring at 1000 mg/kg/day. Based on the results of this study, the No Observed Adverse Effect Level (NOAEL) for the adult animals was 1000 mg/kg/day (the highest dosage tested). The No Observed Adverse Effect Level for reproduction and for the growth, development and survival of the offspring was also considered to be 1000 mg/kg/day. ***Data Quality:*** ECHA 1 (reliable without restriction)							

II.2 Other Health Effects

Pigment	Endpoint	Outcome	Reference
D&C Violet No. 2	Sensitization	Sensitization: commercial grade No Sensitization: purified grade	Fujii 2003
Test article: D&C Violet No. 2 (CAS# 81-48-1) (commercial and purified grades) ***Dose:*** 0.1, 0.01,0.001, and 1%; (commercial); 0.01, 0.1, and 1% (purified). control. ***Treatment duration:*** 24 or 48hrs. ***Species (strain):*** Guinea pig (strain not reported). ***Gender:*** Female. ***Number per Group:*** 10. ***Control:*** untreated group; 0.03% acetone solution of each substance was negative. ***Method***: Days 0, 1, & 2: Initiation (shaved shoulder, abraded skin, intradermal injection and closed patch) with a duration of 24 h. Day 9: 10% SLS intradermal injection. Day 10: (shaved shoulder and closed patch) with a duration of 48 h. Day 24: (shaved back and closed patch) with a duration of 24 h. ***Adverse Effect(s) (brief):*** Initiation dose = 2.38 mg/kg bw. D&C Violet No. 2 per se induced hypersensitivity in guinea pigs. A structure-activity study indicated that 1-hydroxyl and 4-hydroxyl groups in the anthraquinone structure are key factors in hypersensitivity induction by anthraquinone-related compounds. ***Note:*** Calculation for dose: 0.1 mL x 1g/mL x 1%/0.42 kg guinea pig = 2.38 mg/kg bw. D&C Violet No. 2 do not intrinsically induce hypersensitivity. The reaction to challenge with the commercial-grade dyes may be due to quinizarin (i.e., impurity). ***Data Quality:*** Data quality cannot be accessed because adequate information is not reported. ***Reference:*** *Fujii, S. (2003) Evaluation of hypersensitivity to anthraquinone-related substances. Toxicology, 193, 261–7.*			
D&C Violet No. 2	Sensitization	Positive	ECHA, 2016
Test article: 1-hydroxy-4-[(4-methylphenyl)amino]-9,10-anthraquinone (Purity: 97.0%) . ***Dose:*** Animals were applied 0 (control group), 5, 10 or 25% in dimethylformamide. The application volume, 25 µL/ear/day, was spread over the entire dorsal surface (diameter ~ 8 mm) of each ear once. ***Treatment duration:*** daily for three consecutive days. ***Species (strain):*** Mouse (CBA/Ca) 6-8 weeks ***Gender:*** Females ***Number per Group***: 5/ per sex/ per dose (5 control) ***Control:*** A control group of five mice was treated with the vehicle only. ***Method:*** Three groups each of five female mice were treated with different concentrations of the test item by topical application at the dorsum of each ear once daily each on three consecutive days. A control group of five mice was treated with the vehicle only. Five days after the first topical application, the mice were intravenously injected into a tail vein with radio-labelled thymidine (^{3}H-methyl thymidine; ^{3}HTdR). Approximately five hours after intravenous injection, the mice were sacrificed, and the draining auricular lymph nodes excised and pooled per animal. Single cell suspensions of lymph node cells were prepared from pooled lymph nodes, which were subsequently washed and incubated with trichloroacetic acid overnight. The proliferative capacity of pooled lymph node cells was then determined by the incorporation of ^{3}H-methyl thymidine measured in a β-scintillation counter. ***Adverse Effect(s) (brief):*** No deaths occurred during the study period. No symptoms of local skin irritation at the ears of the animals and no signs of systemic toxicity were observed during the study period. Redness of the ear skin could not be examined, due to the color of the test item. The body weight of the animals, recorded prior to the first application and prior to treatment with ^{3}HTdR, was within the range commonly recorded for animals of this strain and age. The measured ear weight of all animals treated was recorded on test day 6 (after necropsy). A relevant increase in ear weights was not observed. The measured ear thickness of all animals treated was recorded prior to the 1st application, on study day 3 and prior to necropsy (day 6). A relevant increase in ear thickness was not observed. In this study stimulation indices of 2.1, 2.6, and 3.5 were determined with the test item at concentrations of 5, 10, and 25% in DMF. A dose response was observed. The EC3 value calculated was 16.7 %. The results indicate 1-hydroxy-4-[(4-methylphenyl)amino]-9,10-anthraquinone is positive for having skin sensitizing potential. ***Data Quality:*** ECHA Reliability 1 (reliable without restriction)			
D&C Violet No. 2	Sensitization	Positive	ECHA, 2016
Test article: 1-hydroxy-4-[(4-methylphenyl)amino]-9,10-anthraquinone (Purity: 97.0%) . ***Dose:*** Animals were applied 0 (control group), 5, 10 or 25% in dimethylformamide. A control group of five mice was treated with the vehicle only. The application volume, 25 µL/ear/day, was spread over the entire dorsal surface (diameter ~ 8 mm) of each ear once. ***Treatment duration:*** daily for three consecutive days. ***Species (strain):*** Mouse (CBA/Ca) 6-8 weeks ***Gender:*** Females ***Number per Group***: 5/ per sex/ per dose **Control:** Untreated/ 5 control. ***Method:*** Three groups each of five female mice were treated with different concentrations of the test item by topical application at the dorsum of each ear once daily each on three consecutive days. A control group of five mice was treated with the vehicle only. Five days after the first topical application, the mice were intravenously injected into a tail vein with radio-labelled thymidine (^{3}H-methyl thymidine; ^{3}HTdR). Approximately five hours after intravenous injection, the mice were sacrificed, and the draining auricular lymph nodes excised and pooled per animal. Single cell suspensions of lymph node cells were prepared from pooled lymph nodes, which were subsequently washed and incubated with trichloroacetic acid overnight. The proliferative capacity of pooled lymph node cells was then determined by the incorporation of ^{3}H-methyl thymidine measured in a β-scintillation counter. ***Adverse Effect(s) (brief):*** No deaths occurred during the study period. No symptoms of local skin irritation at the ears of the animals and no signs of systemic toxicity were observed during the study period. Redness of the ear skin could not be examined, due to the color of the test item. The body weight of the animals, recorded prior to the first application and prior to treatment with ^{3}HTdR, was within the range commonly recorded for animals of this strain and age. The measured ear weight of all animals treated was recorded on test day 6 (after necropsy). A relevant increase in ear weights was not observed. The measured ear thickness of all animals treated was recorded prior to the 1st application, on study day 3 and prior to necropsy (day 6). A relevant increase in ear thickness was not observed.			

Pigment	Endpoint	Outcome	Reference
In this study stimulation indices of 2.1, 2.6, and 3.5 were determined with the test item at concentrations of 5, 10, and 25% in DMF. A dose response was observed. The EC3 value calculated was 16.7 %. The results indicate 1-hydroxy-4-[(4-methylphenyl)amino]-9,10-anthraquinone is positive for having skin sensitizing potential. ***Data Quality:*** ECHA Reliability 1 (reliable without restriction) ***Note:*** Original report			
Cytotoxicity			
D&C Violet No. 2	Cytotoxicity	Cytotoxic	Karmaus et al. 2016
Test article: D&C Violet 2. ***Dose:*** NA ***Treatment duration:*** NA ***Species (strain):*** Variety. ***Control:*** NR ***Methods***: Median AC_{50} from 35 ToxCast assays measuring cytotoxicity was used to define a "cytotoxicity center" for each chemical. To calculate a cytotoxicity center, chemicals must have elicited a significant effect in at least three cytotoxicity assays. ***Adverse Effect(s) (brief):*** 3 active cytotoxicity assays were positive from 14 cytotoxicity assays evaluated with a cytotoxicity center of 1.75 µM. ***Data Quality:*** NR Evaluation of ToxCast data. ***Reference:*** *Karmaus, A.L. et al. (2016) Evaluation of food-relevant chemicals in the ToxCast high-throughput screening program. Food Chem. Toxicol., 92, 188–196.*			
Irritation (skin)			
D&C Violet No. 2	Dermal irritation	No observable adverse effect reported	ECHA, 1983
Test article: 1-hydroxy-4-[(4-methylphenyl)amino]-9,10-anthraquinone. ***Dose:*** Animals were applied 500 mg of the test substance in water. ***Treatment duration:*** Four hours; followed by a 7-day observation period. ***Species (strain):*** Rabbit (New Zealand White) 6-8 weeks ***Gender:*** Males (3.5 kg) ***Number per Group***: 3. ***Control:*** Controls were not required. ***Method:*** One day before the application of the test substance, an area of 6 x 6 cm of the animals was clipped. Three animals were applied 500 mg of the test substance under semiocclusive conditions for 4 hours. After 4 hours the skin areas were washed with water. After 1, 24, 48, 72 hours and 7 days the skin areas were scored for erythema and edema according to Draize. ***Adverse Effect(s) (brief):*** NOEAL=500 mg. Following treatment, the erythema and edema score was = 0 at any time point (24/48/72 hours). 1-Hydroxy-4-(p-toluidino)anthraquinone is not irritating to the skin in rabbits. ***Data Quality:*** ECHA Reliability 1 (reliable without restriction)			
Eye irritation			
D&C Violet No. 2	Eye irritation	No observable adverse effect reported	ECHA, 1983b
Test article: 1-hydroxy-4-[(4-methylphenyl)amino]-9,10-anthraquinone. ***Dose:*** Animals were applied 500 mg of the test substance in water. ***Treatment duration:*** 24hrs, followed by a 7-day observation period. ***Species (strain):*** Rabbit (New Zealand White) 6-8 weeks ***Gender:*** Males (3.5 kg) ***Number per Group***: 3. ***Control:*** The other eye served as control. ***Method:*** Three animals were applied 100 µg of the test substance into the conjunctival sac of one eye. The other eye served as control. The treated eye was washed after 24 hours with physiologic saline. After 1, 24, 48, 72 hours and 7 days the eyes were scored for cornea, iris, conjunctivae and chemosis according to Draize. ***Adverse Effect(s) (brief):*** **NOEAL=500 mg.** Cornea opacity, iris, conjunctivae and chemosis scores were 0 at any time point (24, 48 and 72 hours). 1-hydroxy-4-(p-toluidino)anthraquinone is not irritating to the eyes in rabbits. ***Data Quality:*** ECHA Reliability 1 (reliable without restriction)			

Section III. Dose-Response Assessment

Provisional Tolerable Intake Values

Insufficient toxicological data is available to derive a tolerable intake value for Solvent Violet 13. Therefore, the threshold of toxicological concern (TTC) is applied. Experimental data supports Solvent Violet 13 is mutagenic; therefore, the TTC value (i.e., 1.5 µg/kg/day) is recommended.

Contact Duration	Route of Exposure	Patient	pTI
Permanent	Parenteral	All	0.0015 mg/kg/day

pTI is derived from Kroes et al. 2005 TTC (*i.e.*, 90 µg/day ÷ 60 kg body weight). Body weight (60 kg) is in accordance with Kroes et al. 2005.

Note: When a supplier-specific color additive safety data sheet (SDS) does not report the presence of a carcinogen or non-carcinogen impurity, and SDS complies with Globally Harmonized System (GHS) hazard communication limits (i.e., <0.1% and <1%, respectively), then this pTI is low enough to be protective for naturally occurring impurities which could be present in the color additive. Processes used to color a polymer system involve diluting the pigment and its impurities by at least 50x (i.e., the CHRIS calculator is valid when the maximum concentration of pigment in a colored polymer is 2%, i.e., 1 ÷ 2% = 50); therefore, the concentration of naturally occurring impurities in the finished colored polymer will be significantly lower compared to the concentration of these substances in the pigment (i.e., 0.002% (i.e., 0.1% ÷ 50) for carcinogens and 0.02% (i.e., 1% ÷ 50) for non-carcinogens). Unknown impurities are addressed by the CHRIS calculator.

Attachment D

Additional Toxicological Considerations for the Color Additives

Chemical toxicity, or lack there-of, is a consequence of interactions between the chemical substance and the body, as well as, the impact of the body on the chemical substance. These interactions are influenced by, but not limited to, the chemical substance molecular structure and physicochemical characteristics. The following information is a summary of physicochemical determinants that influences the toxicity of the color additives included in this document. Additionally, hazardous situations applicable to the color additives in this document are also discussed briefly.

Physicochemical determinants of color additive toxicity

The ability of a color additive to chemically interact with biological tissue and elicit toxicity can be addressed by supporting evidence, which includes physicochemical properties.[Error! Bookmark not defined.,95] The t ypes of physicochemical properties that generally indicates whether or not pigments and dyes could elicity toxicity are listed in Table 5.

Table 6. Physicochemical determinants of CA toxicity (examples)

Pigments	Dyes
particle size particle shape particle surface area-to-mass ratio reactivity	solubility/lipophilicity molecular weight alerting structural groups, e.g., reactivity metabolism toxicity

Solubility

Solubility of an organic molecule in an aqueous (e.g., water) versus lipophilic (e.g., octanol) environment plays a significant role in the likelyhood that the molecule will (a) dissolve to the lowest possible molecular size and (b) diffuse through cellular membranes.[95,96] The lipophilic nature of nonionic organic molecules is correlated with its bioaccumulation in aquatic organisms.[97] For example, Solvent Violet 13 (CAS # 81-48-1) has a predicted water:octanol coefficient (i.e., Log_{kow}), a measure of an organic molecule lipophilicity, value of 5.25 that indicates this color additive prefers a lipophilic environment compared to a polar environment. Thus, this dye is expected to diffuse through cellular membranes when dissolved in a non-polar environment. In contrast, pigments are insoluble in polar and non-polar environments; therefore, pigments are not expected to diffuse through cellular membranes.

Molecular weight and particle size

The U.S. EPA (1995) considers the ability of an organic molecule to pass through membranes generally decreases with increasing molecular weight.[98] EPA applied this principle and determined that a molecular weight of 10,000 daltons, which includes oligomeric substances, to be the cut-off value below which an

[95] Locher, M., 2020. Characterization of Physicochemical Parameters in Toxicology, in: Reichl, F.-X., Schwenk, M. (Eds.), Regulatory Toxicology. Springer, Berlin, Heidelberg, pp. 1–8. https://doi.org/10.1007/978-3-642-36206-4_30-2

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[98] EPA, 1995. 40 CFR Part 723. Premanufacture Notification exemptions; Revisions of Exemptions for Polymers; Final Rule. The U.S. Federal Register 60, 16316-16336.

organic molecule could be absorbed (i.e., cross cellular membranes). Dyes are low molecular weight organic molecules (i.e., <1,000 daltons). In contrast, pigments (i.e., 0.1 to 1000 nm) are much, much larger (i.e., >600 kilodaltons). The molecular weight of Solvent Violet 13 (CAS # 81-48-1) is 329.355 g/mol; therefore, the molecular weight of Solvent Violet 13 is sufficiently low to permit diffusion through cellular membranes. Unlike dyes, the physical size of pigments prevents these substances from diffusing through cellular membranes.

Molecular structural alerts

For organic molecules, structural groups can alert that a chemical could elicit toxicity.[99, 100, 101] The molecular structure of Solvent Violet 13 (CAS # 81-48-1) contains structural alerts for genotoxicity/carcinogenicity. Because pigments exist as particles, rather than molecules, alerting structural groups do not apply to pigments.

Particle shape and surface

Particle shape (e.g., amorphous, coarse, irregular, regular, or spherical) influences particle toxicity.[102, 103, 104, 105, 106] The shape of all of the pigments evaluated in this report are characterized as regular or spherical, which indicates the shape of these color additives is not a significant determinat of harm and likely explains (at least partially) the unobservable experimentally obtained toxicological data reported in the literature.

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[103] Warheit, D.B., McHugh, T.A., Hartsky, M.A., 1995. Differential pulmonary responses in rats inhaling crystalline, colloidal or amorphous silica dusts. Scandinavian journal of work, environment & health 19–21.

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Surface area:mass ratio and surface reactivity also influence particle toxicity.**Error! Bookmark not defined.**[107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117] The potential for surface reactivity toxicological effects (e.g., photoactivity) of pigments is mitigated by surface treatments, which are required to achieve the intended coloring effect in a polymer system (e.g., dispersion that results in homogenous distribution and fastness). Surface treatments include coating pigment particles with inorganic oxides (e.g., aluminum hydroxide, silica oxide), organic molecules (e.g., polyols, octylsiloxanes, dimethicone, or other polymeric material), or both.**Error! Bookmark not defined.**[118, 119, 120, 121,10, 122, 123, 124, 125] Surface treatment materials that are stable and u nreactive are not expected to adversely impact the toxicity of the pigment (Warheit and Brown 2019).**Error! Bookmark not defined.**,

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Hazardous Situations

The toxicological tenet, dose makes the poison, is Centuries old and still applicable today.[126] Application of this well-known toxicological principle requires knowledge of the dose that causes harm. However, we derived color additive pTI values that are intended for general application and represent an exposure dose below which any appreciable harm will not occur. Therefore, conclusions of the probability that harm will occur based on an exposure that exceeds these color additve pTI values requires expert judgement. Common criteria to consider when making judgements of whether or not these color additives could be a hazardous situation are presented in the following table.

Table 7. Criteria for judging CA exposure is or isn't a hazardous situation

Common Criteria	Pigment Specific Criteria (10 in Table 5)	Dye Specific Criteria (i.e., Solvent Violet 13)
Clinical relevance of exposure estimate	case-by-case (i.e. exposure estimation method)	
Physicochemical property	type of surface coating(s)	solubility of the dye in device fluid/tissue
Biological removal processes	tissue macrophage activity	tissue specific metabolic pathways
Dose at which harm will occur	color additive specific (see tox profile)	

Clinical relevance of exposure estimate

Exposure estimation methods, of varying clinical relevance, are described in ISO 10993-18 and in the literature.[Error! Bookmark not defined.,127] In general, exposure estimation methods that are of greater clinical r elevance permit more definitive conclusions that contact between the body and a pigment or dye is or is not a hazardous situation.

Physicochemical property

Additional evidence that further supports exposure to a specific color additive presents a hazardous situation include those physicochemical properties that are specific to the color additive or applicable to the specific contact scenario. For example, a novel surface coating that could be unstable or chemically react with the body could indicate exposure to a pigment could be hazardous. Conversely, insolubility in an externally communicating fluid or biological tissue could reduce the probability that a dye elicits harm.

Biological removal processes

Knowledge of presence or absence of biological processes that remove a color additive from the site of contact or other target site could also indicate whether or not exposure to the color additive is a hazardous situation (i.e., limited duration of exposure to the color additive).

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For pigments, the biological removal process is cellular phygocytosis (e.g., macrophages).[128,129,130] Thus, exposure to a pigment that occurs in tissue/organ with resident phagocytic macrophages (e.g., peritoneal cavity and lung) has a greater capacity to remove pigments compred to tissue/organ with fewer phagocytic macrophages.[131,132,108,109,111]

For organic dyes, the biological removal process includes metabolic pathways that results in lesser toxic metabolites. The hazard situation criteria in Table 6 should only be applied after supporting evidence specific to the color additive and device intended use is obtained.

Dose at which harm will occur

The dose at which harm occurs are those that exceed the color additive specific TI value. The specific harmful dose is unique for each color additive. In general, doses reported to represent a LOAEL or LOAEC are harmful doses. The same uncertainty factor approach used to derive a TI value may be used to extrapolate a harmful dose from an animal study to humans. In general, a clinically relevant exposure estimate equal to or exceeds a harmful dose indicates exposure is a hazardous situation.

Note: Based on the latest data cited previously, dose expressed as surface area to mass ratio (rather than mass alone) should be considered when estimating risk of exposure to a harmful dose of a particle.

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