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Pigment Yellow 12, Pigment Yellow 13, Pigment Yellow 83

MAK Value Documentation – Translation of the German version from 2021

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Keywords

Pigment Yellow; solubility; lung; granular biopersistent dusts; bioavailability; inhalation; carcinogenicity; maximum concentration at the workplace; MAK value

Abstract

The German Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission) summarized and evaluated the data for Pigment Yellow 12 [6358-85-6], Pigment Yellow 13 [5102-83-0] and Pigment Yellow 83 [5567-15-7] to derive a maximum concentration at the workplace (MAK value) considering all toxicological end points. Relevant studies were identified from a literature search and also unpublished study reports were used. The pigments are mainly used as colourants in inks, cosmetics and industrial paints and varnishes. Depending on their intended use, the pigments are produced in different sizes with a d50 ranging from 15 to 250 nm. In technical applications, particles smaller than 100 nm are called nanoparticles. By definition, pigments are not soluble in application media. Pigment Yellow 12, 13 and 83 were found to be insoluble in the body and in phagolysosomal solution in vitro, which simulates the lung environment. The carcinogenic 3,3′-dichlorobenzidine was not released. Systemic toxicity was not observed in oral studies in rats and mice with exposure periods of up to 2 years. Therefore, these 3 pigments are insoluble and not bioavailable. If inhaled, insoluble particles can accumulate in the lung. In two 5-day inhalation studies of Pigment Yellow 83 and two 21-day inhalation studies of Pigment Yellow 13 in rats, local effects in the lungs were observed, which were similar to those observed after exposure to granular biopersistent dusts. No long-term inhalation toxicity studies have been carried out with the pigments. Long-term exposure to pigment concentrations which exceed the lung clearance capacity is assumed to result in particle-induced carcinogenic effects in the lungs. Therefore, in analogy to granular biopersistent dusts, Pigment Yellow 12, 13 and 83 have been classified in Carcinogen Category 4. A maximum concentration at the workplace (MAK value) has been established in analogy to that for granular biopersistent dusts taking into account the lower agglomerate density of particles below 100 nm. This results in a MAK value of 0.3 mg/m^3 × (mass density of the pigment × 0.5). In analogy to granular biopersistent dusts, the pigments have been assigned to Peak Limitation Category II with an excursion factor of 8. No developmental toxicity studies are available for Pigment Yellow 12, 13 and 83. In analogy to granular biopersistent dusts, and based on the findings of two developmental toxicity studies with orally applied nanoparticles, titan dioxide and amorphous silica, which did not induce any effects on the foetus, Pigment Yellow 12, 13

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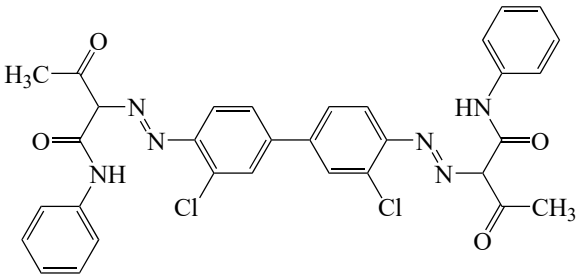
and 83 have been assigned to Pregnancy Risk Group C. As the pigments are not soluble or bioavailable, genotoxicity and mutagenicity were not observed. The pigments are not sensitizing to the skin or airways and do not penetrate the skin in toxicologically relevant amounts.

MAK value (2020)	**0.3 mg/m³ R (respirable fraction) × (material density × 0.5)**[a)]
Peak limitation (2020)	**Category II, excursion factor 8**
Absorption through the skin	–
Sensitization	–
Carcinogenicity (2020)	**Category 4**
Prenatal toxicity (2020)	**Pregnancy Risk Group C**
Germ cell mutagenicity	–
BAT value	–
Synonyms	Pigment Yellow 12: CI 21090 2,2′-[(3,3′-dichloro[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[3-oxo-*N*-phenylbutanamide] Pigment Yellow 13: CI 21100 2,2′-[[3,3′-dichloro[1,1′-biphenyl]-4,4′-diyl]bis(azo)]bis[*N*-(2,4-dimethylphenyl)-3-oxobutanamide] Pigment Yellow 83: CI 21108 2,2′-[(3,3′-dichloro[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[*N*-(4-chloro-2,5-dimethoxyphenyl)-3-oxobutanamide]
Chemical name (IUPAC)	Pigment Yellow 12: 2-[[4-[4-[(1-aniline-1,3-dioxobutane-2-yl)diazenyl]-3-chlorophenyl]-2-chlorophenyl]diazenyl]-3-oxo-*N*-phenylbutanamide Pigment Yellow 13: 2-[[2-chloro-4-[3-chloro-4-[[1-(2,4-dimethylaniline)-1,3-dioxobutane-2-yl]-diazenyl]phenyl]phenyl]diazenyl]-*N*-(2,4-dimethylphenyl)-3-oxobutanamide Pigment Yellow 83: 2-[[2-chloro-4-[3-chloro-4-[[1-(4-chloro-2,5-dimethoxyaniline)-1,3-dioxobutane-2-yl]diazenyl]phenyl]phenyl]diazenyl]-*N*-(4-chloro-2,5-dimethoxyphenyl)-3-oxobutanamide
CAS number	Pigment Yellow 12: 6358-85-6 Pigment Yellow 13: 5102-83-0 Pigment Yellow 83: 5567-15-7

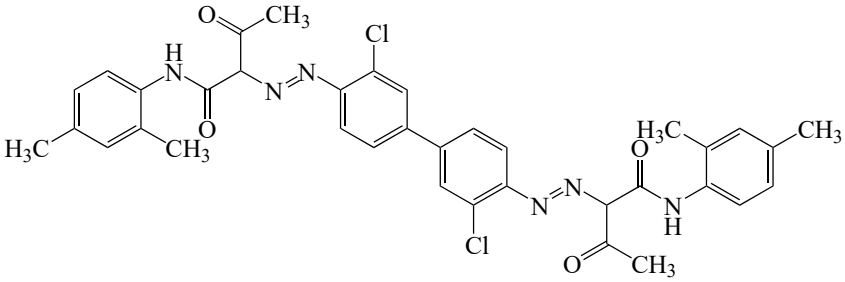
Structural formula



Molecular formula

Pigment Yellow 12: $C_{32}H_{26}Cl_2N_6O_4$

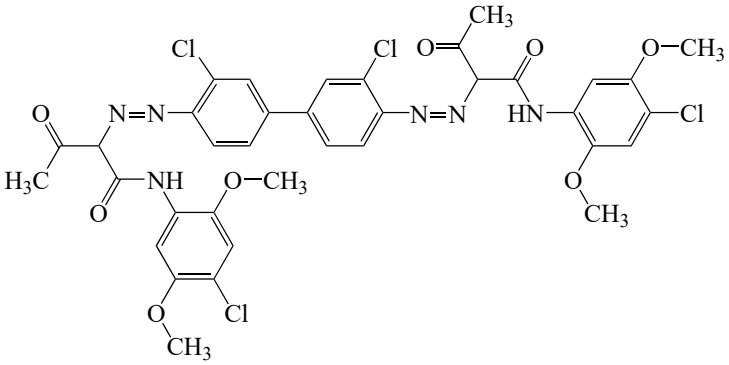
Structural formula



Molecular formula

Pigment Yellow 13: $C_{36}H_{34}Cl_2N_6O_4$

Structural formula



Molecular formula

Pigment Yellow 83: $C_{36}H_{32}Cl_4N_6O_8$

Molar mass

Pigment Yellow 12: 629.51 g/mol
Pigment Yellow 13: 685.61 g/mol
Pigment Yellow 83: 818.50 g/mol

Melting point

Pigment Yellow 12:
306 °C; decomposes at 310 °C (ECHA 2017 a)
Pigment Yellow 13:
decomposes at 330 °C (ECHA 2017 b)
Pigment Yellow 83:
decomposes at 300 °C (ECHA 2017 c)

Boiling point

–

Vapour pressure at 25 °C

Pigment Yellow 12:
2.9×10^{-17} hPa (calculated; OECD 2003)
Pigment Yellow 13:
6.2×10^{-19} hPa (calculated; OECD 2003)
Pigment Yellow 83:
2.4×10^{-21} hPa (calculated; OECD 2003)

Density	Pigment Yellow 12: 1.39 g/cm^3 (ECHA 2017 a) Pigment Yellow 13: 1.36 g/cm^3 (ECHA 2017 b) Pigment Yellow 83: 1.5 g/cm^3 (ECHA 2017 c)
log K_{OW}	Pigment Yellow 12: 2.1 at 23 °C (calculated; ECHA 2017 a; OECD 2003) Pigment Yellow 13: 1.8 at 24 °C (calculated; ECHA 2017 b; OECD 2003) Pigment Yellow 83: 0.02 at 24 °C (calculated; ECHA 2017 c; OECD 2003)
Solubility	Pigment Yellow 12: 0.4 µg/l water at 24 °C, pH 7 (ECHA 2017 a; OECD 2003) Pigment Yellow 13: 0.35 µg/l water at 24 °C, pH 7 (ECHA 2017 b; OECD 2003) Pigment Yellow 83: 8.1 µg/l water at 23 °C, pH 7 (ECHA 2017 c; OECD 2003)
Purity	Pigment Yellow 12: > 96% (ECHA 2017 a) Pigment Yellow 13: > 96% (ECHA 2017 b) Pigment Yellow 83: > 96% (ECHA 2017 c)
Impurities	water < 1%; 3,3′-dichlorobenzidine < 25 mg/kg, normal content typically < 5 mg/kg; primary aromatic amines < 500 mg/kg (no other details; ECHA 2017 a; OECD 2003) Pigment Yellow 12: acetoacetanilide [102-01-2] (OECD 2003) Pigment Yellow 13: 2′,4′-dimethylacetoacetanilide [97-36-9] (OECD 2003) Pigment Yellow 83: 4′-chloro-2′,5′-dimethoxyacetoacetanilide [4433-79-8] (OECD 2003)
Production	diazotization of 3,3′-dichlorobenzidine and coupling with an appropriately substituted acetoacetanilide; particles precipitate out as a fine powder, some of the particles are below 100 nm in size (Schmidt et al. 2007)

[a)] (material density × 0.5) corresponds to an assumed agglomerate density at a packing factor of 50%

Cited unpublished toxicological studies from companies have been made available to the Commission.

Colourant is the general term used to refer to all organic or inorganic substances that impart colour to products. Dyes are soluble colourants; pigments are, by definition, insoluble in the application media (BAuA 2001; Wesendorf 2018).

This documentation is based on a group evaluation carried out as part of the ICCA-HPV Programme (High Production Volume (HPV) Chemicals Programme of the International Council of Chemical Associations (ICCA)) (OECD 2003), the dataset publicly available through REACH (ECHA 2017 a, b, c), a group evaluation carried out by the Australian authorities (NICNAS 2017) and unpublished studies from companies which have been made available to the Commission. The particle size distribution of Pigment Yellow 12, 13 and 83 extends into the ultrafine particle range below 100 nm (Schmidt et al. 2007). In technical applications, particles that are smaller than 100 nm are called nanoparticles. According to the

manufacturer's information, the d50 values for Pigment Yellow 12 and 13 are in the range from 15 to 100 nm and the d50 values for Pigment Yellow 83 range from 15 to 250 nm (Verband der Mineralfarbenindustrie e.V. 2019).

In the cosmetic industry, the pigments (only Pigment Yellow 13 and 83) are used in products such as bath salts, face makeup, mascara, hair dye, nail polish and showering products. All 3 pigments are used as colourants in industrial and decorative paints, varnishes, printing inks and plastics (polymers), in the reproduction of recorded sound, image and data storage media, and in chalks, textiles (only Pigment Yellow 12), modelling clays and synthetic resin lacquers (only Pigment Yellow 13) (NICNAS 2017; OECD 2003). The use of several of the yellow pigments as tattoo inks has been reported, but these colourants are not manufactured or recommended for this application (OECD 2003).

Group evaluation

Pigment Yellow 12, 13 and 83 are structurally similar. All contain chloro-substituted biphenyl moieties, azo moieties and keto groups as well as substituted or non-substituted phenyl rings at both ends of the molecule, which are connected to the rest of the molecule via an amide bond. They are similar in molar mass, stability and physico-chemical properties; for example, they are practically insoluble in water. For this reason, hydrolysis of the amide bond is not to be expected upon contact with water. A reduction of the azo bond did not occur in various test systems. Cleavage of the azo bond or hydrolysis of the substances were not observed in vivo. On the basis of the available data, the 3 pigments are assumed to have a similar toxicological profile and are not expected to be bioavailable (NICNAS 2017; OECD 2003). Pigment Yellow 12, Pigment Yellow 13, Pigment Yellow 83 "opaque" and Pigment Yellow 83 "transparent" were found to be insoluble in phagolysosomal simulant fluid in static and dynamic solubility tests (BASF SE 2019 b).

The number and identity of the substituents on the phenyl rings of Pigment Yellow 12, 13 and 83 are unlikely to have a significant effect on their chemical behaviour (OECD 2003).

1 Toxic Effects and Mode of Action

As Pigment Yellow 12, 13 and 83 are not bioavailable and do not undergo cleavage after oral administration and in phagolysosomal simulant fluid in vitro, local effects on the airways were observed only after inhalation exposure.

In two 5-day inhalation studies in rats that investigated 2 different sizes of Pigment Yellow 83 particles, hypertrophy and hyperplasia were observed in the bronchial epithelium and the histiocytes in the tracheobronchial lymph nodes were loaded with particles. These effects were observed after exposure to Pigment Yellow 83 "opaque" at a concentration of 30 mg/m^3 and after exposure to Pigment Yellow 83 "transparent" at concentrations as low as 10 mg/m^3. Another effect observed after exposure to Pigment Yellow 83 "transparent" was neutrophil infiltration in the bronchi.

In two 3-week inhalation studies that investigated 2 different formulations of Pigment Yellow 13, yellowish-brown deposits in the alveoli and interstitium and the accumulation of foam cells in the alveoli were observed at all 3 concentrations of about 53, 150 and 400 mg/m^3. The lung weights were found to be increased at the higher concentrations; this is regarded as a reaction to particle deposition.

In a developmental toxicity study with Pigment Yellow 12 that was carried out in rats according to OECD Test Guideline 422, substance-related effects were not observed in the dams and offspring at 1000 mg/kg body weight and day. Pigment Yellow 12, 13 and 83 were not found to be genotoxic in vitro or in micronucleus and sister chromatid exchange (SCE) tests in vivo. In an oral carcinogenicity study, Pigment Yellow 12 and Pigment Yellow 83 were not found to induce carcinogenic effects in rats or mice.

2 Mechanism of Action

Pigment Yellow 12, 13 and 83 are not systemically available. Inhalation exposure leads to accumulation in the lungs at concentrations that overload alveolar clearance. Particle effects were observed in 5-day and 21-day inhalation studies in rats (Section 5.2.1; BASF SE 2018, 2019 a; Ciba-Geigy 1979 a, b). Long-term exposure may cause chronic inflammation

which, in turn, leads to the development of carcinogenic effects in the respiratory tract. However, chronic inhalation studies with the requisite scope are not available.

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

3.1.1 Inhalation and intratracheal exposure

Studies that analysed the quantitative uptake of the 3 pigments by inhalation are not available. On the basis of the physico-chemical properties of the pigments, particularly their log K_{OW} and molar mass, no or only minimal absorption is assumed to occur after exposure by inhalation. This was confirmed by the findings of two 5-day inhalation studies that investigated Pigment Yellow 83 (2 different products) and two 21-day inhalation studies that investigated Pigment Yellow 13 (2 different products). The studies did not detect systemic effects, but slight local effects on the respiratory tract were observed (BASF SE 2018, 2019 a; Ciba-Geigy 1979 a, b; see Section 5.2.1).

An earlier study investigated the absorption and metabolism of Pigment Yellow 83 and the structurally similar Pigment Yellow 17 after intratracheal administration to determine whether the azo bonds of the pigment molecules, like those of the azo dyes, are cleaved in vivo, leading to the release of the carcinogenic metabolite 3,3′-dichlorobenzidine (CAS No. 4531-49-1; Figure 1).

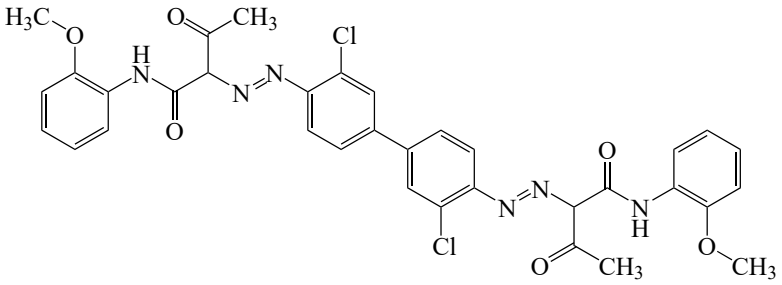


Fig. 1 Structural formula of Pigment Yellow 17

Groups of 6 male Wistar rats per concentration and examination time point were given 10 or 20 mg of Pigment Yellow 83 or Pigment Yellow 17 in suspension or 3,3′-dichlorobenzidine by intratracheal instillation 5 times over a period of 4 weeks and then observed for 4 weeks following the end of exposure. The 3,3′-dichlorobenzidine levels in the urine, faeces and haemoglobin were determined by sensitive and specific gas chromatography-mass spectrometry (GC-MS). The parent substance was analysed for impurities. Pigment Yellow 17 was found to contain amino monoazo compounds in a concentration of 11 mg/kg pigment; these compounds were detected in Pigment Yellow 83 at levels below 10 mg/kg pigment. Pigment Yellow 17 and Pigment Yellow 83 contained 3,3′-dichlorobenzidine in concentrations of 9 and 1.7 µg/kg pigment, respectively. The pigments occurred in microcrystalline form as short rods with an edge length of 1 µm. Their level of solubility was less than 20 µg/l water or octanol. For both pigments, the amount of pigment determined in the lungs on day 31 was between 50% and 75% of the amount of pigment administered; this amount did not decrease during the observation period. During treatment with Pigment Yellow 83, very small amounts of 3,3′-dichlorobenzidine were found in the urine, in some animals also in the faeces and bound to haemoglobin (Table 1). On day 30, the bioavailable fraction determined in a 24-hour sample in relation to the total amount of pigment in the lungs was 0.0012 mole percent; on day 56, it was below 0.00006 mole percent. In 2 of 5 samples, the maximum amount still bound to haemoglobin at the end of the observation period was 0.13 ng/g. On the basis of the amounts excreted with the urine, it was calculated that 0.001% to 0.005% of the theoretical amount of 3,3′-dichlorobenzidine that could be

released from the total amount of substance administered was recovered within each 24-hour period (BAuA 2001). The study found that the 3,3′-dichlorobenzidine did not originate solely from contamination by 3,3′-dichlorobenzidine and the monoazo compound because the calculated amount of released substance was slightly higher than the determined level of contamination. However, these findings were later disproven by other studies that investigated bioavailability (see the analysis of solubility in phagolysosomal simulant fluid; BASF SE 2019 b).

Another study administered 7.3 mg of 3,3′-dichlorobenzidine to each test animal according to the same method and found that about 3% of the administered amount was excreted within 24 hours in the form of free amines or as conjugates. Pigment Yellow 17 was likewise administered according to the same method at a dose of 20 mg per animal. The amounts of 3,3′-dichlorobenzidine that were excreted with the urine and faeces and bound to haemoglobin were below the limit of detection. The limits of detection for this method were a 3,3′-dichlorobenzidine concentration of 0.02 ng/ml urine and a 3,3′-dichlorobenzidine concentration of 0.05 ng/g haemoglobin. In the exposed animals, the lymph nodes associated with the lungs were found to be yellow and enlarged and pigment was detected in the macrophages by microscope. "Slight breathing sounds and moderately lethargic behaviour" lasting 4 to 6 hours after application were attributed to the treatment (BAuA 2001).

Tab. 1 3,3′-Dichlorobenzidine in the urine, faeces and in the form of haemoglobin adducts after intratracheal administration of 5 doses of 10 or 20 mg Pigment Yellow 83 to rats within a 4-week period followed by an observation period of 4 weeks (BAuA 2001)

Amount, examination day	Urine	Faeces	Haemoglobin adducts
10 mg, day 2/26/30	3.9–4.2 ng/24 hours	detectable amounts in only 2 of 30 samples: 2.2 and 4.4 ng, respectively, per 24 hours	day 31 in 3/5 samples: max. 0.24 ng/g
20 mg, day 2	4.6 ng/24 hours		–
day 30	8.8 ng/24 hours		4/5 samples: max. 6.66 ng/g
day 56	–	–	2/5 samples: max. 0.13 ng/g

3.1.1.1 Systemic availability of nano materials after inhalation

Respirable particles are able to pass from the alveolar space into the lymphatic system via the connective tissue in the lungs; particles in the ultrafine range are able to pass also into the capillaries (DFG 2019; Section V). Nanoparticles are able to cross membranes within the organism, reaching secondary organs such as the brain, heart and liver as well as the foetus. The fraction of particles found there is below 0.5% of the amount absorbed, but is conversely dependent on particle size. However, it also depends on the material, morphology, hydrophilic and lipophilic properties, surface charge, surface ligands and their possible exchange with different body fluids. The systemic distribution in humans is expected to be similar to that in rats (Kreyling 2013).

3.1.1.2 Investigations of the solubility of the pigments

To investigate whether fractions of Pigment Yellow 12, Pigment Yellow 13 and two Pigment Yellow 83 products with differently sized particles, Pigment Yellow 83 "transparent" and Pigment Yellow 83 "opaque", are soluble in the lungs after exposure by inhalation, a static and a dynamic in vitro study were carried out in phagolysosomal simulant fluid at a pH of 4.5. This corresponds to the milieu inside the phagolysosomes of the alveolar macrophages. The exact specifications of the pigments, the study method and results are shown in Table 2. Before testing, the pigments were washed once with a mixture of methanol/toluene (80/20), once with 1-octanol and once with methanol to remove impurities. Then, 125 mg of pigment per litre of phagolysosomal simulant fluid were suspended and then dispersed by shaking for 7 days in a laboratory shaker or by sonication for 24 hours in an ultrasound bath. The fluid was then analysed for dissolved substance. The limit of detection was 0.1 ng/cm^2 and hour for UV-VIS (ultraviolet-visible) analysis and 0.034 ng/cm^2 and hour for LC-MS (liquid chromatography-mass spectrometry) analysis. The concentration in the sample solution determined by UV-VIS analysis was in the range of or below the limit of detection of 0.01% dissolved substance; the concentration determined by LC-MS analysis was always below the limit of detection. In the dynamic assay using a continuous flow system, the concentrations determined by both analytical techniques were below the

limits of detection. Additionally, particles of Pigment Yellow 83 "transparent" and "opaque" were analysed by transmission electron microscopy (TEM) before and after a week in the flow-through cell. The particles were found to be rod-shaped; some had a cross-sectional diameter in the nm-range and occurred as agglomerates. Pigment Yellow 83 "transparent" decreased in size by 6%, Pigment Yellow 83 "opaque" increased by 23%. According to the authors, the changes in particle size were not statistically significant as the small number of analysed particles and the large variation in particle sizes led to an inaccuracy in the determinations of particle size of about 20%. The smooth, even surface morphology of both particles remained unchanged, even after the solubility tests, which is evidence of the stability of the particle surface. Thus the studies did not find evidence for the solubility of Pigment Yellow 83 "transparent" and "opaque" or Pigment Yellow 12 and Pigment Yellow 13 (BASF SE 2019 b).

Tab. 2 Static and dynamic solubility testing with Pigment Yellow (BASF SE 2019 b)

	Pigment Yellow 83 "transparent"	**Pigment Yellow 83 "opaque"**	**Pigment Yellow 12**	**Pigment Yellow 13**
production date	12/2014	10/2014	06/2006	09/2004
purity	98.7%	99.7%	98.05%	98.9%
mass-specific surface area (BET)	63 m^2/g	17 m^2/g	31 m^2/g	38 m^2/g
volume-specific surface area (VSSA)	93 m^2/cm^3	25 m^2/cm^3	43 m^2/cm^3	53 m^2/cm^3
static solubility testing in water with Na_2HPO_4, NaCl, Na_2SO_4, $CaCl_2 \times 2\ H_2O$ at 37°C and a pH of 4.5: 125 mg/l shaken for 7 days or 24 hours in ultrasound bath, followed by filtration and analysis of the fluid				
UV-VIS analysis (LOD 0.01%)	0.013% (shaken) 0.014% (ultrasound)	n. d. (shaken or ultrasound)	n. d. (shaken or ultrasound)	n. d. (shaken) 0.016% (ultrasound)
LC-MS analysis (LOD 20 µg/l = 0.016%)	n. d.	n. d.	n. d.	n. d.
continuous flow system: 1 mg solid per flow cell at a fluid flow rate of 2 ml/hour; solvent: water with Na_2HPO_4, NaCl, Na_2SO_4, $CaCl_2 \times 2\ H_2O$, glycine (organic acid), potassium hydrogen phthalate (ion trap) at 37°C and pH 4.5, 7 days				
UV-VIS analysis (defined as insoluble: at < 1 ng/cm^2/hour)	0.072 ng/cm^2/hour → insoluble	0.049 ng/cm^2/hour → insoluble	0.039 ng/cm^2/hour → insoluble	0.065 ng/cm^2/hour → insoluble
LC-MS maximum concentration (LOD 20 µg/l)	n. d.	n. d.	n. d.	n. d.
LC-MS dynamic dissolution rate (LOD dependent on surface)	n. d. (LOD 0.009 ng/cm^2/hour)	n. d. (LOD 0.034 ng/cm^2/hour)	n. d. (LOD 0.028 ng/cm^2/hour)	n. d. (LOD 0.029 ng/cm^2/hour)
TEM: particle size before and after solubility testing	difference −6% (not significant as methodological uncertainty of about 20%)	difference +23% (not significant as methodological uncertainty of about 20%)	not tested	not tested
mean size	before: 37.5 nm after: 35.7 nm	before: 173 nm after: 210 nm	not tested	not tested
median size	before: 37.0 nm after: 34.6 nm	before: 159 nm after: 196 nm	not tested	not tested
minimum size	before: 19.7 nm after: 15.5 nm	before: 67.5 nm after: 75.5 nm	not tested	not tested
maximum size	before: 63.8 nm after: 57.0 nm	before: 358.5 nm after: 458 nm	not tested	not tested

after: after 7 days in the flow-through cell; before: before entering the flow-through cell; LOD: limit of detection; n. d.: not detected; TEM: transmission electron microscopy

3.1.2 Oral administration

To study the absorption of ^{14}C-Pigment Yellow 12 and its sulfonated water-soluble derivative (synthesized from U-^{14}C-(3,3′-dichlorobenzidine)), male F344 rats were given a single oral dose of 1.24 to 2.65 µmol/kg body weight (about 0.7 to 1.7 mg/kg body weight). Radioactivity was not detected in the blood after 10 minutes or after 8 hours and the radioactivity in the tissues was in the range of the background exposure. After 8 hours, the total administered amount of radioactivity had been recovered in the faeces and in the contents of the intestines. Similar findings were observed after administration of the sulfonated derivative, with the exception that after 1 day 0.02% of the radioactivity was recovered in the urine. After repeated oral administration of "larger amounts" of Pigment Yellow 12, the mucous membranes of the digestive tract were found to be discoloured, suggesting that traces of the substance or its impurities may have been absorbed. On the other hand, this may have been caused by contamination during the preparation for necropsy. A developmental toxicity screening study with gavage administration of Pigment Yellow 12 revealed slight discoloration of the skin in a small number of offspring at the high dose of 1000 mg/kg body weight and day. This was caused by contamination during necropsy. Neither Pigment Yellow 12 nor the administered water-soluble derivatives were metabolized in vivo by intestinal bacteria (OECD 2003).

The absorption, distribution and elimination of ^{14}C-Pigment Yellow 12 (11 µCi/µmol; vehicle: Emulphor EL-620: ethanol: water in a ratio of 1:1:8; (v/v)) was investigated in groups of 3 to 6 male F344 rats given a single gavage dose of 1.11 mg/kg body weight (equivalent to 3.89 µCi/rat or 0.354 µmol/rat). No radioactivity was detected in the blood, liver or urine up to 8 hours after exposure; 104% was recovered in the faeces and appendix. The authors concluded that absorption did not occur after oral administration of the substance to rats (Decad et al. 1983).

A study from 1989 analysed the levels of urinary metabolites in male rats (Tif:RAIf) given a single gavage dose of Pigment Yellow 12. The pigment was administered in oil (no other details, not radioactively labelled) at doses of 0, 40 or 400 mg/kg body weight. A total of 30 animals were tested; however, the report does not include data for the number of animals per dose group. The urine was collected over a period of 48 hours following administration of the substance. No metabolites of 3,3′-dichlorobenzidine were detected in the urine. In a positive control group given 3,3′-dichlorobenzidine (no other details), 2% to 4% of the administered dose was recovered in the urine within 24 hours and another 0.2% to 1% between 24 and 48 hours after administration of the substance. The limit of detection in the urine was 5 µg/l urine. According to the authors, the findings in vivo after oral administration do not provide evidence of the cleavage of Pigment Yellow 12 to form 3,3′-dichlorobenzidine (ECHA 2017 a).

Groups of 5 male and 5 female Sprague Dawley rats were given a single gavage dose of Pigment Yellow 13 of 400 mg/kg body weight. No metabolites of 3,3′-dichlorobenzidine were detected in the urine within 48 hours (ECHA 2017 a).

In rabbits given a single oral dose (no other details) of Pigment Yellow 13 of 50 mg/kg body weight, the substance was not detected in the urine after 24 to 72 hours (OECD 2003).

Groups of 6 female Wistar rats were given normal feed or feed containing 0.2% Pigment Yellow 13 (3,3′-dichlorobenzidine: < 0.1 mg/kg, monoazo compounds: 220 mg/kg) or Pigment Yellow 17 (3,3′-dichlorobenzidine: < 0.1 mg/kg, monoazo compounds: 21 mg/kg) for 4 weeks. This was equivalent to pigment doses of 170 and 165 mg/kg body weight and day, respectively. The 4 female rats in the positive control group were given drinking water with 0.06% Direct Red 46 (3,3′-dichlorobenzidine: 19 mg/kg), an azo dye with known bioavailability. This was equivalent to a dose of 69 mg/kg body weight and day. No signs of toxicity were observed in the animals; the body weight gains were similar to those of the control animals. Steady-state 3,3′-dichlorobenzidine–haemoglobin adduct levels were determined by GC/MS analysis and 3,3′-dichlorobenzidine–DNA adduct levels in the liver were determined by ^{32}P-postlabelling. For purposes of comparison, the DNA and haemoglobin adduct levels were determined in rats given 0.00024%, 0.0012% or 0.006% 3,3′-dichlorobenzidine with the drinking water for 4 weeks. In the animals treated with 3,3′-dichlorobenzidine, a dose-dependent increase in adduct levels from 8.1 ng/g haemoglobin to 160 ng/g haemoglobin and from 2.6 ng/g DNA (relative adduct level 3.3×10^{-9}) to 45.4 ng/g DNA (relative adduct level 56.1×10^{-9}) was observed. After exposure to Pigment Yellow 13, 3,3′-dichlorobenzidine-equivalent adduct levels of 0.18 and 0.2 ng/g haemoglobin were determined in 2 of 6 animals and adduct levels of 0.3 and 0.15 ng/g DNA were obtained in 2 of 9 samples (limit of detection 0.1 ng/g haemoglobin and 0.08 ng/g DNA). The theoretical release of 3,3′-dichlorobenzidine from Pigment Yellow 13 in doses of

0.01 to 0.02 mg/kg body weight and day was extrapolated. However, contamination by the monoazo compound needs to be taken into consideration, which could lead to the theoretical release of a 3,3′-dichlorobenzidine dose of 0.02 mg/kg body weight and day. This suggests that contamination led to the formation of the detected 3,3′-dichlorobenzidine adducts. Haemoglobin or DNA adducts were not found in the animals exposed to Pigment Yellow 17. In the animals of the positive control group with exposure to Direct Red 46, total adduct levels of 17.7 ng/g haemoglobin and 5.2 ng/g DNA (relative adduct level 6.4×10^{-9}) were determined (Sagelsdorff et al. 1996).

3,3′-Dichlorobenzidine was not found in the urine of rats, rabbits or monkeys given oral doses of Pigment Yellow 13 (NICNAS 2017; OECD 2003) or in oral carcinogenicity studies with rats and mice given Pigment Yellow 12 and Pigment Yellow 83 (Leuschner 1978).

3.1.3 Dermal application

^{14}C-Pigment Yellow 12 was not absorbed after occlusive application to the skin of rats for 1 day. The radioactivity was recovered at the application site, on the dressing and on the tip of the pipette used for application (OECD 2003).

3.1.4 Summary

There is no evidence that oral, dermal and intratracheal exposure lead to the cleavage of the azo groups of the pigments and the release of 3,3′-dichlorobenzidine. Several studies found 3,3′-dichlorobenzidine in the urine or in the form of haemoglobin and DNA adducts after administration of Pigment Yellow 12 and 13; these findings were attributed to contamination of the pigments by 3,3′-dichlorobenzidine or the respective monoazo compounds. Two 5-day inhalation studies with Pigment Yellow 83 (2 different products) and two 21-day inhalation studies with Pigment Yellow 13 (2 different products) did not reveal systemic effects.

Pigment Yellow 83 “transparent” and “opaque” and Pigment Yellow 12 and 13 were found to be insoluble in phagolysosomal simulant fluid (BASF SE 2019 b). For this reason, Pigment Yellow 12, 13 and 83 are regarded as insoluble in the lungs.

3.2 Metabolism

As no evidence of absorption was found, possible metabolic processes are unlikely to play a major role in the development of systemic effects. Oral and intratracheal exposure did not lead to cleavage of the azo bond in vivo. In vitro studies did not find evidence of metabolism by the intestinal flora (OECD 2003). Metabolism to 0.005 mole percent 3,3′-dichlorobenzidine is assumed to be the worst case for Pigment Yellow 83 (Section 3.1).

4 Effects in Humans

The concentrations of 3,3′-dichlorobenzidine or *N*-acetyl-3,3′-dichlorobenzidine were determined in the urine of the workers of a pigment manufacturing company during and after the shift. No differences were found between the workers with and those without exposure. However, no exposure data were given (OECD 2003).

Findings in humans are available only for allergenic effects.

Allergenic effects

After handling chalk dyes for many years, a patient (50 years old) had recurring episodes of eczematous changes on the face, the back of the hands, the wrists and the skin between the fingers that lasted for a period of 2 months. A patch test yielded 2+ reactions to 1% and 10% formulations of yellow chalk and 10% formulations of red chalk. The patient additionally reacted to the Pigment Yellow 12 contained in the yellow chalk (no other details), producing 1+ and 2+ reactions in the patch test with 1% and 10% formulations, respectively (Lovell and Peachey 1981).

Positive results were not obtained in patch tests with 2% formulations of Pigment Yellow 12 and Pigment Yellow 13 in petrolatum in any of the 32 patients with sensitization to *p*-aminoazobenzene who had produced positive reactions to 0.25% *p*-aminoazobenzene (Thierbach et al. 1992).

In an incompletely documented repeated insult patch test, Pigment Yellow 13 was applied occlusively 8 times for induction, each of the treatments lasting 48 hours. A reaction was not observed in any of the 200 volunteers after repeated treatment. The challenge treatment was carried out 12 days after the last induction treatment. However, the test concentrations, substance specifications and vehicle were not documented (ECHA 2021 a).

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

Rats (no other details) were exposed once for 4 hours to Pigment Yellow 13 in concentrations of 1384, 2237 or 4448 mg/m^3. The substance was administered in the form of a dust with an inhalable fraction of 81%, 98% and 94%. Rapid breathing, dyspnoea, protruding eyes, ruffled fur and hunched posture were observed. All animals recovered. None of the animals died and no substance-related findings were determined at necropsy. The LC_{50} was thus above 4448 mg/m^3. It is assumed that similar findings would be obtained for Pigment Yellow 12 and 83 (OECD 2003).

In a study carried out using a method similar to that of OECD Test Guideline 403, groups of 10 male and 10 female Tif:RAIf rats were exposed nose-only for 4 hours to Pigment Yellow 13 at a concentration of 4250 (± 128) mg/m^3. The substance was administered in the form of an aerosol with particle sizes of 1 to 7 µm. The rats were observed for 14 days after the end of exposure. Dyspnoea, hunched posture and ruffled fur were detected during the last hour of exposure. The animals recovered within 4 days. No gross-pathological findings were determined (ECHA 2017 b).

5.1.2 Oral administration

Studies of acute oral toxicity are shown in Table 3.

A study carried out according to OECD Test Guideline 401 reported an oral LD_{50} for Pigment Yellow 12 above 2000 mg/kg body weight in male and female Wistar rats. None of the animals died and there were no symptoms of toxicity and no substance-related pathological findings. The faeces were dyed orange for up to 2 days after substance administration (Hoechst AG 1995 c).

The oral LD_{50} in Sprague Dawley rats and Wistar rats was above 3000 mg/kg body weight for Pigment Yellow 13 and above 1750 mg/kg body weight for Pigment Yellow 83. The only substance-related effect observed 24 hours after substance administration was discoloration of the faeces. The purity of the substances was not specified. Other studies that were reported by the authors to have been carried out according to OECD Test Guideline 401 used formulations and substance amounts ranging from 30% to 72%. The other substances contained within the test formulations were not specified. A Pigment Yellow 12 formulation (containing 62% to 72% of substance) yielded an LD_{50} for rats of above 2000 mg/kg body weight. None of the animals died during the studies, nor were symptoms of toxicity reported. In LD_{50} studies in rats, doses of up to 17 000 mg/kg body weight were tolerated without mortality (OECD 2003).

Tab. 3 Acute oral toxicity of Pigment Yellow 12, 13, 83

Species, strain	LD_{50}	Comments	References
Pigment Yellow 12			
rat, Wistar, ♀	> 15 000 mg/kg body weight	not lethal, no symptoms of toxicity, no gross-pathological findings	Hoechst AG 1975 a, 1976 b

Tab. 3 *(continued)*

Species, strain	LD_{50}	Comments	References
rat, Wistar, ♂	> 15 000 mg/kg body weight	difficulties breathing directly after application	Hoechst AG 1976 a
rat, Wistar, ♀	> 5000 mg/kg body weight	not lethal, no symptoms of toxicity, no gross-pathological findings	Hoechst AG 1982 a
rat, Wistar, ♂, ♀	> 2000 mg/kg body weight	high stepping, irregular breathing, no gross-pathological findings	Hoechst AG 1988 c, 1995 c
Pigment Yellow 13			
rat, Wistar, ♀	> 15 000 mg/kg body weight	not lethal, ruffled fur, accelerated breathing	Hoechst AG 1977 b
rat, Wistar, ♀	> 5000 mg/kg body weight	not lethal, standing on hind legs, ruffled fur, narrowed lids	Hoechst AG 1982 b
Pigment Yellow 83			
rat, Wistar, ♀	> 10 000 mg/kg body weight	not lethal, no symptoms of toxicity, no gross-pathological findings	Hoechst AG 1976 c
rat, Wistar, ♀	> 15 000 mg/kg body weight	not lethal, ruffled fur, accelerated breathing	Hoechst AG 1977 a
rat, Wistar, ♀	> 10 000 mg/kg body weight	not lethal, no symptoms of toxicity, no gross-pathological findings	Hoechst AG 1980 a
rat, Wistar, ♀, ♂	> 5000 mg/kg body weight	not lethal, no symptoms of toxicity, no gross-pathological findings	Hoechst AG 1984 a

5.1.3 Dermal application

A dermal LD_{50} of above 3000 mg/kg body weight was determined for Pigment Yellow 13 in Sprague Dawley rats following the application of Pigment Yellow 13 in polyethylene glycol/water (50 : 50) in a concentration of 400 mg/l. No mortality and no symptoms of toxicity were reported (OECD 2003).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

Two 5-day inhalation studies were carried out in groups of 16 male Wistar rats, one with Pigment Yellow 83 "transparent" with primary particles about 37 nm in size, and the other with Pigment Yellow 83 "opaque" with primary particles of about 159 nm. The test concentrations were 0, 3, 10 or 30 mg/m³ (Table 4). Half of the animals were observed for 3 weeks following the end of exposure. No systemic effects were induced. Among the animals exposed to the high concentration of 30 mg/m³ of the "opaque" material, 2 were found to have hypertrophy and hyperplasia in the bronchial epithelium and 3 animals had particle-laden histiocytes in the tracheobronchial lymph nodes; these are typical findings induced by exposure to particles. The no-effect concentration for the "opaque" pigments was 10 mg/m³. In the animals exposed to the "transparent" pigments, effects began to be observed at the lower concentration of 10 mg/m³: 3 animals were found to have hypertrophy and hyperplasia in the bronchial epithelium, in 1 animal neutrophil infiltration was observed in the bronchi, and particle-laden histiocytes were found in the tracheobronchial lymph nodes of all 3 animals. The no-effect concentration for the "transparent" pigments was 3 mg/m³. The effects found in the animals of the observation groups were a slight increase in the number of red blood cells and a not significant increase in the number of neutrophils in the bronchoalveolar lavage fluid (BASF SE 2018, 2019 b).

In 2 inhalation studies published in 1979, groups of 10 male and 10 female RAIf rats were exposed nose-only for 3 weeks to an aerosol containing different Pigment Yellow 13 particles (TK10090 and TK10863; see Table 4); 70% to 80% of the particles were smaller than 7 µm. The 2 test substances were produced under different conditions and underwent different surface treatments. The first substance (TK10090) was made up of finer primary particles treated with 2.5% triethanolamine oleate salt and was coated with a small amount of triethanolamine oleate. The second substance (TK10863) was made up of coarser primary particles treated with 40% Staybelite resin (partially hydrogenated rosin) and had a very thick surface coating of resin. The exposure concentrations were monitored gravimetrically once every hour; the exposure concentrations for TK10090 were determined to be 0, 52, 151 and 401 mg/m³ and those for TK10863 were 0, 54, 157 and 410 mg/m³. Additionally, 5 male and 5 female rats from the control group and from the

high concentration group were observed for 21 days following the end of exposure. The animals did not exhibit any symptoms of toxicity. In the high concentration groups, the body weight gains of the male animals on day 21 and of the female animals on days 3, 7 and 10 were reduced slightly, but with statistical significance. The findings were reversible during the observation period. The polymorphonuclear neutrophils were found to be increased and the lymphocytes decreased. The lungs were slightly enlarged; the increase in lung weights was statistically significant (absolute and relative weights). Yellow deposits were observed in the lungs; these were still noticeable at the end of the observation period. At concentrations of 151 mg/m^3 and above, local effects ranging from foamy macrophages to pneumoconiosis were found in the lungs. The findings in the lungs were not attributed to a specific substance-related effect, but to the particle effect. At concentrations of 52 mg/m^3 and above, the histopathological examination revealed minimal deposits in the lungs and particles in the cytoplasm of the histiocytes in the interstitium, occasionally also in the alveoli and in the lumen of the small bronchi and in isolated cases also in the peribronchial lymphatic tissue. However, these were not accompanied by symptoms of inflammation or other adverse reactions. The NOAEC (no observed adverse effect concentration) was lower than the lowest concentration tested (Ciba-Geigy 1979 a, b).

The local effects observed in the lungs are regarded as particle effects.

Influence of the surface treatment of the pigments on their toxicity

Pigments undergo different surface treatments depending on the intended application. Two surface treatments were studied with Pigment Yellow 13: a resin that acts as an adhesive for pigments and treatment with 2% to 3% ethanolamine. The almost identical 3-week inhalation studies in rats described above reported similar findings in the lungs (see Table 4; Ciba-Geigy 1979 a, b). These studies did not detect significant differences in the effects of the 2 types of particle treatment; rather, the effects of the particles themselves were the decisive factor.

In the two 5-day inhalation studies carried out in rats with Pigment Yellow 83 "transparent" (primary particle size: about 37 nm, agglomerate MMAD (mass median aerodynamic diameter): 0.4 to 0.6 µm) and "opaque" (primary particle size: about 159 nm, agglomerate MMAD: 0.3 to 0.4 µm), the "transparent" pigments induced slightly severer effects and were thus found to be more reactive (BASF SE 2018, 2019 a). This is possibly attributable to the size of the particles: the primary particles of the "transparent" pigments were smaller than those of the "opaque" pigments. Again, these findings would suggest that the particles themselves were responsible for the effects.

Tab. 4 Effects of specific Pigment Yellows after repeated inhalation exposure

Species, strain, number per group	Exposure	Findings	References
rat, Wistar, 16 ♂	**Pigment Yellow 83 "transparent"**, 5 days, nose only, 0, 3, 10, 30 mg/m^3, 6 hours/day, 5 days/week, 8 animals/group: observation for 3 weeks, 98.7% purity, produced 12/2014, primary particles about 37 nm, agglomerates 94% < 3 µm aerodynamic size, MMAD 0.4–0.6 µm with GSD 2.9–3.8	on days 5 and 26, necropsy of 3 animals per group: organs weighed and stored, histopathological examination of the respiratory tract only, on days 8 and 29, 5 animals per group: clinico-chemical parameters examined in the blood and bronchoalveolar lavage fluid (cytological, biochemical parameters and selected antigens); **3 mg/m^3**: **NOAEC**; **10 mg/m^3**: in 1 animal slight neutrophilic infiltration in the bronchiolar epithelium, in 3 animals hyperplasia/hypertrophy in the bronchial epithelium; **30 mg/m^3**: absolute and relative lymphocyte, neutrophil and monocyte counts increased in the bronchoalveolar lavage fluid, monocyte chemoattractant protein (MCP-1) increased in the bronchoalveolar lavage fluid, slight neutrophilic infiltration in the bronchiolar epithelium; animals in the observation group: **10 mg/m^3 and above**: red blood cell count ↑, number of neutrophils in the bronchoalveolar lavage fluid not significantly ↑; findings in the respiratory tract, see Table 5	BASF SE 2018

Tab. 4 *(continued)*

Species, strain, number per group	Exposure	Findings	References
rat, Wistar, 16 ♂	**Pigment Yellow 83 "opaque"**, 5 days, nose only, 0, 3, 10, 30 mg/m³, 6 hours/day, 5 days/week, 8 animals/group: observation for 3 weeks, 99.7% purity, produced 10/2014, primary particles about 159 nm, agglomerate MMAD 0.3–0.4 µm with GSD 3.0–4.6	on days 5 and 26, necropsy of 3 animals/group: organs weighed and stored, histopathological examination of the respiratory tract only, on days 8 and 29, 5 animals/group: clinico-chemical parameters in the blood and bronchoalveolar lavage fluid (cytological, biochemical parameters and selected antigens); **10 mg/m³**: **NOAEC**; **30 mg/m³**: in 2 animals hyperplasia/hypertrophy in the bronchial epithelium, in 3 animals particles in the tracheobronchial lymph nodes; for the findings in the respiratory tract, see Table 5; no substance-induced systemic effects	BASF SE 2019 a
rat, RAIf, 10 ♂, 10 ♀	**Pigment Yellow 13**, TK10090: finer primary particles coated with a small amount of triethanolamine oleate, 3 weeks, nose only, aerosol, 0, 52, 151, 401 mg/m³, 70%–80% particle mass < 7 µm, 6 hours/day, 5 days/week, 5 ♂, 5 ♀ additionally 0, 401 mg/m³ and observed for 21 days	**52 mg/m³ and above**: concentration-dependent increase in the number of yellow particles in the lungs, small yellowish-brown, lipid-soluble particles in the interstitium of the lungs and in some cases in the alveoli; no symptoms of inflammation at this concentration; **151 mg/m³ and above**: yellow discoloration of the lungs, yellowish-brown, lipid-soluble particles in the alveoli and interstitium of the lungs, in 8/20 rats focal accumulation of foam cells in several alveoli of the lungs; **401 mg/m³**: body weight gains (♂) ↓, lungs: polymorphonuclear neutrophils ↑, lymphocytes ↓, lung size ↑, relative and absolute lung weights ↑, focal accumulation of basophilic material, number of foam cells in the alveoli ↑, animals with pneumoconiosis, in frozen sections: yellowish-brown particles 1–3 µm in size in the alveolar lumen and cytoplasm of foam cells in the lumen of the small bronchi and in the macrophages of the interstitium, slight focal lymphohistiocytic infiltration in the interstitium, focal pneumoconiosis, yellowish-brown particles, in some cases also in the intrapulmonary lymphoid tissue; **401 mg/m³ and follow-up**: body weights normalized, all local findings in the lungs and pneumoconiosis still noticeable, isolated yellowish-brown particles in the intrapulmonary lymphoid tissue	Ciba-Geigy 1979 a; ECHA 2017 b
rat, RAIf, 10 ♂, 10 ♀	**Pigment Yellow 13**, TK10683: coarser primary particles with a very thick surface coating of resin, 3 weeks, nose only, aerosol, 0, 54, 157, 410 mg/m³, 70%–80% particle mass < 7 µm, 6 hours/day, 5 days/week, 5 ♂, 5 ♀ additionally 0, 410 mg/m³ and observed for 21 days	**54 mg/m³ and above**: concentration-dependent increase in the number of yellow particles in the lungs, focal accumulation of yellowish-brown birefringent particles in the cytoplasm of the histiocytes in the interstitium, occasionally in the alveoli and in the lumen of the small bronchi, in isolated cases in the peribronchial lymphatic tissue, no symptoms of inflammation, no foam cells in the alveoli; **410 mg/m³**: body weight gains (♂, ♀) ↓, relative and absolute lung weights ↑, animals with pneumoconiosis, yellowish-brown particles 2–4 µm in size (birefringent) in the lumen of many alveoli and small bronchi, in many histiocytes in the interstitium and peribronchial lymphatic tissue, focal accumulation of foamy pneumocytes in the alveoli, focal lymphohistiocytic infiltration; **410 mg/m³ and follow-up**: only body weights normalized, all local findings in the lungs and pneumoconiosis still noticeable	Ciba-Geigy 1979 b; ECHA 2017 b

GSD: geometric standard deviation; MMAD: mass median aerodynamic diameter

Tab. 5 Incidence (severity) of the findings in the respiratory tract of male Wistar rats after inhalation exposure for 5 days to Pigment Yellow 83 "transparent" or "opaque" (BASF SE 2018, 2019 a)

Exposure concentration [mg/m³] number of examined animals	**0 n = 3**	**3 n = 3**	**10 n = 3**	**30 n = 3**
	incidence (severity)	incidence (severity)	incidence (severity)	incidence (severity)
Pigment Yellow 83 "transparent"				
98.7% purity, produced 12/2014, primary particles about 37 nm, agglomerates, 94% < 3 µm aerodynamic size, MMAD 0.4–0.6 µm with GSD 2.9–3.8				
lungs		**NOAEC**		
alveolar histiocytosis and particles in the histiocytes	0 (–)	3 (1)	3 (2)	3 (3)
hypertrophy/hyperplasia in the bronchial epithelium	0 (–)	0 (–)	3 (1)	3 (2)
neutrophilic infiltration in the bronchi	0 (–)	0 (–)	1 (1)	3 (3)
particles in the histiocytes of the BALT	0 (–)	2 (2)	3 (3)	3 (3)
tracheobronchial lymph nodes				
particles in the histiocytes	0 (–)	not examined	not examined	3 (2)
animals in the observation group				
lungs				
alveolar histiocytosis and particles in the histiocytes	0 (–)	0 (–)	3 (1)	3 (2)
particles in the alveolar histiocytes	0 (–)	3[a)]	0 (–)	0 (–)
particles in the histiocytes of the BALT	0 (–)	3 (1)	3 (1)	3 (1)
Pigment Yellow 83 "opaque"				
99.7% purity, produced 10/2014, primary particles about 159 nm, agglomerates, MMAD 0.3–0.4 µm with a GSD 3.0–4.6				
lungs			**NOAEC**	
alveolar histiocytosis and particles in the histiocytes	0 (–)	0 (–)	2 (1) 1 (2)	3 (2)
particles in the histiocytes	0 (–)	3[a)]	0 (–)	0 (–)
hypertrophy/hyperplasia in the bronchial epithelium	0 (–)	0 (–)	0 (–)	2 (1)
particles in the histiocytes of the BALT	0 (–)	0 (–)	3 (1)	3 (1)
tracheobronchial lymph nodes				
particles in the histiocytes	0 (–)	not examined	not examined	3 (2)
animals in the observation group				
lungs				
alveolar histiocytosis and particles in the histiocytes	0 (–)	0 (–)	3 (1)	1 (1) 2 (2)
particles in the alveolar histiocytes	0 (–)	3[a)]	0 (–)	0 (–)
particles in the histiocytes of the BALT	0 (–)	3 (1)	2 (1)	3 (1)

[a)] "present", no data for severity

BALT: bronchus-associated lymphoid tissue; GSD: geometric standard deviation; MMAD: mass median aerodynamic diameter

5.2.2 Oral administration

A number of studies that investigated the oral exposure of rats and mice for periods of up to 2 years did not reveal systemic effects that were attributable to the substance (see Table 6).

In a combined study of toxicity and developmental toxicity carried out according to OECD Test Guideline 422, groups of 10 male and 10 female Wistar rats were given gavage doses of Pigment Yellow 12 in polyethylene glycol 400 of 0, 50, 200 or 1000 mg/kg body weight and day; the males were exposed for 4 weeks, the females for 7 weeks. Exposure of the animals began 2 weeks before mating; exposure of the females continued up to lactation day 6. All animals, including the animals of the control group, developed diarrhoea. Diarrhoea occurs frequently after administration of polyethylene glycol and is therefore not considered an effect induced by Pigment Yellow 12. In all groups that were exposed to the pigment there were a number of animals with faeces that were yellowish-green and body parts that were yellow. Lethargy, hunched posture, chromodacryorrhea (red tears), laboured breathing, salivation, hair loss and reddish-brown faeces were observed in isolated cases; however, these were not found to be dependent on the dose. No substance-related effects were determined by clinical observation, functional observational battery, the analysis of body weight gains, feed consumption and organ weights, clinical pathology, gross pathology and histopathological examination. The NOAEL (no observed adverse effect level) was the highest dose tested of 1000 mg/kg body weight and day (NOTOX B.V. 2001).

In an 8-week range-finding study for a carcinogenicity study, groups of 5 male and 5 female F344 rats or B6C3F1 mice were given Pigment Yellow 12 in concentrations of 0, 300 (only mice), 1000, 3000, 10 000 or 30 000 mg/kg feed (0, about 90, 270, 900, 2700 mg/kg body weight and day for rats, conversion factor 0.09 according to EFSA (2012); 0, about 60, 200, 600, 2000, 6000 mg/kg body weight and day for mice, conversion factor 0.2 according to EFSA (2012)). No effects on feed consumption, body weight gains and mortality were observed and no substance-related effects were found during the pathological examination of the organs and tissues. The animals appeared yellow in colour. The mucous membranes of the gastrointestinal tract likewise appeared yellow as a result of contact with the pigment (NTP 1978).

As the range-finding study did not find substance-related effects in F344 rats and B6C3F1 mice, the carcinogenicity study was carried out in rats and mice up to the highest concentration recommended by the test guideline of 5% Pigment Yellow 12 or 50 000 mg/kg feed. Groups of 50 male and 50 female animals were given Pigment Yellow 12 in concentrations of 0, 25 000 or 50 000 mg/kg feed (rats: about 0, 1250, 2500 mg/kg body weight and day; mice: about 0, 3750, 7500 mg/kg body weight and day) for 78 weeks and then observed for 28 weeks (rats) or 19 weeks (mice). All treated animals appeared yellow in colour. The conjunctivae were likewise faintly yellow and most organs and internal mucosal surfaces were yellow in colour. In the mice, the body weight gains were reduced in the exposed animals from week 36 onwards (see Table 6). In the treated rats, the number of basophilic cells in the liver was increased (♂: 0/50, 5/49, 11/50; ♀: 2/49, 42/49, 40/48 (in each case: 0, medium, high dose)). No other substance-related findings were observed in the rats or mice (NTP 1978). The reduced body weight gains observed in the mice at doses of 3750 mg/kg body weight and day and above are not regarded as effects specifically induced by the substance because 2.5% to 5% of the feed consisted of pigment, which does not have any nutritional value and is excreted in unchanged form.

In a carcinogenicity study, groups of 50 male and 50 female NMRI mice were given Pigment Yellow 12 or 83 with the feed for 104 weeks in concentrations of 0, 1000, 3000 or 9000 mg/kg feed. The amounts absorbed were: Pigment Yellow 12 in doses of 0, ♂ 214 and ♀ 219, ♂ 649 and ♀ 681 or ♂ 1957 and ♀ 2030 mg/kg body weight and day and Pigment Yellow 83 in doses of 0, ♂ 213 and ♀ 210, ♂ 652 and ♀ 642 or ♂ 1936 and ♀ 1961 mg/kg body weight and day. No systemic or local effects were observed (Leuschner 1978).

Summary: Rats and mice given repeated oral doses of Pigment Yellow 12 and 83 from different batches over a period of up to 2 years did not exhibit any substance-related effects. The highest administered doses were 2500 mg/kg body weight and day in rats and 7500 mg/kg body weight and day in mice, in each case over a period of 2 years.

Tab. 6 Effects of Pigment Yellow 12 and 83 after repeated oral administration

Species, strain, number per group	Exposure	Findings	References
rat, Wistar, 10 ♂, 10 ♀	**Pigment Yellow 12**, OECD Test Guideline 422, 4 (♂) – 7 (♀) weeks, 0, 50, 200, 1000 mg/kg body weight and day in polyethylene glycol 400, gavage	**1000 mg/kg body weight**: **NOAEL**	NOTOX B.V. 2001
rat, no other details	**Pigment Yellow 12**, 30 days, 0%, 0.2%, 1%, 5% in the feed (0, 80, 400, 2000 mg/kg body weight and day)	**2000 mg/kg body weight**: **NOAEL**	OECD 2003
rat, F344, 5 ♂, 5 ♀	**Pigment Yellow 12**, 8 weeks, 0%, 0.1%, 0.3%, 1%, 3% in the feed (0, 1000, 3000, 10 000, 30 000 mg/kg feed; 0, about 90, 270, 900, 2700 mg/kg body weight and day[a])	**all doses**: yellow fur, yellow faeces, yellow mucous membranes in the gastrointestinal tract; **2700 mg/kg body weight**: **NOAEL**	NTP 1978
rat, F344, 50 ♂, 50 ♀	**Pigment Yellow 12**, 78 weeks and observation for 28 weeks, 0%, 2.5%, 5% in the feed (0, 25 000, 50 000 mg/kg feed; 0, about 1250, 2500 mg/kg body weight and day[b])	**all doses**: yellow fur, yellow faeces, yellowish conjunctiva, yellowish internal mucous membranes; all doses: dose-dependent increase in the number of basophilic cells in the liver: ♂: 0/50, 5/49, 11/50; ♀: 2/49, 42/49, 40/48; **2500 mg/kg body weight**: **NOAEL**	NTP 1978
rat, Sprague Dawley, 50 ♂, 50 ♀	**Pigment Yellow 12**, 104 weeks, 0, 68, 205, 630 mg/kg body weight and day, with the feed	**68 mg/kg body weight and above**: yellow faeces; **630 mg/kg body weight**: **NOAEL**, after 6 and 23 months: no metabolites detected in the urine samples of 10 animals per sex	Leuschner 1978
rat, Sprague Dawley, 50 ♂, 50 ♀	**Pigment Yellow 83**, 104 weeks, 0, 68, 205, 630 mg/kg body weight and day, with the feed	**68 mg/kg body weight and above**: yellow faeces; **630 mg/kg body weight**: **NOAEL**, after 6 and 23 months: no metabolites detected in the urine samples of 10 animals per sex	Leuschner 1978
rat, Sprague Dawley, 50 ♂, 50 ♀	**Pigment Yellow 83**, containing 3,3′-dichlorobenzidine in a concentration of 20 mg/kg, 104 weeks, 0, 68, 205, 630 mg/kg body weight and day, with the feed	**68 mg/kg body weight and above**: yellow faeces; **630 mg/kg body weight**: **NOAEL**, after 6 and 23 months: 3,3′-dichlorobenzidine detected in the urine samples of 10 animals per sex	Leuschner 1978
mouse, B6C3F1, 5 ♂, 5 ♀	**Pigment Yellow 12**, 8 weeks, 0%, 0.03%, 0.1%, 0.3%, 1%, 3% in the feed (0, about 60, 200, 600, 2000, 6000 mg/kg body weight and day[c])	**all doses**: yellow fur, yellow faeces, yellow mucous membranes in the gastrointestinal tract; **6000 mg/kg body weight**: **NOAEL**	NTP 1978
mouse, B6C3F1, 50 ♂, 50 ♀	**Pigment Yellow 12**, 78 weeks and observation for 19 weeks, 0%, 2.5%, 5% in the feed (0, 25 000, 50 000 mg/kg feed; 0, about 3750, 7500 mg/kg body weight and day[d])	**all doses**: yellow fur, yellow faeces, yellowish conjunctiva, yellowish internal mucous membranes; **3750 mg/kg body weight and above**: body weight gains decreased from week 36 onwards, at the end of the study: body weights decreased by about 12% (♀) and 7% (♂) – based on data from figure; **7500 mg/kg body weight**: at the end of the study: body weights decreased by about 25% (♀) and 13% (♂) – based on data from figure	NTP 1978

Tab. 6 *(continued)*

Species, strain, number per group	Exposure	Findings	References
mouse, NMRI, 55 ♂, 55 ♀	**Pigment Yellow 12**, 104 weeks, 0, 215, 650, 1960 mg/kg body weight and day, with the feed	**215 mg/kg body weight and above**: yellow faeces; **1960 mg/kg body weight**: **NOAEL**, after 6 and 23 months: no metabolites detected in the urine samples of 10 animals per sex	Leuschner 1978
mouse, NMRI, 55 ♂, 55 ♀	**Pigment Yellow 83**, 104 weeks, 0, 215, 650, 1960 mg/kg body weight and day, with the feed	**215 mg/kg body weight and above**: yellow faeces; **1960 mg/kg body weight**: **NOAEL**, after 6 and 23 months: no metabolites detected in the urine samples of 10 animals per sex	Leuschner 1978
mouse, NMRI, 55 ♂, 55 ♀	**Pigment Yellow 83**, containing 3,3′-dichlorobenzidine in a concentration of 20 mg/kg, 104 weeks, 0, 215, 650, 1960 mg/kg body weight and day, with the feed	**215 mg/kg body weight and above**: yellow faeces; **1960 mg/kg body weight**: **NOAEL**, after 6 and 23 months: 3,3′-dichlorobenzidine detected in the urine samples of 10 animals per sex	Leuschner 1978

[a] conversion factor 0.09 according to EFSA (2012)
[b] conversion factor 0.05 according to EFSA (2012)
[c] conversion factor 0.2 according to EFSA (2012)
[d] conversion factor 0.15 according to EFSA (2012)

5.2.3 Dermal application

No studies are available that are considered relevant for the evaluation.

The yellow pigments are used also in tattoo inks. However, the pigments are neither produced nor recommended for such use. They can be broken down by photodegradation to form carcinogenic amines such as 3,3′-dichlorobenzidine (NICNAS 2017).

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

Studies of the effects induced in the skin of rabbits found that Pigment Yellow 12, Pigment Yellow 13 and Pigment Yellow 83 did not cause irritation or, at most, only slight irritation. Erythema developed after occlusive application, but had regressed by the end of the observation period (Table 7).

Tab. 7 Effects of formulations of Pigment Yellow 12, 13 and 83 on the skin of rabbits

Strain, number per group, method	Dose or concentration	Findings and evaluation	References
Pigment Yellow 12			
Himalayan, 6, no other details	0.5 ml of a 5% or 10% solution in sesame oil, 24 hours, occlusive	single: 5% solution: tolerated without symptoms, 10% solution: 3 animals with very mild erythema, 2 animals with very mild oedema (index 0.42); 5 doses: 5% solution: tolerated without symptoms, 10% solution: from day 3 onwards, slight redness and desquamation observed in all animals, **slightly irritating**	Hoechst AG 1975 b

Tab. 7 *(continued)*

Strain, number per group, method	Dose or concentration	Findings and evaluation	References
Himalayan, 6, no other details	500 mg undiluted, 24 hours, occlusive	no evaluation possible because of interference from dye after 24 hours, no findings after 48 and 72 hours, **not irritating**	Hoechst AG 1975 b
New Zealand White, 6, no other details	500 mg undiluted, made into a paste with polyethylene glycol, 24 hours, occlusive	irritation index 2.7; skin dry, chapped, hardened, surface cracked after 48 and 72 hours, **slightly irritating to the skin**	Hoechst AG 1982 c
New Zealand White, 6, no other details	500 mg undiluted, made into a paste with polyethylene glycol, 24 hours, occlusive	irritation index 2.5; skin dry, chapped, hardened after 48 and 72 hours **slightly irritating to the skin**	Hoechst AG 1982 d
New Zealand White, 3, no other details, similar to OECD Test Guideline 404	per dressing 500 mg undiluted, made into a paste with polyethylene glycol, 4 hours, occlusive	no oedema; erythema and scabbing 0.9 of 4, all findings reversible after 7 days, **not irritating**	Hoechst AG 1985 b
New Zealand White, 3, no other details, similar to OECD Test Guideline 404	per dressing 500 mg undiluted, made into a paste with polyethylene glycol, 4 hours, occlusive	erythema and scabbing 1.6 of 4, after 72 hours and 7 days: skin dry, chapped, hardened, oedema 0.6 of 4, all findings reversible after 7 days, **not irritating**	Hoechst AG 1988 b
New Zealand White, 3, no other details, similar to OECD Test Guideline 404	per dressing 500 mg undiluted, made into a paste with isotonic saline solution, 4 hours, occlusive	erythema maximally 1.7, 1.7, 1.5 of 4, oedema 0 of 4, skin dry, chapped, hardened, all findings reversible after 14 days, **not irritating**	Hoechst AG 1995 a
Pigment Yellow 13			
New Zealand White, 6, no other details	per dressing 500 mg undiluted, 24 hours, occlusive	no evaluation possible because of interference from dye after 24 hours, no symptoms of irritation after 48 hours, **not irritating**	Hoechst AG 1977 c
New Zealand White, 6, no other details	per dressing 500 mg undiluted, made into a paste with polyethylene glycol, 24 hours, occlusive	maximum irritation index 1.1, **not irritating**	Hoechst AG 1982 e
New Zealand White, 3, no other details, similar to OECD Test Guideline 404	per dressing 500 mg undiluted, made into a paste with physiological saline solution, 4 hours, occlusive, neutral pH in water	1 animal: very mild erythema after 1 hour, no other findings, **not irritating**	Hoechst AG 1989 c
Pigment Yellow 83			
Himalayan, 6, no other details	per dressing 500 mg undiluted–yellow powder, 24 hours, occlusive	all animals: irritation index 0, **not irritating**	Hoechst AG 1976 e
New Zealand White, 6, no other details	per dressing 500 mg undiluted–yellow powder, 24 hours, occlusive	no evaluation possible because of interference from dye after 24 hours, no symptoms of irritation, **not irritating**	Hoechst AG 1977 d
Himalayan, 6, no other details	0.5 ml of liquid substance, 24 hours, occlusive, yellow paste	maximum irritation index 0.4, **not irritating**	Hoechst AG 1980 b

Tab. 7 *(continued)*

Strain, number per group, method	Dose or concentration	Findings and evaluation	References
New Zealand White, 3, no other details, similar to OECD Test Guideline 404	per dressing 500 mg undiluted, made into a paste with polyethylene glycol – yellow powder, 4 hours, occlusive	erythema maximally 1.7 of 4, oedema maximally 0.3 of 4, **slightly irritating**	Hoechst AG 1992 a
New Zealand White, 3, no other details, similar to OECD Test Guideline 404	per dressing 500 mg undiluted, made into a paste with polyethylene glycol – yellow powder, 4 hours, occlusive, neutral pH in water	erythema maximally 1.3 of 4, oedema maximally 0 of 4, **slightly irritating**	Hoechst AG 1996 a

5.3.2 Eyes

In most studies, no or very slight irritation was observed in the eyes of rabbits after exposure to Pigment Yellow 12, 13 and 83 (Table 8). Only formulations that contained 24% to 45% monoamines or diamines or 10% uric acid induced irritation or corrosive effects. In these cases, the additives were considered responsible for these effects (Hoechst AG 1988 a, 1989 a, 1992 b, 1995 b, 1996 b; NICNAS 2017; OECD 2003).

Tab. 8 Effects of formulations of Pigment Yellow 12, 13 and 83 in the eyes of rabbits

Strain, number per group, method	Dose or concentration	Findings and evaluation	References
Pigment Yellow 12			
Himalayan, 8, no other details	0.1 cm^3 undiluted in 1 eye of each animal, 3 animals: rinsed out after 5 minutes; 5 animals: rinsed out after 24 hours	in all animals: slight conjunctival redness (grade 1 of 3), in 5 animals: slight conjunctival swelling (grade 1 of 4), all animals symptom-free after 72 hours, **not irritating**	Hoechst AG 1975 b
Himalayan, 6, no other details	100 mg undiluted, 0.1 ml 10% in sesame oil	undiluted dye: 24-hour irritation index: 58.6 of 110, 72-hour irritation index: 100 of 110, **severely irritating** 10% solution: maximum irritation index: 2.6 of 110 after 1 hour, **not irritating**	Hoechst AG 1976 d
New Zealand White, 6, no other details	100 mg undiluted, made into a paste with polyethylene glycol	maximum irritation index: 14 of 110 after 7 hours, **slightly irritating**	Hoechst AG 1982 c
New Zealand White, 6, no other details	100 mg undiluted, made into a paste with polyethylene glycol, after 24 hours rinsed with physiological saline solution	maximum irritation index: 15 of 110 after 7 hours, **slightly irritating**	Hoechst AG 1982 d
New Zealand White, 3, no other details, OECD Test Guideline 405	100 mg undiluted, made into a paste with polyethylene glycol, eye rinsed after 24 hours	mean values for all animals: corneal opacity 0 of 4, iritis 0.1 of 2, conjunctival swelling 0.9 of 4, conjunctival redness 1.7 of 3, **not irritating**	Hoechst AG 1985 a
Pigment Yellow 13			
New Zealand White, 6, no other details	100 mg undiluted	maximum irritation index 10 of 110 after 1 and 7 hours, **not irritating**	Hoechst AG 1977 c
New Zealand White, 6, no other details	100 mg undiluted, made into a paste with polyethylene glycol	maximum irritation index 10 of 110 after 1 hour, **not irritating**	Hoechst AG 1982 e

Tab. 8 *(continued)*

Strain, number per group, method	Dose or concentration	Findings and evaluation	References
New Zealand White, 3, no other details, OECD Test Guideline 405	100 mg undiluted, neutral pH in water	mean indices for all animals: corneal opacity 0.0 of 4, iritis maximally 0.2 of 2, conjunctival redness maximally 1.3 of 3, conjunctival swelling maximally 0.7 of 4, **slightly irritating**	Hoechst AG 1989 b
Pigment Yellow 83			
Himalayan, 6, no other details	100 mg undiluted	maximum irritation index 14 of 110 after 7 hours, **slightly irritating**	Hoechst AG 1976 e
New Zealand White, 6, no other details	100 mg undiluted	maximum irritation index 16 of 110 after 1 hour, **slightly irritating**	Hoechst AG 1977 d
Himalayan, 6, no other details	0.1 ml liquid substance, eye rinsed after 24 hours	maximum irritation index 2 of 110 after 7 hours, **not irritating**	Hoechst AG 1980 b
New Zealand White, 3, no other details, OECD Test Guideline 405	100 mg undiluted–yellow powder, eye rinsed after 24 hours	marked redness and swelling after 1 hour, no findings detected after 48 hours, **not irritating**	Hoechst AG 1984 b

5.4 Allergenic effects

5.4.1 Sensitizing effects on the skin

In a local lymph node assay carried out in female CBA mice according to OECD Test Guideline 429, Pigment Yellow 13 was classified as not sensitizing. In practice, treatment of the animals with pigment concentrations of 2.5%, 5% and 10% in suspension in 3 different vehicles yielded the following stimulation indices: 1.7, 1.3 and 2.3 (propylene glycol); 1.1, 1.4 and 1.7 (ethanol/water (7:3)) and 1.5, 1.7 and 2.1 (acetone/olive oil (4:1)). Higher concentrations were not tested (ECHA 2021 b).

Pigment Yellow 83 yielded negative results in a local lymph node assay carried out according to OECD Test Guideline 429 in 4 female CBA mice per dose group. The treatment of the animals with pigment concentrations of 2.5%, 5% and 10% in suspension in ethanol/water (7:3, v/v) yielded stimulation indices of 1.1, 1.4 and 1.7; the highest attainable concentration was 10% in suspension (ECHA 2021 c).

A Buehler test carried out with Pigment Yellow 12 and 13 in female Hsd/Poc:DH guinea pigs likewise yielded negative results. The animals were treated for induction either with a 60% formulation of Pigment Yellow 12 or with a 75% formulation of Pigment Yellow 13. Corn oil was chosen as the vehicle. Groups of 20 animals were challenged with either 3%, 10%, 30% and 60% formulations (Pigment Yellow 12) or 3%, 10%, 30% and 75% formulations (Pigment Yellow 13) in the same vehicle; no reactions were observed (ECHA 2021 a, b).

5.4.2 Sensitizing effects on the airways

There are no data available.

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

The combined study of toxicity and developmental toxicity described in Section 5.2.2 was carried out in Wistar rats according to OECD Test Guideline 422 with gavage doses of Pigment Yellow 12 in polyethylene glycol 400 of 0, 50, 200 or 1000 mg/kg body weight and day. The NOAEL for the parent animals was the highest dose tested of 1000 mg/kg body weight and day. All animals, including the animals of the control group, developed diarrhoea. As diarrhoea

occurs in most animals given polyethylene glycol, this is not considered an effect induced by Pigment Yellow 12. As no effects on reproduction and the number of offspring were observed, the NOAEL for maternal toxicity and fertility was the highest dose tested of 1000 mg/kg body weight and day (NOTOX B.V. 2001).

No effects on the reproductive organs were found in studies investigating repeated exposure (OECD 2003).

5.5.2 Developmental toxicity

In the combined study of toxicity and developmental toxicity described in Section 5.2.2, the NOAEL for the dams and for developmental toxicity induced by Pigment Yellow 12 was determined to be the highest dose tested of 1000 mg/kg body weight and day. No effects on the number of offspring, weight, sex ratio, survival and the development of the offspring were observed. The foetuses of the high dose group appeared yellow in colour, an effect that was caused by contamination during necropsy (NOTOX B.V. 2001). However, the study protocol included only an examination of the offspring for external changes and not for visceral and skeletal variations/malformations. For this reason, these types of studies are not suitable for the evaluation of developmental toxicity. However, all 3 pigments were found to be insoluble and not bioavailable.

Systemic availability in the foetus after oral administration of nano materials to the mother animal

The pigments may contain particles smaller than 100 nm in size and therefore the data for nanomaterials are likewise taken into consideration.

Two reviews reported teratogenic effects induced by multi-walled and single-walled carbon nanotubes and TiO_2-nanoparticles administered to mice during early gestation. The effects may have been transmitted via the yolk sac. Prenatal administration of several different nanomaterials led to an increase in the number of reactive oxygen species in the placenta in addition to histological and functional abnormalities, increased prenatal and postnatal mortality, delays in growth, changes in behaviour, increased sensitivity for allergies and other effects (Ema et al. 2010, 2016). Both reviews evaluated a number of studies that used non-standardized tests. In 1 example taken from the reviews, CD1 mice (TiO_2: 11 to 14 animals per group; silver: 12 to 18 animals per group) were given either TiO_2 (50 nm) or silver nanoparticles (20 nm) by gavage on gestation day 9 at doses of 0, 10, 100 or 1000 mg/kg body weight. The examination carried out on gestation day 19 after caesarean section found an increased percentage of non-viable foetuses at a TiO_2 dose of 1000 mg/kg body weight (7.6%; controls: 1.7%). An increased number of 'morphological defects' were observed in the foetuses at TiO_2 doses of 100 mg/kg body weight and above (percentage of foetuses with effects: controls: 0%; 10 mg/kg body weight: 2.2%; 100 mg/kg body weight: 5.3%; 1000 mg/kg body weight: 2.5%). The defects were pooled and included exencephaly (6 foetuses), open eyelids (7 foetuses), leg defects (2 foetuses) and tail defects (1 foetus). These types of effects were not observed in the groups exposed to the silver nanoparticles. Treatment with either of the 2 substances did not lead to adverse effects on litter size, maternal and foetal body weights or the number of resorptions (Philbrook et al. 2011). The evaluation was not based on foetuses or litters and was not described in detail; the number of animals tested was smaller than the number required by the OECD test guideline.

However, there are 2 prenatal developmental toxicity studies available that were carried out according to OECD Test Guideline 414. In these studies, no effects of maternal or developmental toxicity were observed in rats given 3 different types of ultrafine titanium dioxide particles or nanostructured amorphous silica by gavage up to the limit dose of 1000 mg/kg body weight and day (Hofmann et al. 2015; Warheit et al. 2015; Table 9). The size of the primary particles of all 3 types of titanium dioxide tested (42 to 47 nm) and the shape of the primary particles of one of the titanium dioxide types (rod-shaped) are similar to those of Pigment Yellow 83 "transparent" (median size: 37.0 nm; range: 19.7 to 63.8 nm; see Section 3.1).

Summary: Studies with nanoparticles of other substances that were carried out according to OECD Test Guideline 414 did not lead to developmental toxicity.

Tab. 9 Developmental toxicity studies with nanomaterials carried out according to OECD Test Guideline 414

Substance	Species	Exposure	Findings	References
titanium dioxide: uf-1: 89% anatase and 11% rutile, size: 43 nm, shape: irregular, agglomeration index (10 mg/ml; 200 mg/ml): 42, 41 uf-2: 100% anatase, size: 42 nm, shape: irregular, agglomeration index: 9.5, 6.2 uf-3: 100% rutile, size: 47 nm, shape: rod-shaped, agglomeration index: 52, 53	**rat**, Wistar and Crl:CD(SD), groups of 22 ♀	GD 5–19 (Wistar), GD 6–20 (SD), OECD Test Guideline 414, 0, 100, 300, 1000 mg/kg body weight and day, gavage, vehicle: sterile water, examination on GD 21	**1000 mg/kg body weight**: **NOAEL** maternal and developmental toxicity; statistically significant effects not found at any dose, no teratogenicity, other studies with rats found slight or no absorption of TiO_2 from the gastrointestinal tract	Warheit et al. 2015
synthetic amorphous silica: 96.5% silica, size of the primary particles: 10–25 nm, addition of 10% foetal calf serum to prevent agglomeration, determined particle sizes: 40 nm–3000 nm (agglomerates)	**rat**, Wistar (Crl:WI[Han]), groups of 25 ♀	GD 6–19, OECD Test Guideline 414, 0, 100, 300, 1000 mg/kg body weight and day, gavage, vehicle: highly deionised water, examination on GD 20	**1000 mg/kg body weight**: **NOAEL** maternal and developmental toxicity; statistically significant effects not found at any dose, no teratogenicity, from a kinetics study with rats given a single oral dose of colloidal SiO_2 particles of 500 or 1000 mg/kg body weight and day: oral absorption 6.6%–9.7%	Hofmann et al. 2015

agglomeration index: the higher the index, the more extensive the agglomeration; GD: gestation day

5.6 Genotoxicity

5.6.1 In vitro

The studies are shown in Table 10. Pigment Yellow 12, 13 and 83 did not cause mutations in the Salmonella typhimurium strains TA98, TA100, TA102, TA1535 and TA1537 or in Escherichia coli either with or without metabolic activation from the hamster or rat liver. Negative results were likewise obtained with the Prival modifications used specifically for azo dyes. In the comet assay, Pigment Yellow 12 led to an increase in DNA strand breaks in freshly isolated rat hepatocytes at 20 µg/ml. Pigment Yellow 12 and Pigment Yellow 83 did not induce chromosomal aberrations in CHO cells (a cell line derived from Chinese hamster ovary). However, no cytotoxicity data were provided. Pigment Yellow 12, Pigment Yellow 13 and Pigment Yellow 83 yielded negative findings in the $TK^{+/-}$ mutation test in mouse lymphoma cells. Again, no cytotoxicity data were provided (ECHA 2017 a, b, c; NICNAS 2017; OECD 2003). The pigment particles in the hepatocyte solution may have influenced the results of the comet assay. Additionally, the test method had not yet been fully validated. As a result, the findings of the study are of only limited relevance for the evaluation (OECD 2003).

Tab. 10 Genotoxicity of Pigment Yellow 12, 13 and 83 in vitro

End point	Test system	Concentration [µg/plate][a)]	Effective concentration[a)]	Cytotoxicity[a)]	Findings		References
					−m. a.	+m. a.	
gene mutation	**Pigment Yellow 12** Salmonella typhimurium TA98, TA100, TA1535, TA1537	0, 100, 333, 1000, 10 000 ± S9 from rat and hamster liver treated with Aroclor	–	–	–	–	ECHA 2017 a; Zeiger et al. 1987
	Pigment Yellow 12 Salmonella typhimurium TA98, TA100, TA1535, TA1537, Prival modification	0, 3–5000 ± S9 from untreated hamster liver; precipitation at 1000 and above, but evaluation possible	–	–	–	–	ECHA 2017 a

Tab. 10 *(continued)*

End point	Test system	Concentration [µg/plate][a)]	Effective concentration[a)]	Cytotoxicity[a)]	Findings		References
					−m. a.	+m. a.	
	Pigment Yellow 12 Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	0, 8–5000 or 4–2500 ± S9 from untreated rat liver	–	–	–	–	Hoechst AG 1981 a, b, c, 1982 g
	Pigment Yellow 12 Salmonella typhimurium TA98, TA100, TA1535, TA1537, Prival modification	0, 3–5000 ± S9 from untreated hamster liver; precipitation at 1000 and above, but evaluation possible	–	–	–	–	ECHA 2017 a
	Pigment Yellow 12 Salmonella typhimurium TA98, TA100, TA102, TA1535, TA1537, Prival modification	0, 50–5000 ± S9 from untreated rat liver; precipitation, but evaluation possible	–	–	–	–	ECHA 2017 a
	Pigment Yellow 12 Escherichia coli WP2 uvr A, Prival modification	0, 3–5000 ± S9 from untreated hamster liver, precipitation at 1000 and above, but evaluation possible	–	–	–	–	ECHA 2017 a
	Pigment Yellow 12 Escherichia coli WP2 uvr A	0, 8–5000 or 4–2500 ± S9 from untreated rat liver	–	–	–	–	Hoechst AG 1981 a, b, c
	Pigment Yellow 12 Escherichia coli WP2 uvr A, Prival modification	0, 3–5000 ± S9 from untreated hamster liver, precipitation at 1000 and above, but evaluation possible	–	–	–	–	ECHA 2017 a
	Pigment Yellow 13 Salmonella typhimurium TA98, TA100, TA1535, TA1537	0, 100, 333, 1000, 10 000 ± S9 from rat and hamster liver treated with Aroclor	–	–	–	–	ECHA 2017 b; Zeiger et al. 1987
	Pigment Yellow 13 Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	0, 4–2500 ± S9 from untreated rat liver	–	–	–	–	Hoechst AG 1982 f
	Pigment Yellow 13 Salmonella typhimurium TA98, TA100, TA1535, TA1537, Prival modification	0, 3–5000 ± S9 from untreated hamster liver	–	–	–	–	ECHA 2017 b
	Pigment Yellow 13 Escherichia coli WP2 uvr A, Prival modification	0, 3–5000 ± S9 from untreated hamster liver	–	–	–	–	ECHA 2017 b
	Pigment Yellow 13 Escherichia coli WP2 uvr A	0, 4–2500 ± S9 from untreated rat liver	–	–	–	–	Hoechst AG 1982 f

Tab. 10 *(continued)*

End point	Test system	Concentration [µg/plate][a)]	Effective concentration[a)]	Cytotoxicity[a)]	Findings		References
					−m. a.	+m. a.	
	Pigment Yellow 83 Salmonella typhimurium TA98, TA100, TA1535, TA1537	0, 50–5000 ± S9 from rat and hamster liver treated with Aroclor, precipitation, but evaluation possible	–	–	–	–	ECHA 2017 c
	Pigment Yellow 83 Salmonella typhimurium TA98, TA100, TA1535, TA1537	0, 100, 333, 1000, 10 000 ± S9 from rat and hamster liver treated with Aroclor, precipitation at all concentrations, but evaluation possible	–	–	–	–	ECHA 2017 c; Haworth et al. 1983; Zeiger et al. 1987
	Pigment Yellow 83 Salmonella typhimurium TA98, TA100, TA1535, TA1537	0, 3–5000 ± S9 from untreated hamster liver or treated rat liver, precipitation at 1000 and above, but evaluation possible	–	–	–	–	ECHA 2017 c
	Pigment Yellow 83 Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	0, 8–5000 ± S9 from untreated rat liver	–	–	–	–	Hoechst AG 1981 b
	Pigment Yellow 83 Salmonella typhimurium TA98, TA100, TA1535, TA1537, Prival modification	0, 3–5000 ± S9 from untreated hamster liver or treated rat liver, precipitation at 333 and above, but evaluation possible	–	TA1535 +m. a.: 5000, TA1537 +m. a.: 1000	–	–	ECHA 2017 c
	Pigment Yellow 83 Escherichia coli WP2 uvr A	0, 3–5000 ± ± S9 from untreated hamster liver or treated rat liver, precipitation at 1000 and above, but evaluation possible	–	–	–	–	ECHA 2017 c
	Pigment Yellow 83 Escherichia coli WP2 uvr A	0, 8–5000 ± ± S9 from untreated rat liver	–	–	–	–	Hoechst AG 1981 b
	Pigment Yellow 83 Escherichia coli WP2 uvr A, Prival modification	0, 3–5000 ± ± S9 from untreated hamster liver or treated rat liver; precipitation at 333 and above, but evaluation possible	–	–	–	–	ECHA 2017 c
DNA strand breaks (comet assay)	**Pigment Yellow 12** rat hepatocytes	0, 10, 20 µg/ml 40 minutes incubation	20 µg/ml	–	not tested	+ at 20 µg/ml	Møller 2001; Møller et al. 1998
chromosomal aberrations	**Pigment Yellow 12** CHO cells	0, 1.6–50 µg/ml	–	no data	–	–	ECHA 2017 a
	Pigment Yellow 83 CHO cells	−m. a.: 0, 1.6–160 µg/ml +m. a.: 0, 0.5–50 µg/ml ± S9 from ♂ rat liver treated with Aroclor, −m. a. 8–12 hours, +m. a. 2 hours exposure, 10 hours growth, 2 hours selection, 12 hours fixation	–	no data	–	–	ECHA 2017 c

Tab. 10 *(continued)*

End point	Test system	Concentration [µg/plate][a)]	Effective concentration[a)]	Cytotoxicity[a)]	Findings		References
					–m. a.	+m. a.	
gene mutations in mammalian cells	**Pigment Yellow 12** $TK^{+/-}$, mouse lymphoma cells L5178Y	–m. a.: 0, 0.0312–0.5 µg/ml +m. a.: 0, 0.1–0.5 µg/ml ± S9 from rat liver treated with Aroclor, 4 hours exposure, 48 hours growth, 10–12 days selection	–	–	–	–	ECHA 2017 a
	Pigment Yellow 12 $TK^{+/-}$, mouse lymphoma cells L5178Y	0, 62.5–1000 µg/ml ± S9 from rat liver treated with Aroclor; precipitation at 62.5 and above, but evaluation possible	–	–	–	–	ECHA 2017 a
	Pigment Yellow 13 $TK^{+/-}$, mouse lymphoma cells L5178Y	–m. a.: 0, 0.0312–0.5 µg/ml +m. a.: 0, 0.1–0.5 µg/ml S9 from rat liver treated with Aroclor, 4 hours exposure, 48 hours growth, 10–12 days selection	–	–	–	–	ECHA 2017 b
	Pigment Yellow 13 $TK^{+/-}$, mouse lymphoma cells L5178Y	0, 62.5–1000 µg/ml ± S9 from rat liver treated with Aroclor; precipitation at 62.5 and above, but evaluation possible; 3 hours exposure, 24 hours growth, 10–14 days selection	–	–	–	–	ECHA 2017 b
	Pigment Yellow 83 $TK^{+/-}$, mouse lymphoma cells L5178Y	–m. a.: 0, 0.0312–0.5 µg/ml +m. a.: 0, 0.1–0.5 µg/ml S9 from rat liver treated with Aroclor, 4 hours exposure, 48 hours growth, 10–12 days selection	–	–	–	–	ECHA 2017 c
	Pigment Yellow 83 $TK^{+/-}$, mouse lymphoma cells L5178Y	0, 62.5–1000 µg/l ± S9 from rat liver treated with Aroclor; precipitation at 62.5 and above, but evaluation possible; 3 hours exposure, 24 hours growth, 10–14 days selection	–	–	–	–	ECHA 2017 c

[a)] unless specified otherwise, concentrations expressed as [µg/plate]
–m. a.: without the addition of a metabolic activation system; +m. a.: with the addition of a metabolic activation system

5.6.2 In vivo

Pigment Yellow 12

In a poorly documented study, sister chromatid exchange was not observed in the bone marrow of groups of 5 or 6 male Balb-c mice given single intraperitoneal injections of Pigment Yellow 12 or Pigment Yellow 83 at doses of 5, 10, 50, 100, 200 or 400 mg/kg body weight. Data were not provided for the purity of the substance and the solvent control (dimethyl sulfoxide). A negative control was not included; the positive control yielded the expected results. Only 20 instead of 50 metaphases per animal were analysed (ECHA 2017 a).

In a micronucleus test carried out in 1994 according to OECD Test Guideline 474, groups of 5 male and 5 female ICR mice were given single intraperitoneal injections of Pigment Yellow 12 at dose levels of 1250, 2500 or 5000 mg/kg body weight. All animals were examined after 24 hours; the animals of the high dose group also after 48 and 72 hours. However, only 1000 instead of 2000 cells were evaluated. Clinical signs of toxicity in the form of hunched posture and

lethargy were observed, but the incidence of micronuclei in the bone marrow was not increased. Likewise, the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) in the exposed animals did not differ with statistical significance from the ratios determined for the controls (ECHA 2017 b). It is unclear whether the substance reached the bone marrow.

Pigment Yellow 13

In a micronucleus test carried out in vivo according to OECD Test Guideline 474, groups of 5 male and 5 female ICR mice were given single intraperitoneal doses of Pigment Yellow 13 of 1250, 2500 or 5000 mg/kg body weight. Investigation of the bone marrow after 24, 48 or 72 hours yielded negative results. However, only 1000 instead of 2000 immature erythrocytes were evaluated per animal. No cytotoxicity data were provided (ECHA 2017 b). It is unclear whether the substance reached the bone marrow.

Pigment Yellow 83

In a micronucleus test carried out in 1994 according to OECD Test Guideline 474, groups of 5 male and 5 female ICR mice were given single intraperitoneal doses of Pigment Yellow 83 of 1250, 2500 or 5000 mg/kg body weight. All animals were examined after 24 hours; those in the high dose group also after 48 and 72 hours. Only 1000 instead of 2000 cells were evaluated per animal and no cytotoxicity data were provided. The incidence of micronuclei in the bone marrow was not increased. At 5000 mg/kg body weight, hunched posture was observed in 1 animal and another was lethargic. Other signs of toxicity were not observed. Likewise, the PCE to NCE ratio in the exposed animals did not differ with statistical significance from the ratios determined for the controls (ECHA 2017 c). It is unclear whether the substance reached the bone marrow.

Summary: Pigment Yellow 12, 13 and 83 administered by intraperitoneal injection did not induce micronuclei in the erythrocytes of mice. It is unclear whether the substances reached the bone marrow.

5.7 Carcinogenicity

5.7.1 Oral administration

In a carcinogenicity study, groups of 50 male and 50 female NMRI mice or 50 male and 50 female Sprague Dawley rats were given Pigment Yellow 12 or Pigment Yellow 83 in concentrations of 0, 1000, 3000 or 9000 mg/kg feed over a period of 104 weeks. The particles were in the range from 0.1 to 1 µm. Another group was given Pigment Yellow 83 to which 20 mg 3,3′-dichlorobenzidine/kg pigment had been added. Mice received average doses of 0, 215, 650 or 1960 mg/kg body weight and day and rats received average doses of 0, 68, 205 or 630 mg/kg body weight and day. The maximum doses of 3,3′-dichlorobenzidine absorbed from the Pigment Yellow 83 mixed with 3,3′-dichlorobenzidine were 0.04 mg/kg body weight and day in mice and 0.01 mg/kg body weight and day in rats. None of the studies reported an increased incidence of tumours or symptoms of toxicity (Leuschner 1978).

A carcinogenicity study was carried out in rats and mice up to the highest dose permitted by the test guideline of 5% in the feed (this is equivalent to a Pigment Yellow 12 concentration of 50 000 mg/kg feed). Groups of 50 male and 50 female rats or mice were given Pigment Yellow 12 with the feed in concentrations of 25 000 or 50 000 mg/kg (equivalent to Pigment Yellow 12 doses of 0, 1250 and 3750 or 2500 and 7500 mg/kg body weight and day, respectively; see Section 5.2.2) for 78 weeks and were observed for another 28 weeks (rats) or 19 weeks (mice). No local or systemic effects or increased tumour incidences were observed (NTP 1978).

5.7.1.1 3,3′-Dichlorobenzidine

Reports are available from long-term studies of 3,3′-dichlorobenzidine that were carried out from 1959 to 1978. Many of these studies tested only one dose. The different studies investigated different species and found different tumour localizations (see Table 11) (Henschler 1993; NTP 1990). These were not observed following oral exposure to the pigments.

Tab. 11 Carcinogenicity studies with 3,3′-dichlorobenzidene (Henschler 1993)

Species, number per group, administration route, dose	Tumour incidences of exposed animals (control animals)
CD rats, 50 ♂ and 50 ♀, **oral**, 0, 1000 mg/kg feed (about 50 mg/kg body weight and day)	mammary adenocarcinomas ♂: 7/44 (0/44), ♀: 26/44 (3/44); mammary fibroadenomas ♂: 4/44 (1/44), ♀: 7/44 (17/44); granulocytic leukaemia ♂: 9/44 (2/44), ♀: 0/44 (0/44); Zymbal gland carcinoma ♂: 8/44 (0/44), ♀: 1/44 (0/44)
rats, 35 ♂ and 15 ♀ (controls 130), 10–20 mg/day, **oral** (paste)	Zymbal gland 7/29 (0); mammary glands 7/29 (0); skin 3/29 (0); bladder 3/29 (0); haematopoietic organs 3/29 (0); connective tissue 2/29 (0); ileum 2/29 (0); salivary glands 2/29 (0), no other data
rats, 20 ♀ (controls 140 ♀), 30 mg 3,3′-dichlorobenzidene-dihydrochloride/day, **oral** (gavage), every 3rd day, 10 doses in all, administration for 1 month, observation for 9 months	14 surviving animals; mammary glands 0/20 (3/132), no other data
mice, 51 ♂ and 22 ♀, total dose 127–135 mg/animal; **oral** with the feed, 10 months	liver 4, lungs 1, no other data
mice, 18 animals, 12 months, **oral**, 0.1% in the feed	all 18 animals on average 18 hepatomas/animal (2/21 control animals 2 hepatomas each), no other data
beagle dogs, 6 ♀ (controls 6), **oral** (capsule), 100 mg 3 × per week and 5 × per week (9.1–12.8 mg/kg body weight/dose)	7 years: transitional cell carcinomas of the bladder 4/4 (0/6), hepatocellular carcinomas 3/4 (0/6), mammary adenocarcinomas and carcinosarcomas 0/4 (4/6), no other data
rats, 36 ♂ and 25 ♀ (controls 130), **subcutaneous** (paste), 0, 20–120 mg/animal and week, 6 months, then 20 mg/animal/week, total dose 1620–3000 mg/animal	Zymbal gland 10/35 (0); application site 7/35 (0); mammary glands 6/35 (0); skin 5/35 (0); connective tissue 2/35 (0); haematopoietic organs 2/35 (0); salivary glands 1/35 (0), no other data
mice 36 ♂ and 31 ♀, **subcutaneous**, 130 mg/animal; 8 ♂ and 15 ♀, 130, 265 mg/animal, no other details	130 mg/animal: application site 1, liver 5, lungs 2, no other data; 265 mg/animal: application site 2, liver 3, lungs 1, no other data

5.7.2 Inhalation

There are no carcinogenicity studies available that used inhalation exposure. As Pigment Yellow 12, 13 and 83 are insoluble in water and not absorbed, the particles could potentially induce local effects on the respiratory tract. This is supported by the findings of a 21-day inhalation study that detected local effects on the lungs (see Section 5.2.1).

6 Manifesto (MAK value/classification)

The particle size distribution of Pigment Yellow 12, 13 and 83 varies depending on the intended use and may extend into the ultrafine range below 100 nm (Schmidt et al. 2007). In technical applications, particles smaller than 100 nm are called nanoparticles. According to the manufacturer's information, the d50 values for Pigment Yellow 12 and 13 are in the range from 15 to 100 nm and the d50 values for Pigment Yellow 83 range from 15 to 250 nm (Verband der Mineralfarbenindustrie e.V. 2019). However, most study reports do not include data for particle size distribution or the associated agglomeration behaviour.

The critical effects induced by long-term inhalation exposure to the dusts of the water-insoluble Pigment Yellow 12, 13 and 83 are local effects on the respiratory tract.

MAK value. Pigment Yellow 12, 13 and 83 are insoluble in water and in phagolysosomal simulant fluid, which simulates the intracellular environment of the lungs. The pigments are thus not bioavailable and particle effects are expected to occur in the lungs after inhalation exposure.

In some cases, the findings observed after inhalation exposure to Pigment Yellow 13 for 21 days were similar to those characteristic for lung overload caused, for example, by biopersistent granular dusts (Hartwig 2014). A long-term

inhalation study of the pigments is not available. After long-term exposure at concentration levels that overload lung clearance, the particles are expected to cause inflammation, which in turn leads to carcinogenic effects. For this reason, the general threshold limit value for dust is applied for these pigments. However, because the pigments also occur in nanosizes and in the respective agglomerates, the value is corrected by the agglomerate density.

The solubility tests in phagolysosomal simulant fluid included the analysis of 2 Pigment Yellow 83 formulations by transmission electron microscopy (TEM). The particles were found to be rod-shaped with some having a cross-sectional diameter in the nanometre range and occurring in agglomerate form. The smooth, even surface morphology remained unchanged even after the solubility tests, which is evidence for the stability of the particle surface. The nanoscale and microscale pigments did not differ in this respect (BASF SE 2019 b). However, the increased agglomeration behaviour of the ultrafine particles and thus the agglomerate density is to be taken into consideration for the derivation of the limit value (Hartwig 2014). The limit value of micrometre-sized biopersistent granular dusts is based on a material density of 1 g/cm^3. As Pigment Yellow 12, 13 and 83 occur as agglomerates of ultrafine particles, however, the limit value is based on the agglomerate density. Assuming a minimal packing factor of 50% of the volume of an agglomerate unit (meaning that 50% of the volume of the agglomerate is filled with particles), the agglomerate density is equal to half of the material density and the MAK value is derived based on the equation: 0.3 mg/m^3 × (material density of the pigment × 0.5).

Taking into account that Pigment Yellow 12, 13 and 83 have a density of 1.39, 1.36 and 1.5 g/cm^3, respectively, and assuming a respiratory volume of 10 m^3 per working day and 100% deposition of the respirable fraction after inhalation, this would be equivalent to a lung burden of 2.1, 2.05 or 2.25 mg of pigment, respectively, per day.

Peak limitation. Like the biopersistent granular dusts, Pigment Yellow 12, 13 and 83 have been classified in Peak Limitation Category II with an excursion factor of 8.

Carcinogenicity. The water-insoluble Pigment Yellow 12, 13 and 83 were not absorbed after oral and intratracheal administration, they were not cleaved systemically and they yielded negative findings in oral carcinogenicity studies in 2 rat and 2 mouse strains. Carcinogenic effects are not expected to be induced by metabolism to 3,3′-dichlorobenzidine (see Section 3).

Two 21-day inhalation studies of Pigment Yellow 13 and two 5-day inhalation studies of Pigment Yellow 83 revealed effects in the lungs that are similar to the effects characteristic for biopersistent granular dusts (Hartwig 2014). A long-term inhalation study is not available. As the 3 pigments evaluated in this documentation are insoluble in water, a high particle burden over an extended period of time is expected to lead to carcinogenic effects in the respiratory tract resulting from inflammation. This has been demonstrated in rats with other biopersistent granular dusts. For this reason, in analogy to these dusts, Pigment Yellow 12, Pigment Yellow 13 and Pigment Yellow 83 have been classified in Carcinogen Category 4.

Dose comparison with 3,3′-dichlorobenzidine

A publication suggested that rats given Pigment Yellow 83 by intratracheal administration are able to metabolize at most 0.005 mole percent of the pigment to produce 3,3′-dichlorobenzidine in a 24-hour period (20 mg of Pigment Yellow 83 administered = 24 400 nmol Pigment Yellow 83; maximum amount excreted with the urine = 8.8 ng of 3,3′-dichlorobenzidine (BAuA 2001)). The findings of this study were later disproved by bioavailability studies; metabolism was not found to occur. Nevertheless, a comparative analysis is made using the above dose. As only 3% of 3,3′-dichlorobenzidine given by intratracheal administration is excreted with the urine in the form of 3,3′-dichlorobenzidine, 8.8 ng in the urine is equivalent to the formation of 293 ng of 3,3′-dichlorobenzidine after intratracheal administration of Pigment Yellow 83 = 1.2 nmol = 0.005 mole percent. Assuming that the same applies to humans, about 35 ng of 3,3′-dichlorobenzidine (molar mass of 3,3′-dichlorobenzidine: 253 g/mol, Pigment Yellow 83: 818 g/mol) would form for an exposure at the MAK value (maximum uptake: 2.25 mg Pigment Yellow 83). At 70 kg body weight, this would be equivalent to a 3,3′-dichlorobenzidine dose of 0.5 ng/kg body weight. A 20 000-fold 3,3′-dichlorobenzidine dose of 10 000 ng/kg body weight and day, administered as an impurity of Pigment Yellow 83, did not induce tumours in rats

(Leuschner 1978). However, the amount of 3,3′-dichlorobenzidine that could potentially form as a metabolic product of Pigment Yellow 83 was not taken into consideration (about 10 000 ng/kg body weight; in sum, therefore, a 30 000-fold dose of 3,3′-dichlorobenzidine). As the findings of a large number of studies do not provide any evidence of the metabolism to 3,3′-dichlorobenzidine (see Section 3), it is not assumed that carcinogenic effects develop by this mechanism.

Germ cell mutagenicity. There are no in vivo studies with germ cells available. The findings of mutagenicity studies in vitro were negative, as were the results of in vivo micronucleus tests in the bone marrow of mice after intraperitoneal administration. However, it was not possible to determine whether the bone marrow was reached. On the basis of the negative results of the in vitro and in vivo genotoxicity studies and the lack of bioavailability, Pigment Yellow 12, 13 and 83 have not been classified in any of the categories for germ cell mutagenicity.

Prenatal toxicity. No prenatal developmental toxicity studies of the 3 pigments are available. A screening study carried out according to OECD Test Guideline 422 yielded a NOAEL for maternal and perinatal toxicity of Pigment Yellow 12 of 1000 mg/body weight and day, the highest dose tested (NOTOX B.V. 2001).

All 3 pigments are insoluble and not bioavailable.

Microscale fractions, which are biopersistent granular dusts and thus, by definition, not available systemically, are classified in Pregnancy Risk Group C (General threshold limit value for dust (R fraction) (Biopersistent granular dusts); Hartwig 2014).

In order to account for the nanoscale fractions (< 100 nm), 2 studies of titanium dioxide and amorphous silica nanoparticles that were carried out according to OECD Test Guideline 414 were included in the evaluation. Three different types of ultrafine titanium dioxide particles and nanoscale amorphous silica were given by gavage to rats up to the limit dose of 1000 mg/kg body weight and day. Maternal and developmental toxicity were not observed in either of the studies (Hofmann et al. 2015; Warheit et al. 2015).

On this basis, Pigment Yellow 12, Pigment Yellow 13 and Pigment Yellow 83 have been classified in Pregnancy Risk Group C.

Absorption through the skin. Only one animal study is available for the evaluation of the absorption of the pigments through the skin. This study was carried out in rats with radioactively-labelled Pigment Yellow 12. After occlusive application for 1 day, no radioactivity was detected in systemic compartments. Relevant levels of absorption through the skin are not to be expected because the pigments have a high molar mass that exceeds 600 Da. For this reason, Pigment Yellow 12, 13 and 83 have not been designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. The clinical findings in humans are insufficient to evaluate the skin sensitizing effects of Pigment Yellow 12, 13 and 83. Studies in mice and guinea pigs reported only negative results. There are no findings of sensitizing effects in the respiratory tract available. For this reason, Pigment Yellow 12, 13 and 83 have not been designated with "Sh" or "Sa" (for substances which cause sensitization of the skin or airways).

Notes

Competing interests

The established rules and measures of the Commission to avoid conflicts of interest (www.dfg.de/mak/conflicts_interest) ensure that the content and conclusions of the publication are strictly science-based.

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